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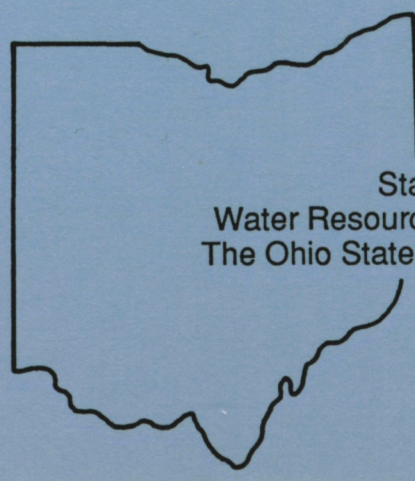
Report No. G-1607-02

FISCAL YEAR 1990 PROGRAM REPORT

Robert C. Stiefel
Director

United States
Geological Survey



State of Ohio
Water Resources Center
The Ohio State University

Report No.
G-1607-02

FISCAL YEAR 1990 PROGRAM REPORT

Grant No. 14-08-0001-G1607

for

U. S. Department of the Interior
Geological Survey

by

Water Resources Center
The Ohio State University
Columbus, Ohio 43210

Robert C. Stiefel, Director

September, 1991

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ABSTRACT

Water is one of Ohio's most important natural resources, and the State has an adequate supply to meet its immediate needs. Most of Ohio's water problems are associated with water quality. Of primary concern are the sediments, nutrients and acids in the surface waters from urban, agricultural and mining areas, and the toxic and hazardous wastes that threaten the ground and surface waters. The focus of the 1990 State Water Research Program was directed at some of these needs. One project explored the design criteria for an innovative two-stage fluidized bed bioreator in which the three major processesofcell immobilization, biodegradation, and biofilm control were combined in a single unit. Thisinnovative, reliable biological wastewater treatment process and design provides an efficient and environmentally safe waste water treatment system. Two projects explored the fate and transport of agricultural chemicals. One studied the potential impacts that interactions and reactions between herbicides and existing humic materials as they move through the soils toward the groundwater table. The other project studied the behavior of Nitrogen-heterocyclic compounds as they breakdown in the soil and their persistence in an aquifer. The other project studied the Scioto River buried valley aquifer. This research developed a ground-water management model for predicting water-quality changes associated with ground-water abstraction.

Training was provided to four students enrolled in three disciplines and two colleges at The Ohio State University.

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WATER PROBLEMS AND ISSUES OF OHIO

Water is one of Ohio's most important natural resources. Ohio is bounded on the north by Lake Erie and on the south by the Ohio River and contains other extensive ground and surface waters. Ohio has an adequate supply of water to meet its immediate needs. However, the combination of large, heavily industrialized urban centers; extensive agricultural activities; high volume coal production and large coal reserves; and the demands associated with new energy production continue to cause concern for water quality and water management.

In addition, extreme hydrologic events cause localized problems of both excessive water and water deficiencies at times.

Surface Water

The northern twenty-five percent of Ohio's area drains into Lake Erie, while the remaining area drains into the Ohio River. Runoff from Ohio's streams and rivers averages about 25 billion gallons a day. The state also receives nearly a billion gallons of runoff daily from the neighboring state of Indiana which drains through the Maumee River to Lake Erie; Ohio also has access to additional flows past its boundaries in Lake Erie and the Ohio River that total well over 150 billion gallons of water a day.

Last year, more than 16 billion gallons of water were withdrawn from Ohio's surface sources each day to meet the demands for municipal supplies; rural needs for domestic and livestock purposes; irrigation; and self-supplied industrial needs including cooling water for thermo-electric power generation. Each day these demands account for 60 percent of the available surface waters in the state's streams. Localized shortages develop only during certain dry seasons and periodic droughts.

The combined length of all the streams in Ohio approaches 44,000 miles, which means that there is approximately one mile of stream for each square mile of surface area in the state. In addition, there are more than 50,000 lakes, ponds and reservoirs within the state which have a combined surface area of 200,000 acres. About 6,700 acres occur naturally, while the remainder are man-made impoundments that range in size from small farm ponds to large multipurpose reservoirs.

The reservoirs in the state are used to provide water for many different purposes including municipal, agricultural and industrial supplies; stream flow augmentation; flood control; and recreation. No impoundments in Ohio, other than those on the main stem of the Ohio River, provide water for downstream navigation or hydro-electric power generation. However, there is extensive navigation on both Lake Erie and the Ohio River, and consideration is being given to the installation of low-head hydro-electric generators at several developed dam sites throughout the state.

Flooding is a major problem in Ohio and it affects both urban and agricultural areas. It has been estimated that nearly two million acres of land in Ohio are flood prone. This represents more than seven percent of the total area of the state and includes nearly four percent of those areas classified as urban regions. Average annual flood damages in Ohio vary from year-to-year but amount to several millions of dollars annually.

Ground Water

Ground water is an important part of Ohio's water resources. Ground water underlies most of the state but is predominant in the glacial drift in the northwest, in the ice-contact and outwash deposits in river valleys along the border of the glaciated areas, and in the bedrock of the western portions of the state. Ground water supplies are largest in the glacial valley-train deposits in those drainage basins which border the Ohio River including the Ohio, Miami, Little Miami, Scioto, Hocking and Muskingum Rivers. Well yields from these deposits often exceed 500 gallons per minute (gpm), while aquifers in the glacial drift in the northwest and west-central parts of the state produce yields between 100 and 500 gpm. Isolated aquifers in the northeast, northwest and southwest have yields between 25 and 200 gpm, while much of the northeast contains aquifers whose yield is between 5 and 25 gpm. With the exception of the valleys along the major streams, most of the aquifers in the area that is tributary to the Ohio River have yields less than 5 gpm.

Seventy-five percent of Ohio's 650 public water supply systems use ground water as their source. In terms of volume withdrawn, however, a lesser share of these supplies comes from ground water, almost a half billion gallons of ground water are withdrawn daily for public water supply purposes, while more than a billion gallons come from surface water sources. However, ground water supplies nearly 80 percent of the rural water needs in Ohio, 32 percent of the irrigation waters and 21 percent of the industrial water demands. Nearly one billion gallons of ground water are withdrawn in the state each day to meet these needs.

Water Quality

It is the water quality, rather than its quantity, that is the more critical and limiting condition associated with the use of both ground and surface waters in Ohio. The ground waters of the state frequently have relatively high, natural mineral contents; but, except for a few local areas, most of these waters are free from man-related contamination. Most complaints are related to increased levels of turbidity, bacterial populations and other substances from improperly sited or poorly constructed or maintained wells. Other problems are related to the spillage and leakage of brines and petroleum at oil wells in the southeastern part of the state; the mis-application of pesticides, herbicides and insecticides in agricultural areas: and the improper siting and operation of solid and liquid waste disposal facilities. Some minor ground water problems are also associated with the excessive use or improper storage of highway de-icing salts.

The dissolved solids concentrations in Ohio's streams range from 120 and 2,500 milligrams per liter (mg/l). The higher concentrations are found in the Tuscarawas, Cuyahoga and Grand Rivers and in other stream reaches below major municipal and industrial outfalls or in areas subjected to diffuse source runoff.

Of the 23,000 miles of principal rivers downstream of major urban areas in the state that have been monitored, 16,000 miles, or 70 percent of these streams, meet the current water quality standards. Where problems do exist, they are frequently caused by inadequate municipal wastewater treatment at facilities that need to be upgraded or expanded, or by combined sewer overflows. Sub-stantial improvements in surface water quality have resulted from the development of pretreatment regulations for industrial waste discharges to municipal sewerage systems. Violations of the state's water quality standards occur most often in dissolved oxygen levels; ammonia nitrogen concentrations; the numbers of fecal coliforms; and the levels of heavy metals such as lead, zinc, and cadmium.

Acid mine drainage is a major cause of water quality problems throughout the Appalachian Coal Basin in the eastern United States. In Ohio this region extends in a band approximately 50 miles wide in a southwesterly direction from the east-central to the south-central parts of the state. Acid drainage from abandoned and improperly operated or reclaimed coal mined lands causes a loss of water for domestic and industrial uses; the degradation of water quality for recreational purposes; a lethal impact on the aquatic life in a stream; and an accelerated deterioration of highways, railroad bridges and electrical transmission lines and towers. Drainage from abandoned coal mines, both surface and under ground, has impacted around 1,500 miles of streams in 27 counties in southeastern Ohio. Approximately 370,000 acres of abandoned strip mines, 7,000 acres of coal refuse piles and 3,000 underground mines are contributing to this problem. It has been estimated that four billion dollars would be needed to reclaim the abandoned mines and refuse piles throughout Ohio. Projected revenues from severance taxes earmarked for abandoned mine reclamation come to about ten million dollars annually. Obviously, the technologic problems and the economic costs associated with the control of acid mine drainage will continue to keep this a major problem of water quality in southeastern Ohio for years to come.

Little detailed information is available on the impacts that diffuse sources of pollution, such as agricultural and urban stormwater drainage, have on the quality of water in Ohio's inland streams. One concern with non-point pollution is the sediment that is dislodged from the land surface and carried to the streams. Of greater concern are the pollutants, such as the nutrients, heavy metals and toxic organic substances, that enter the streams attached to the sediments. There has been no need for intensive, non-point source control programs to meet water quality standards where Ohio waters drain into the Ohio River; but several studies are underway in the Lake Erie drainage basin to define the role of agricultural drainage on the water quality in the lake. Much more research and many more demonstration projects on the best management practices for agriculture, silviculture, mining and urban run off control must be conducted before this problem is fully understood and control measures can be instituted.

The trophic status of several lakes and reservoirs in Ohio has been studied; and the results suggest that the lakes and reservoirs in the sandstone bedrock areas of the state generally have lower trophic levels than those in the limestone bedrock areas or glaciated regions. Water quality was generally good to excellent in most of the lakes and reservoirs surveyed. However, excessive concentrations of copper and other heavy metals, bacteria and other pollutants that are normally associated with urban activities were identified in some of the lakes.

Recent studies on Lake Erie indicate that there has been a reduction in several key pollutants and a gradual, but steady, improvement in the water quality in the Lake during the past few years. Phosphorus is a major pollutant which results in the exces-sive growth of algae and other aquatic plants. As these plants die and decay, they deplete the oxygen resources of the Lake. The construction of facilities to remove phosphorus at the municipal wastewater treatment plants which discharge directly to Lake Erie has been a major factor in the reduction of phosphorus loadings and of the subsequent reduction of the anoxic areas within the Lake. Additional work on the control of phosphorus from both diffuse sources and point sources needs to be accomplished, but a significant start has been made.

Bacteria levels have been reduced in the nearshore zones where municipal wastewater treatment facilities have been constructed. This has permitted regulatory agencies to re-open bathing beaches which were often closed during the period between 1960 and 1970. Concentrations of mercury and pesticides have been reduced substantially, principally because of the federal bans that have been instituted on their manufacture, use and disposal. PCB remains a major challenge, as does the control of sediment and the nutrients, fertilizers and organic chemicals that are attached to it.

Fish populations, including the walleye pike, are beginning to increase again in the lake; but the quality and diversity of fish is still far from what they were in the past. Thermal pollution is a localized problem in some near-shore areas. However, as closed cycle cooling is required on all power generation facilities, the extent of this problem will diminish.

PROGRAM GOALS AND PRIORITIES

The Water Resources Center at The Ohio State University encourages and supports research that is directed at providing information needed to solve the major water problems at the local, state, regional and national levels. The research program at the Center includes basic or fundamental research, problem oriented or applied research, and information dissemination and technology transfer activities.

During FY 1982, the Center, in cooperation with several groups of water-related agencies and officials throughout the State prepared a prioritized list of Ohio's major water resources problems. Based upon this analysis, the following ranking of these problems was developed:

1. POLLUTION FROM DIFFUSE SOURCES - including agricultural runoff; urban runoff; runoff from on-site waste disposal systems; runoff from active, reclaimed or abandoned coal and strip mines.

2. CONTAMINATION OF DRINKING WATER SUPPLIES including surface and ground waters for both urban and rural uses by diffuse and point sources, and by the disposal of toxic and hazardous wastes on the land.

3. TOXIC AND HAZARDOUS WASTE DISPOSAL - including their control, treatment, disposal and impact upon land, water and air resources.

4. POLLUTION FROM POINT SOURCES - including municipal and industrial sources not yet in compliance with their NPDES permits.

5. IMPACTS OF FLOODING AND DRAINAGE - including flood damages, the use of flood plains and alternative structural and non-structural means of controlling floods and reducing flood damages.

6. IMPACTS OF WATER RESOURCES DEVELOPMENTS - including the impacts on various land uses caused by structural and non-structural water resources developments such as the extension of water mains and sewers into rural areas; flood control projects; hydro-electric power generation; water-based recreation; etc.

7. INSTREAM FLOWS NEEDS - including interrelationships among water quality, water quantity and land use practices on the instream flow needs for fish, wildlife, and recreation and the optimum development and protection of these instream uses.

8. IMPACTS OF SYNTHETIC FUEL DEVELOPMENT- including requirements for water and impacts of the disposal of wastes from these processes into waters and onto the land.

9. IMPACTS OF ATMOSPHERIC POLLUTION - including the effects of acid precipitation and atmospheric fallout on water quality and the environment.

10. ALLOCATION OF WATER RESOURCES- including the development of contingency plans for the allocation and conservation of limited water supplies among competing water users during periods of low stream flows.

Subsequently, the Directors of the Water Resources Research Institutes in the Great Lakes, Upper Mississippi and Ohio River Basin's met to identify their State problems the major water resources research priorities for the Region. A listing of these priorities is included at the end of this Section of this Report. The focus of the 1990 Ohio Water Research Institute Program was directed at some of these crucial needs.

The project by L. S. Fan entitled "Optimatization of a Novel Two-Stage Fluidized Bed Bioreactor Involving Immobilized Living Cells for Water Treatment" explored the design criteria for an innova-tive two-stage fluidized bed bioreactor in which the three major processes of cell immobilization, biodegradation and biofilm control were combined in a single unit. This innovative, reliable biological wastewater treatment process and design provides an efficient ,less costly and environmentally safe wastewater treatment system.

Drs. Traina and Logan of The Ohio State University Agronomy Department were specifically studying the potential impacts that interactions and reactions between herbicides and existing humic materials have on the fate and transport of the herbicides in the groundwater.

Drs. Sims and Traina of The Ohio State University Agronomy Department studied the fate and transport of agricultural chemicals by studying the behavior of Nitrogen-heterocyclic compounds in the subsurface environment.

Ohio State University researchers, Ward of Agricultural Engineering and Bair of Geology, studied the Scioto River Valley buried aquifer and designed a model to determine the possible contamination of the aquifer by agricultural chemicals. This project was the seed grant which lead to the Ohio MESA program, and should provide nearly $2 million for research annually.

The technology transfer programs of the Water Resources Center continued to disseminate information about the water resources of Ohio to local and state decision-makers and provided technical assistance.

Training on these research projects was provided at Ohio State University to four graduate students in the disciples of Agricultural Engineering, Agronomy, and Chemical Engineering.

REGIONAL RESEARCH PRIORITIES

Great Lakes - Upper Mississippi - Ohio River Region

A. Groundwater contamination

1. Track pollutants through the vadose zone to the ground water and determine their rate of dissipation in the aquifer.

2. Assess the impacts of the disposal of municipal and industrial wastes and effluents on ground water systems.

3. Evaluate sources of recharge of the principal aquifers in the region.

4. Determine the effects of the storage of waste heat in aquifers on groundwater quality.

B. Pollution of lakes and streams from non-point sources

1. Assess relative effectiveness of non-point pollution control "best management practices" to meet the demands of P.L. 92-500.

2. Evaluate the effects of atmospheric fallout and precipitation (acids, toxic metals and hazardous trace organics) on public health and the aquatic environment.

3. Estimate the effects of drainage from land use activities in urban areas on surface water quality.

4. Model sediment transport processes and devise techniques for determining sediment delivery ratios.

5. Determine the relative effectiveness of voluntary programs enhanced by various incentives and regulation as mechanisms of implementing non- point pollution control.

6. Predict the impacts that agricultural technologies will have on surface and groundwater resources.

C. Adverse water resources impacts of energy production and mining.

1. Evaluate the impacts that drainage from mining activities will have on the incursion of acids, toxic metals, radio nuclides and hazardous organic compounds into the environment.

2. Assess atmospheric and aquatic pollution from coal-fired electric generation plants.

3. Assess legal, economic, environmental and social impacts and develop means for resolving water user conflicts associated with siting, constructing and operating energy conversion facilities and mining operations.

4. Examine the potential benefits, public and environmental, from the reclamation of heated waters from power generation.

D. Potential insufficiency of waters for agricultureand rural communities

1. Determine optimal water requirements for crop production and develop practical methods for irrigation scheduling.

2. Evaluate criteria for establishing minimum requirements for the drainage of imperfectly drained soils of the region.

3. Develop water conservation practices and methods for holding and temporarily storing surface and drainage waters for reuse in periods of seasonal suboptimal precipitation.

E. Loss and degradation of water based fish and wildlife habitat

1. Define the functional and economic value of wetlands including ecological and hydrological mechanisms that influence their integrity.

2. Develop acceptable mechanisms, including incentives and legislation, for preserving publicly and privately owned wetlands.

3. Determine the quality and quantity of instream flow necessary to maintain an active and viable aquatic biota.

4. Determine the potential and incentives needed to increase wildlife and waterfowl production on private lands.

F. Miscellaneous

Develop the relationship between commercial/commodity and recreational use of the major lake and river systems of the region. Research emphasis should be placed on development of sufficient water-based recreational facilities in urban settings.

SYNOPSIS

Project Number: 02

Start: 07/89 (actual)
End: 06/91 (actual)

Title: Optimatization of a Novel Two-Stage Fluidized Bed Bioreactor Involving Immobilized Living Cells for Water Treatment

Investigator: Fan, Liang-Shih, The Ohio State University, Columbus

COWRR: 05D **Congressional District:** Fifteenth

Descriptors: Optimatization, waste water treatment, fluidized bed, immobilized cell, phenols, biodegradation

Problem and Research Objectives:

Stricter regulations for drinking water quality and industrial effluent pollution will require wastewater treatment systems that are more efficient and economical than present methods. Fluidized bed bioreactors are proving to be such systems. This system uses microorganisms to cleanse the water while conserving land and protecting the environment. The problems encountered on earlier fluidized bed bioreactor systems are the time required for biofilm cultivation before start-up; the need for frequent monitoring of particle replenishment; and quality control of the biofilms attached to the particle carriers.

For this two-year research project a novel two-stage, three-phase fluidized bed bioreactor was developed which minimizes the problems of conventional bioreactors and enhances the efficiency of the waste water treatment process. This research project studied the effects of several operating variables including liquid flow rate, gas flow rate, inlet substrate concentration and temperature on the bioreactor. The ultimate goal of this research was to obtain the optimum operating conditions required for waste water treatment applications.

Methodology:

The overall reactor performance is characterized by the biological activity of the immobilized cells, bioparticle separation, biofilm regulation, and reactor stability. The optimum operating conditions are obtained based on (1) the efficiency of the particle separation between stages of the bioreactor, (2) the characteristics of the biofilm removal device, (3) the overall biodegradation rate, and (4) the bioreactor's stability during various operating conditions. Experiments at both steady-state and dynamic conditions were conducted. Evaluation was done on the effects of the operating conditions, including the air and liquid flow rates, bioparticle loading, particle size, biofilm thickness, influent-phenol concentration, on the overall biodegradation rate of the two-stage fluidized bed bioreactor.

Phenol was selected as the model substrate because of its inhibitory properties representing toxic pollutants. Measurements of biofilm thickness, particle holdup, and substrate concentra-tions in both stages were needed to study the behavior of the two-stage bioreactor. Particle holdups in both stages were obtained by the free flow method. Biofilm thickness was measured by microscopic visualization. Phenol concentrations were determined by the 4-aminoantipyrine colorimetric method.

Principal Findings and Significance:

Experiments were performed at various operating conditions to evaluate overall removal rates of the two-stage fluidized bed bioreactor. The optimum operating conditions for phenol biodegradation were obtained based on the results of the phenol removal rates and the bioreactor's stability under loading shock situations.

Optimal bioparticle separation is found when the biofilm thickness demarcation is set at 50-70 μm by examining the bioparticle size distributions in both stages of the bioreactor at various liquid flow rates and separation device configurations. It is observed that the efficiency drastically decreases when the demarcated biofilm thickness is less than 50 μm. It is impractical to select a demarcation larger than 70 μm, as the overall phenol removal rate is reduced by substrate deficiency in the inner part of the biofilms.

Efficient biofilm removal occurs in the reactor when the flow rate is increased. The efficiency depends on the thickness of the biofilm of the particles to be treated. For a given flow rate the efficiency is almost constant for large biofilm thicknesses, whereas, for small biofilm thickness the efficiency decreases. When the thickness is below a minimum value, however, no biofilm removal takes place. This minimum value decreases with increasing air flow rate.

Phenol degradation using biological means was conducted in the two-stage fluidized bed bioreactor under different solid holdups and inlet phenol concentrations. As a steady state, the outlet phenol concentrations below 1 ppm with 4.89 kg-phenol/m^3. day removal rate were

obtained for inlet concentrations up to 200 ppm; with 0.64 m^3/day liquid flow rate at 4.0 volume percent bioparticle loading. However, at the same influent phenol concentration and liquid flow rate with 1.0 volume percent bioparticle loading was 3ppm of effluent phenol. This result is higher than the current EPA requirement for phenol waste effluent. The steady-state results also reveal that the degradation rates in the two-stage fluidized bed bioreactor are superior to those in a single-stage fluidized bed bioreactor.

Under dynamic situations, two phenol removal periods were identified-adsorption and biodegradation. In the adsorption period, phenol removal was primarily achieved by adsorption through the large internal surface area of activated carbon particles. In the dynamic experiments this large adsorption capacity of activated carbon particles tolerated sharp loading changes. After the adsorption period, the outlet phenol concentration increases to a maximum level and then decreases to a steady state value. For example, if the influent concentration is less than 200 ppm, for a sharp increase in phenol loading of about 50 ppm, the steady state outlet concentration is less than 1 ppm, which is attained within 40 hours after the loading increase. For 250 ppm influent concentration, the outlet concentration do not return to a satisfactory level. This reaction is probably due to the phenol inhibition on the cell activity.

In summary, the research completed during this year focused on bioreaction engineering and process optimization aspects of the two-stage fluidized bed bioreactor for wastewater treatment. The two primary considerations in the design of this bioreactor are the stability of the bioreactor and the longevity of operation. The optimization of the operating conditions for the bioreactor is examined in terms of the overall biological removal rate with the efficiencies of bioparticle separation between stages and of the biofilm removal. Results show that the two-stage bioreactor outperforms one-stage bioreactors in the aspects of (1) overall pollutant removal rates, (2) reactor stability, and (3) the continuous operation with less human intervention.

Publication and Professional Presentations:

(1) "Characteristics of Fluidized Bed Bioreactors with Immobilized Cells for Anaerobic Wastewater Treatments," paper to be presented at the AIChE Annual Meeting, Los Angeles, November 12-17, 1991.

Training

M.S. theses: None

Ph. D. dissertation:

(1) "Study of Fluidized Bed Reactor-Fluid Dynamics and Bioreactor Applications," Jing-Wen Tzeng, Dissertation, The Ohio State University, Columbus, OH, December, 1991

SYNOPSIS

Project Number: 03

Start: 07-01-89 (actual)
End: 06-30-91 (actual)

Title: Effect of Sorption On Biodegradation Of Nitrogen-Heterocyclic Compounds In Subsurface Materials

Investigators: Sims, Gerald and Traina, Samuel, The Ohio State University, Columbus

COWRR Category: 05B **Congressional District:** Fifteenth

Descriptors: Groundwater quality, organic compounds, pollutants, soil microbiology, trace organics

Problem and research objectives:

The purpose of the proposed research is to determine the role of sorptive processes in the biodegradation of N-hetrocyclic compounds in subsurface environments. Contamination of groundwater by organic pollutants has become a serious environmental problem. One-fourth of all freshwater in the U. S. comes from groundwater and groundwater is the major course of drinking water for rural families. Contamination of groundwater reserves has become much more common in the last decade. For example, in 1986 seventeen pesticides were detected in the groundwater of twenty-three states. Other pollutants, including heterocyclic aromatic compounds have been detected in appreciable quantities in groundwater. Attempts to predict persistence and transport of organic pollutants in the environment have recently been proven unsuccessful in some cases. As a result we are now finding compounds originating from a variety of agricultural and industrial activities in surface and groundwaters,. Once in the subsurface environment, even the most labile organic compounds tend to become persistent, and therefore require some remedial action for removal. Remediation of contaminated aquifers is difficult, especially when there is limited data on the behavior of toxic substances in the subsurface environment, as well as organisms which may detoxify them.

The pollutants under investigation include N-heterocyclic compounds (pyridine, alkylepyridines, and quinolines) which are organic pollutants detected in groundwater in the United States, Europe, Asia, and Australia. Understanding the fate of these compounds, and the potential for their removal from aquifers, depends upon understanding the factors which control biological degradation, the primary route for natural detoxification of organic pollutants. This research specifically addressed the role of sorption in the biological degradation of organic pollutants in contaminated subsurface materials. The results of these investigations will be directly applicable for the refinement of existing fate/transport models used to predict the movement of pollutants to groundwater, as well as prediction of die-off kinetics for contaminants in polluted aquifers.

The long range significance of this research will be the development of a knowledge base which will lead to understanding the environmental fate of organic pollutants in subsurface environments. Specifically addressed will be the role of sorption in the bioavailability and hence the biodegradation of pollutants by indigenous microorganisms in subsurface sediments. *In situ* biodegradation is among the most frequently proposed means for remediation of polluted aquifers. Generally, intervention consists mostly of preventing offsite migration of the contaminant plume, and some form of stimulation of the indigenous microbial community, based on overcoming factors limiting the activities of the organisms. Unfortunately, little is known of the factors limiting microbial activity in the subsurface, and almost nothing is known of the mechanisms limiting biodegradation of organic compounds at the low concentrations normally present in the interstitial water of an aquifer. Our laboratories are presently involved in an investigation to determine what limits biodegradation of pyridine derivatives in an aquifer which has been contaminated with relatively large concentrations of these compounds for a number of years. One of the most important leads we have found for determining how to stimulate microbial activity toward organic pollutants is that in many cases, the potential of the soil or sediment matrix to adsorb or complex an organic compound appears to affect the rate of biodegradation of the compound in the environment. Missing from the literature are experiments which clearly link sorption to decreases in bioavailability, particularly with respect to subsurface materials. At the very least, the results of these experiments, will be useful in predicting kinetics of degradation of strongly adsorbing pollutants in surface and subsurface environments, and will also be used to develop procedures to increase the availability of sorbed pollutants to microorganisms, in order to enhance kinetics of biodegradation. These experiments are unique in the use of subsurface aqueous and solid materials from a well described contaminated aquifer.

Methodology:

Sediments and groundwater samples were contributed by Rielly Industries as a part of a related project which examines in-situ biodegradation of pyridines in a contaminated aquifer at Indianapolis, Indiana. Sediments were taken from both upgradient and downgradient of the source of contamination, and therefore provided both acclimated and non-acclimated microbial communities. Sediments were obtained under a nitrogen blanket to protect organisms from excessive exposure to the atmosphere, and were kept chilled until arrival at Pennsylvania State University, where they were split into subsamples for use at Penn State University and Ohio State University. Care was taken to minimize infusion of drilling fluids into the sediment samples.

Extraction/concentration procedures. The polar nature, and high volatility of pyridine and methylpyridines limits recovery of the compounds in conventional organic extraction procedures, particularly when a concentration step is involved. Concentration of samples (typically by solvent evaporation) containing pyridines may result in low recoveries due to inherent polarity (low extraction efficiency), or volatility (loss as vapor from the concentration apparatus). Therefore extraction/concentration procedures were developed to provide high recoveries of low concentrations of pyridines in environmental samples.

Pyridine and alkylepyridines were extracted from soil and sediment samples with 2 M KCl + 0.1 M KH_2PO_4/H_3PO_4 (pH 2.5) by shaking (sediment/solution ratio = 1:10 w/v) for 30 minutes. The aqueous extract was centrifuged at 10,000 X g for 15 minutes (Teflon labware). The supernatant was adjusted to pH 8 with KOH (2M), filtered through glass, and loaded onto a C_{18} solid phase extraction column. Salts were washed through the column with organic free water, and the pyridines are eluted with isopropanol (1 ml). The procedure works because pyridines are retained in soils and sediments primarily by ionic interactions of the cationic species with exchange sites on mineral surfaces, and can be displaced by flooding samples with K^+. Adjusting the pH of the extract to 8 dissociates the pyridines (which have pKa values ranging from 5.2 for pyridine to 7.4 for collidines), and forming neutral species that facilitate sorption to the SPE column. The presence of KCl in the extract appears to favor partitioning of the pyridines in the extract onto the SPE phase.

Concentration by solid phase extraction from aqueous samples was chosen over concentration by evaporation of solvent in organic systems due to the high volatility of pyridines. The SPE method yielded recoveries over 90 percent for nearly all of the pyridine derivatives examined. Concentration was necessary for determination of contaminants present below the detection limit of our instruments, especially gc/ms.

Gas Liquid Chromatography

Several column stationary phases have been tested for separation of pyridine derivatives. The greatest success has been obtained with 30 m Carbowax capillary column. This polar phase was

the only one tested which allowed baseline separation of virtually all commercially available methylpyridines. Best separation was achieved with a temperature program starting at 65° and holding for one minute, ramping temperature to 110°C at a rate of 1 or 2 ° per minute, and holding at 110°C for five minutes.

Thermionic specific detection has been extremely successful for analysis of trace quantities of pyridines. Excellent recoveries were obtained from soil samples spiked with 1 ppb of each of 11 pyridines tested. Mass detection has also been employed for analysis of pyridines, however sensitivity was much poorer with this technique. Sensitivity was improved by the use of targeted fragments representative of known contaminants within the matrix. Since fragmentation patterns were similar among pyridines differing in only the position of ring substituents, it was important that baseline separation was achieved for confirmation of identity by retention indices. The use of specific fragments for mass detection is being explored at this time.

High Pressure Liquid Chromatography

Several phases have been tested for their performance in the separation of pyridine and alkylepyridines. These include C_8, C_{18}, and a cation exchange packing. Various mobile phases and gradients have also been employed. Some of these phases (esp. ammonium acetate/ methanol) were compatible with the HPLC/ms system available to us. It should be noted that separation by capillary gc was more successful than HPLC in our laboratory. The most successful HPLC program utilized a gradient of methanol and pH when a C_{18} column (Applied Biosystems Spheri-5 RP-18, 220 X 4.6 mm) was used. The pH/methanol gradient was achieved by varying the ratios of the two mobile phases below.

A: 40% methanol/60% 0.05 M KH_2PO_4, pH 7.5

A: 50% methanol/50% 0.05 M KH_2PO_4, pH 4.0

Biotransforming/biodegradation activity. Concurrent with attempts to isolate heterocyclic ring degraders, a preliminary experiment was initiated to determine if pyridines were being degraded in the sediment samples. Solid samples were mixed to capacity with organic-free water and incubated 25 °C. Incubations were aerobic, in the dark. Samples were taken at intervals (weekly for the first two weeks of the experiment, biweekly thereafter), and contamin-ants determined as follows. Subsamples (1 g) were extracted with acidified KCl (10 ml) as described above, concentrated (to 1 ml) and analyzed by gc. At the beginning and end of the experiment, 50g samples were extracted, and concentrated into one ml of isopropanol. This greater concentration factor facilitated detection of pyridines to at least 0.1 ppb (of sediment), and allowed us to examine changes in the concentrations of trace contaminants in the sediments. It also concentrated major contaminants to adequate concentrations for gc/ms analysis.

Enrichment for pyridine and alkylepyridines degraders. Sediment samples were used as inocula for enrichment media containing pyridine, 2-, 3-, or 4-methylpyridine (100 mg L^{-1}) as sole sources of C and N. Samples were removed periodically and streaked onto agar media containing the compounds of interest. Isolates obtained by this procedure were tested for the ability to degrade the substrates in liquid media. Growth of these cultures was determined by optical density measurements which had been calibrated to cell number and biomass (protein). Degradation of the substrate was determined by gc and HPLC. Isolates were identified by cell morphology and Gram reaction, as well as membrane fatty acid analysis. A 2-methylpyridne degrading bacterium was chosen for further study.

Catabolic activity of 2-methylpyridine degrader. Growth response of isolate R1 was determined over a range of pH and 2-MP concentrations. The isolate was cultured in liquid mineral salts media containing either 2.15 mmol L^{-1} 2-MP or 2.78 mmol L^{-1} d-glucose at pH 4,5,6,7,8, or 9. The glucose medium was amended with 1.37 mmol L^{-1} $(NH_4)_2SO_4$. The isolate was also grown in minimal medium containing 0, 0.054, 0.11, 1.07, 10.74, or 53.68 mmol L^{-1} 2-MP at pH 7. The cultures were incubated at 25 °C on a rotary shaker. The cultures were subsampled at regular intervals for measurement of pH and growth. Growth was determined as a function of optical density (O.D.) measured at 660 nm. The catabolic activity of the isolate was surveyed over a range of substrates including hydroxy and alkylpyridines, polycyclic aromatic *N*-heterocycles (PANHs), selected substituted benzoates and low molecular weight organic acids (Table 1). Substrates were added at a concentration of 100 mg L^{-1} (0.51 to 2.17 mmol L^{-1}) (with the exception of acridine, which has an aqueous solubility limit of approximately 38 mg/l) to liquid mineral salts media containing 0.54 mmol L^{-1} $(NH_4)_2SO_4$, and 30 mg L^{-1} yeast extract. All media were filter sterilized using 0.45 µm Millipore™ Type HA membrane filters. The cultures were incubated at 25 °C. Growth was measured by O.D. at 660 nm. at 24 h intervals.

2-Methylpyridine degradation. Sterile liquid mineral salts medium containing 1.20 mmol L^{-1} 2-MP was placed in seven side-arm erlenmeyer flasks. An eighth flask that contained media without 2-MP was included as a control. Two sources of inocula were used. Cells were cultured on either 2.15 mmol L^{-1} 2-MP or 5.55 mmol L^{-1} glucose in liquid basal salts medium. The glucose medium also contained 1.89 mmol L^{-1} $(NH_4)_2SO_4$ and 0.25 g L^{-1} yeast extract. The cells from both cultures were harvested in log phase by centrifugation at 1.0×10^4 g for 15 min at 25 °C. The cells were washed twice and resuspended in mineral salts medium and diluted to a uniform cell density. Three of the seven flasks were inoculated with the 2-MP grown cells, while another three were inoculated with the glucose grown cells. The flask containing media without 2-MP was inoculated with 2-MP grown cells and served as a control for background growth. The remaining flask was not inoculated. All cultures were incubated at 25 °C with agitation on a rotary shaker. Growth was measured as O.D. 660 nm.at two h intervals. Samples were collected for analysis of 2-MP and ammonium at four h intervals. The concentration of 2-MP in the samples was determined by reverse phase HPLC using an Alltech Econoshpere™ C_{18} 220 by 4.6 mm 5 micron column with UV detection at 262 nm. The system was run under isocratic conditions with a mobile phase consisting of 50:50 (v:v) 50 mmol L^{-1} ammonium acetate (99% Fisher

Scientific):methanol (HPLC grade, Fisher Scientific) at a flow rate of 1 m/min. NH_4 was determined by the indophenol blue method (Keeney and Nelson, 1982); the procedure was scaled down to accommodate small sample volumes.

Characterization of quinoline degrader. An organism capable of growth on quinoline as a sole source of carbon and nitrogen was previously isolated from a mixed inoculum containing several soils and paper mill sludge. Experiments were conducted to identify this organism, and to characterize its growth characteristics and use of quinoline. These experiments were needed to establish condi-tions for the use of this organism in sorption/bioavailability experiments to be conducted in the second year.

Materials and Methods for Isolation and Characterization of Quinoline Degrader

Membrane Fatty Acid Analysis

Fatty acid methyl esters were prepared from bacterial cell membranes and subsequently, separated and quantified by high resolution capillary gas chromatography. The procedure was performed with the Microbial Identification System (MIS; Hewlett-Packard Corp., Avondale, PA.).

Mycolic Acid Analysis

Thin-layer chromatography (TLC) for the presence of mycolic acids in whole-cell methanolysates was performed by the method of Minnikin et al. (1980). Methanolysates were spotted onto silica gel-coated glass plates (Baker Si250). The solvent system for single-dimensional development was petroleum ether (bp 60-90° C) acetone (95:5, v/v).

Electron Microscopy

Succinate-and quinoline-grown cells were fixed in four percent glutaraldehyde and Sorenson's phosphate buffer, washed with buffer and fixed with 1 percent OsO_4. The cells were encased in agar blocks, which were cut into 0.5 mm square fragments, dehydrated with ethanol, and washed in propylene oxide. The samples were embedded in Epon 812 and sectioned with an MT-1 microtome. Transmission electron micrographs of the sections were obtained on a Philips electron microscope.

Growth Characteristics

The optimum pH and temperature for growth were determined in 1000 ug/ml glucose: NH_4^+-N media C:N = 5:1. Growth on inorganic nitrogen sources was tested in 1000 ug/ml glucose-basal salts media supplemented with $(NH_4)_2 SO_4$, KNO_3, or KNO_2 to give a C:N of 5:1. Growth was measured as a function of optical density at 660 nm using a Beckman DU-40 UV-VIS

spectrophotometer. Urease activity was determined by growth on Christensen Urea Agar (Smibert and Krieg, 1981). Cells were assayed for spore-forming ability by growth on A K Agar #2 with subsequent malachite green endospore staining (Kolodziej et al., 1980).

Determination of Quinoline Utilization and Degradation

Cells were cultured in liquid basal salts media buffered at pH 8, containing initial quinoline concentrations ranging from 0 to 1000 ug/ml. Growth was measured as a function of protein concentration determined by the method of Lowry. Aliquots collected from cultures containing 200 ug/ml quinoline were loaded onto C_{18} Solid Phase Extraction (SPE) columns. NH_4^+-N in the eluent was determined colorimetrically by the indophenol blue method (Keeney and Nelson, 1982). The components retained on the column were eluted with isopropanol. Quinoline in the eluent was determined by a Varian 3700 capillary gas chromatograph (GC) with a thermionic specific detector.

Large Scale Study of Quinoline Degradation and Metabolite Production

Large (800 ml) cultures containing 200 ug/ml quinoline adjusted to pH 8 were incubated at 25° C. Samples were collected over time, and quinoline concentrations in the medium were analyzed as described above. Aliquots (100 ml) were concentrated in 1 ml isopropanol by SPE, as described above. Samples were analyzed by C_{18} reverse phase High Performance Liquid Chromatography (HPLC) with a UV detector at 254 nm in a mobile phase containing 30:70 methanol : 0.02 M ammonium acetate (pH 6.9). Fractions were collected from the peak of an unknown compound which coeluted with authentic 2-hydroxyquinoline. UV spectra of the unknown compound and 2-hydroxyquinoline were obtained using a Shimadzu UV-160 Recording Spectrophotometer. Additionally, fluorescence spectra of the unknown and standard were acquired with a Perkin-Elmer LS-5 Fluorescence Spectrophotometer. The solvent was removed from pooled fractions by rotary evaporation. The purified residue was analyzed by Fast Atom Bombardment Mass Spectroscopy (FAB-MS). An exact mass-spectrum (Electron Impact (EI)) was also obtained.

Utilization of Putative Quinoline Metabolites

Growth of the isolate on putative quinoline metabolites was measured by O.D. at 660 nm. The isolate was grown in basal salts media containing 100 ug/ml of one of the following substrates: 2-hydroxyquinoline, 4-hydroxyquinoline, 8-hydroxyquinoline, 2,4-dihydroxyquinoline, or 2,3-dihydroxypyridine.

Estimation of Molecular Weight of Pigment

An estimation of the molecular weight of a green pigment produced during quinoline degradation was accomplished using a Millipore Immersible Molecular Separator.

Effects of Sorption on Bioavailability

The effects of sorption on the biodegradation of pyridines were examined in model systems using reference clay minerals. Due to the availability of a well characterized degrader (isolate R1) 2-methylpyridine was chosen as the model compound for these experiments.

Preparation of clay mineral suspensions. Samples of a poorly-crystallized kaolinite from Washington County, Georgia (KGa-2), illite from Silver Hill, Montana (IMt-1), hectorite from San Bernardino County, California (SHCa-1), and a Na-montmorillonite from Crook County, Wyoming (SWy-1) were obtained from the Clay Minerals Society's Source Clays Repository. A vermiculite from Libby, MO was obtained from Zonolite Co. (a division of W.R. Grace & Co, Travelers Rest, SC). Twenty g samples of each clay were washed with 200 mL of 1 mol L^{-1} NaCl, followed by three washes with 200 mL of 50 mmol L^{-1} NaCl and three washings with 200 mL of distilled, deionized water. To ensure complete dispersion of the kaolinite, the pH of the suspension was raised to 9 with the addition of NaOH. The $\leq$2 mm size fraction of the clays was collected by sedimentation. The $CaCO_3$ contained in the hectorite suspension was removed through successive washing with 200 mL of pH 3, 20 mmol L^{-1} NaCl. The clay suspensions were washed three times with 200 mL of mineral salts medium, essentially that of Houghton and Cain (1979) (3.35 mmol L^{-1} KCl, 1.04 mmol L^{-1} $MgSO_4$, 0.29 mmol L^{-1} K_2HPO_4, 4.70 mmol L^{-1} KH_2PO_4, and 1 mL L^{-1} trace elements solution) diluted four fold, prior to use. Thirty g of Dowex-50W-X8 100-200 mesh (J.T. Baker Chemical Co.), a strongly acidic cation exchange resin, were prepared for use by washing with 500 mL 1 mol L^{-1} NaCl, followed by three washes with 500 mL distilled, deionized water, and two washes in 500 mL ten fold strength mineral salts medium. Prior to use, the resin was washed three times with dilute mineral salts medium (DMSM).

Determination of cation exchange capacity (CEC). CEC values for the clays and Dowex- were obtained using a mechanical extractor as described by Jaynes and Bigham (1986). Briefly, samples of the stock clay suspensions were prepared by freeze drying. One g samples were thoroughly mixed with 0.5 g of Celite powdered paper pulp and transfered to a 60 mL syringe containing 1.0 g of analytical paper pulp. The sample syringes were placed in the mechanical extractor (Concept Engineering Inc, Lincoln, NE) and attached to the extraction syringes. The clays were extracted with sites were saturated with Ca^{2+} through successive extractions with 1 mol L^{-1} $CaCl_2$. The Ca^{2+} was displaced by extraction with 1 mol L^{-1} $MgCl_2$. CEC was determined by analysis of the displaced solution for Ca^{2+} by atomic absorption spectroscopy using a Varian Techtron atomic absorption spectrophotometer model AA6.

Adsorption isotherms. Adsorption isotherms were determined by batch equilibrium. Stock clay suspensions were diluted with DMSM to a density of 6 g solids L^{-1} suspension. The prepared suspensions were adjusted to pH 6.0 with either HCl or NaOH. Four mL aliquots of suspension were placed in 8 mL-capacity, borosilicate, screw-cap test tubes. The suspensions were autoclaved for 20 min at 18 kPa and 121 °C. Stock solutions containing 2-MP (98 percent purity, Aldrich Chemical Co.) in DMSM were prepared at concentrations from 0 to 600 mmol L^{-1}, and

filter sterilized through 0.45 mm Nuclepore® nylon membrane filters (2-MP was added separately to avoid volatilazation losses during autoclaving). When the suspensions had cooled to ambient temperature, 2 mL aliquots of the 2-MP stock solutions were added and the tubes were sealed. All tubes contained 6 mL of suspension with a solids to solution ratio of 4 g L^{-1} and from 0 to 200 mmol L^{-1} total 2-MP. All treatments were replicated. The suspensions were equilibrated for 24 h at 25 °C on a rotary shaker.

After equilibration, subsamples were collected for determination of solution phase 2-MP. One mL of each suspension was placed in a 2.0 mL capacity siliconized polypropylene micro-centrifuge tube. The samples were centrifuged at 1.65×10^4 g for 10 min at ambient temperature in an Eppendorf® 5415 C microfuge (Brinkman Instruments Inc., Westbury, NY). A 0.75 ml aliquot of the supernatant was placed in 2.0-mL autosampler vials with screw-caps and Teflon® lined silicone septa; 0.75 mL 50:50 (v:v) of HPLC grade methanol (Fisher Scientific): and 50 mmol L^{-1} ammonium acetate (99 percent Fisher Scientific) in HPLC grade water (Fisher Scientific) were added to each. To determine the extractability of 2-MP from the clay suspensions, 0.75 mL of each suspension was placed in micro-centrifuge tubes containing 0.75 mL ammonium acetate saturated methanol. The tubes were placed in a sonic bath for 30 min followed by 15 min on an end to end shaker. The samples were centrifuged and placed in vials as described above. The concentration of 2-MP in the samples was determined by reverse phase HPLC using an Alltech Econoshpere C_{18} 220 by 4.6 mm 5 mm column with UV detection at 262 nm. The system was run under isocratic conditions with a mobile phase consisting of 50:50 (v:v) 50 mmol L^{-1} ammonium acetate in HPLC grade water:HPLC grade methanol at a flow rate of 1 ml min^{-1}.

Bioavailability of Adsorbed 2-methylpyridine

Culture of 2-methylpyridine degrading bacterium. An *Arthrobacter sp.* capable of utilizing 2-MP as the sole source of carbon, nitrogen, and energy was isolated from subsurface sediments as described in Chapter III. Cells were cultured in 1 L sterile DMSM with 50 mg yeast extract (added to supply any essential growth factors) and 5.0 mmol L^{-1} 2-MP. The latter was added aseptically after autoclaving. In late log phase, 200 mL aliquots were placed in four 250-mL polyallomer centrifuge bottles. The cells were collected by centrifugation at 6.38×10^3 g at 20 °C for 10 min in a Beckman J2-21 centrifuge. The cells were washed with sterile DMSM and centrifuged again. The pellets were resuspended in 50 mL DMSM, and the cell suspensions were pooled in one bottle and centrifuged. The inoculum was resuspended to a uniform cell density standardized by measurement of optical density at 660 nm. Cell numbers were determined by colony counts on spread plates following serial dilution.

Biodegradation experiments. Stock clay suspensions were diluted with DMSM to a density of 4 g solids L^{-1} suspension. The prepared suspensions were adjusted to pH 6.0 with either HCl or NaOH. Seventy-five mL aliquots of suspension were placed in 125 mL erlenmeyer flasks

stoppered with polyurethane foam plugs. Four flasks were prepared for each clay. Flasks without clay were prepared containing 75 mL DMSM. The flasks were autoclaved for 20 min at 18 kPa and 121 °C. A stock 2-MP solution containing 9.0 mmol L^{-1} was filter sterilized through 0.45 mm Nuclepore nylon membrane filters. When the suspensions had cooled to ambient temperature, 1 mL aliquots of the 2-MP stock solution were added and the suspensions were equilibrated for 24 h at 25 °C on a rotary shaker at 180 rpm. All flasks contained 75 mL of suspension with a solids to solution ratio of 4 g L^{-1} and 120 mmol L^{-1} 2-MP. All treatments were replicated three times.

After equilibration and prior to the addition of inoculum, subsamples were collected from the flasks for analysis of solution phase and total 2-MP in the systems. Aliquots (0.75 mL) of each suspension were placed in a 2.0-mL capacity, siliconized, polypropylene, micro-centrifuge tubes. The samples were centrifuged at 1.65 X 10^4 g for 10 min at ambient temperature in an Eppendorf 5415 C microfuge. A 0.50 mL aliquot of each supernatant was placed into a 2.0 mL capacity autosampler vial with a screw-cap and Teflon-lined silicone septum: 0.50 mL 50:50 (v:v) of HPLC grade methanol): and 50 mmol L^{-1} ammonium acetate in HPLC grade water were added to each vial. To extract 2-MP from the clay suspensions, 0.75 mL of each suspension was placed in micro-centrifuge tubes containing 0.75 mL ammonium acetate saturated methanol. The tubes were placed in a sonic bath for 30 min followed by 15 min on an end to end shaker. The samples were centrifuged and placed in vials as described above. These samples represent the status of the systems at time zero, moments prior to inoculation. For each treatment, three of the four flasks were inoculated with 0.8 mL of the cell suspension prepared above. The uninoculated flasks served as sterile controls. All flasks were incubated at 25 °C in an orbit shaker at 180 rpm. The flasks were sampled at regular intervals as previously described. The concentration of 2-MP in the samples was determined by reverse phase HPLC as described above.

RESULTS

Analysis of Sediment Samples

Analysis of sediment samples by gc revealed a large number of substances eliciting response from the thermionic specific (NP) detector. Since this detector is specific primarily to compounds containing N or P, it was thought likely that these peaks were due to pyridines or related compounds. Over the course of incubation, most peaks disappeared, and some new peaks appeared. Eventually, the chromatogram was nearly flat, suggesting pyridines were being degraded in the sediment.

GC/MS analysis of concentrated samples was performed to reveal the identities of these peaks. Procedures used were as follows:

Sediment (50 g) was extracted with KCl as described above. This extract was concentrated by solid phase extraction, and recovered in 1 ml of isopropanol, e.g. a concentration factor of 50. This sample was analyzed by e.i., gs/msd (HP 5970 instrument). The detection limit for pyridines

in the HP gc/msd is probably around 10 ppm under these conditions. With a concentration factor of fifty, the sediments probably contained around 0.1 ppm or more of each of the compounds identified. Quantitative data from the NP detector suggested that many of them may be present at 1 ppm or more.

The following substances were selected by Wiley database search:

4-methylpyridine	3-methylpyridine
2,5-dimethylpyridine	2,3-dimethylpyridine
2-propylpyridine	2-ethylpyridine
methylpyridinemethanamine	3-ethylpyridine
3-ethyl-4-methylpyridine	benzenemethanamine (2-ethylpyridine)
2-ethenylpyridine	2,6-dimethylpyridine
3-ethyl-4-methylpyridine	3,4-dimethylpyridine
4-propylpyridine	m-ethylanaline
5-ethyl-2-methylpyridine	4-ethyl-3-methylpyridine
5H-2-pyridine,6,7-dihydro-4,	7-dimethyl

It should be noted that some of the matches were less than 50 percent probability. Commonly isomers were selected at the next hierarchy of probability. Some compounds were selected twice, again isomers were selected as second choices. It is likely that the data base may not contain some of the compounds actually present in the sediment.

The second data set was obtained as follows:

The sediment from above was extracted again with methylene chloride (50 ml), and evaporated to a volume of approximately 0.5 ml. This was taken to 1 ml (concentration factor of 50) and analyzed by e.i. gs/msd (HP 5970 instrument).

The following substances were selected by Wiley database search:

2,3-dimethylpyridine	2,3,6-trimethylpyridine
5-ethyl-2-methylpyridine	3-ethylpyridine
3-methylpyridine	2-cyclohexen-1-one
2-cyclohexen-1-ol	cyclopentasiloxane, decamethyl
isoquinoline	heneicosane
docosane	tricosane
dodecanoic acid	benzenedicarboxylic acid

hexanedioic acid, dioctyl ester heptadecane-(8)-carbonic acid

Most of these compounds were selected with >90 percent probability, although some of the matches were less than 60 percent probability. Commonly, isomers were selected at the next hierarchy of probability.

Heterocyclic Ring Degraders

Six isolates were found to be capable of growth on either pyridine or 2-methylpyridine in liquid medium. Isolates were evaluated as to optimal concentrations of substrates for growth, substrate specificity range, and pH optima. Cultures were subjected to fatty acid analysis and Gram reaction for probable identification. Probable identities were as follows: R-1-*Arthrobacter* sp., R-2- *Enterobacter cloacae*, R-3-*Arthrobacter globiformis*, R-4-*Pseudomonas diminuta*, R-5-*Xanthomonas maltiphila*, R-6-*Xanthomonas maltiphila*.

Some of the organisms were able to use heterocyclic substrates other than those on which they were isolated. Generally the organisms were able to use heterocyclic substrates up to 1000 mg/L with growth increasing proportionally as concentration increased. In several cases, the lag period before growth was extended at higher substrate concentrations, presumably due to some toxic effect. Excellent growth rates were obtained at pH 5 and pH 7 for several organisms, opening possibilities for use of these organisms in experiments covering a wide range of pH. Since sorption of the compounds to mineral surfaces is pH-dependent, it should be possible to vary the bioavailability of the substrates by altering pH within the physiological limits for growth of the organisms.

Physiological Response and Catabolic Activity of Isolate R1.

Maximal growth of the isolate on both d-glucose and 2-MP was observed in the range of pH 6-9, peaking at pH 7. However, there were significant differences in the pH-growth response profiles below pH 6 with significant growth on glucose at pH 5 accompanied by nearly complete inhibition of growth on 2-MP. Growth in both systems was completely inhibited at pH 4. Since 2-MP is a pH dependent organic cation with a pK_a of 5.92, at pH 5 the protonated species or 2-methylpyridinium ion predominates. The attenuated growth response on 2-MP at pH 5 may have been the result of a preferential uptake of the neutral species or perhaps the lack of an appropriate ion channel or transport system which would allow the 2-methylpyridinium ion to cross the cell membrane. Glucose utilization by the isolate resulted in a decrease of growth medium pH of up to two pH units from the initial value. In the systems containing 2-MP, the pH profiles of all cultures supporting growth were marked by an initial decrease in pH followed by a more gradual recovery. Since complete mineralization of 2-MP would result in the release of ring nitrogen, it is likely that the gradual increase in pH of the growth medium is the result of the accumulation of ammonium in the media. The pH shifts were more pronounced in the systems whose initial pH deviated markedly fromthepKaof the phosphate buffer (7.23)inthe media. Utilization of selected hydroxy-, carbonyl, carboxy-, and alkylpyridines and PANHs by the isolate was quite limited: only 2-methyl-, 2-ethyl-, and 2-hydroxypyridine were capable of supporting growth. A

2-MP degrading *Arthrobacter sp.* isolated by Shukla, (1974), was capable of utilizing 2-ethylpyridine, however 2-hydroxypyridine was not capable of supporting growth. With the exception of picolinic acid, all other aromatic *N*-heterocycles tested were inhibitory to varying degrees. Many of the organisms which have been found to degrade specific aromatic *N*-heterocycles have shown limited degradative potential for other compounds within this chemical class (Stafford and Callely, 1970; Shukla, 1974; Shukla and Kaul, 1974; Shukla, 1975; Sims et al., 1986; O'Loughlin et al., 1989). Substantial growth was supported by 3- and 4-hydroxybenzoate, 2,5-dihydroxybenzoate (gentisic acid) and 3,4-dihydroxybenzoate (protocatechuic acid), suggesting that the isolate has both a gentisic acid pathway and either meta or ortho fission; both central pathways in the degradation of aromatic ring structures. Marginal growth was observed on 2-hydroxybenzoate and 3,5-dihydroxybenzoate. Phenol, catechol, phthalate, and the remainder of the benzoates surveyed were inhibitory. With the exception of tartrate, maleamate, and glutamine, isolate R1 exhibited marked growth on the low molecular weight organic acids surveyed, though growth on formate was less substantial.

2-Methylpyridine Degradation by Isolate R1

Isolate R1 was able to utilize 2-MP as the sole source of carbon, nitrogen, and energy. Growth increased with increasing 2-MP concentration up to a level of 10.74 mmol L^{-1}. Although it was not possible to accurately predict the optimal 2-MP concentration from the limited number of data points, it is clear that the toxicity threshold was within the range of 10.74 - 53.68 mmol L^{-1}. A pale yellow fluorescent pigment was produced during late log to early stationary phase. This pigment may be similar to the pigments produced during 2-MP degradation by an *Arthrobacter sp.* (Shukla, 1974). The pigments were identified as riboflavin and a pale yellow compound with a bluish fluorescence in the UV range. It was suggested that the latter compound was a riboflavin degradation product rather than an intermediate in the 2-MP degradative pathway. Though no attempt was made to further identify this compound, its physical properties are similar to limichrome, a common degradation product for riboflavin. Overproduction of riboflavin during degradation of pyridine by *Micrococcus luteus* has been reported (Sims and Tuhela, 1988). Pigment production has been observed during the biodegradation of aromatic N-heterocycles by a number of microorganisms (Ensign and Rittenberg, 1963; Shukla and Kaul, 1986; Shukla, 1987; Schwartz et al., 1988; O'Loughlin et al., 1989).

As expected the biodegradation of 2-MP was paralleled by growth of the isolate. The concentration of NH_4 in the medium was strongly correlated to the biodegradation of 2-MP, suggesting that degradation proceeded at lest to the point at which ring nitrogen was released. The non-stoichiometric accumulation of NH4 was attributed to incorporation into biomass and losses from volatilization (This was an open system). Analysis of the growth medium by reverse phase HPLC provided no evidence of intermediates in the degradative pathway. There was an apparent lag in 2-MP degradation for the glucose grown cells relative to the 2-MP grown cells. However the 2-MP was completely degraded within 38 hr with the glucose grown cells as compared to 47 h for the 2-MP grown cells. A similar lag was observed for ammonium

accumulation and in the growth curves. The apparent lags observed for the glucose grown cells suggests that the pathway for 2-MP degradation in this isolate was inducible.

Identification and Characterization of Quinoline Degrader.

Membrane Fatty Acid Analysis

The capillary gas chromatogram of the fatty acid methyl esters from the unknown bacterium showed a similarity quotient in the MIS for *Rhodococcus* and *R. rhodochrous* of 0.682 (data not shown). For this system, a similarity quotient greater than 0.5 denotes a good match. There were no other matches given.

Mycolic Acid Analysis

The TLC of the unknown bacterium yielded a single mycolic acid methylester, $R_f = 0.55$. Similarly, a single mycolic methyl ester, ($R_f = 0.5$) was evident for *Rhodococcus equi*, whereas none was evident for *Arthrobacter crystallopoietes*. Based upon the TLC and MIS results the isolate is either a *Rhodococcus* sp. or a *Nocardia* sp.

Electron Microscopy

Transmission electron micrographs of succinate-grown cells showed polyphosphate and poly - hydroxybutyrate granules (PHB) which are characteristic cell inclusions for *Rhodococci* (Goodfellow, 1986). In addition a polymer coating was evident. Conversely, quinoline-grown cells lacked PHB granules or coatings.

Growth Characteristics

Colonies were light orange when grown on quinoline agar and other solid media used in this study. No aerial mycelia were evident, indicating that the probability of this isolate being a *Nocardia* sp. is very low. The optimum pH for growth was pH 9. The optimum temperature was between 30 and 35° C. The isolate was able to use ammonium, nitrate, and nitrite as nitrogen sources. However, there was a long lag period when the isolate was grown on nitrate relative to ammonium, 55 and 20 hours respectively. A longer lag period is typically seen for inducible metabolic systems. Growth of the isolate on Christensen Urea Agar did not result in the release of ammonium, indicating a lack of urease activity. Examination of endospore-stained cells showed no evidence of endospore formation.

Determination of Quinoline Utilization and Degradation

Growth curves for the isolate at quinoline concentrations between 100 and 200 ug/ml exhibit rapid growth occurring after a lag of 96 hours. Maximal biomass production was observed in

cultures which initially contained 500 ug/ml quinoline. However the lag period was considerably longer than at lower concentrations. Since quinoline is volatile, it is possible that at 500 ug/ml quinoline is inhibitory, and only after enough quinoline has been lost from solution (through volatilization) to drop below some threshold concentration are the cells able to grow. Quinoline at 1000 ug/ml was completely inhibitory. Pigment production occurred during log phase growth, however pigment production was not evident in cultures which had initial quinoline concentration less than 100 ug/ml, though quinoline was utilized for growth. Disappearance of quinoline was concurrent with increasing biomass (protein) and increasing ammonium concentration in the medium. This suggested that degradation of quinoline proceeded at least to the point where the heteroatom ring is cleaved and ring nitrogen is released.

Large Scale Study of Quinoline Degradation and Metabolite Production

Quinoline completely disappeared within 46 hours. A metabolite which coeluted with authentic 2-hydroxyquinoline was detected after 24 hours incubation and reached a concentration of 2.1 ug/ml after 46 hours. UV-spectra of the unknown metabolite and 2-hydroxyquinoline showed identical patterns (l_{max} = 227 nm). Similarly, fluorescence emission spectra of the compounds were the same. The FAB-mass spectrum of the unknown had prominent peaks at m/z 146, 100% $(M)H^+$ and 168, 23% $(M)Na^+$, matching the FAB-MS of 2-hydroxyquinoline. The exact mass of the metabolite (145.0527) was equal to the theoretical exact mass. These results confirm that 2-hydroxyquinoline is the first intermediate in the degradation pathway of quinoline for this strain. The HPLC chromatograms of samples taken later in the incubation (46 and 70 hours) contained other peaks with retention times upstream from the 2-hydroxyquinoline peak, indicating an increase in polarity for these compounds. This would be expected for the products of further degradative reactions. Identification of these potential intermediates is the subject of present research.

Utilization of Putative Quinoline Metabolites

Of the putative quinoline metabolites tested, only 2-hydroxyquino-line was capable of supporting more than minimal growth. The growth observed on the other substrates was comparable to the level of growth which was supported in control cultures containing the yeast extract supplied in the basal salts medium as the only source of carbon, nitrogen, and energy. Though 2-hydroxyquinoline was present at 100 ug/ml, no evidence of pigment production was observed, however pigment was produced when grown on 100 ug/ml quinoline. This suggested that the pigment is produced via a side reaction in addition to the formation of 2-hydroxyquinoline.

Estimation of Molecular Weight of Pigment

The results of the molecular separator indicate that the green pigment has a molecular weight in excess of 10,000 mass units. Due to the size of this molecule it has been speculated that it was

formed through a polymerization of quinoline or a metabolic intermediate. Further studies are required to elucidate the structure of this polymer(s).

Adsorption of 2-methylpyridine on clay minerals and Dowex

The adsorption isotherms of 2-MP on specimen clays and Dowex cation exchange resin were developed. The curves for kaolinite, illite, vermiculite, and Dowex were convex relative to the solution concentration axis. Isotherms with this characteristic are often described as L-curve isotherms. L-isotherms result from the combined effects of the decrease of available adsorption sites as the solution concentration increases, and the high affinity of the adsorbate (2-MP in this instance) for the solid phase relative to the solvent (water) (Sposito, 1984; Calvet, 1989). The isotherms for hectorite and montmorillonite were characterized by curves initially concave to the solution concentration axis; such curves are often described as S-curve isotherms. This behavior is believed to be the result of cooperative interactions among sorbed organic species which act to stabilize the adsorbate on the surface and enhance the affinity of the surface for the adsorbed species.

Similar L- and S-isotherms have been reported for other aromatic N-heterocycles. Adsorption of fluridone by soils with high montmorillonite clay and low organic carbon contents, and by Ca-montmorillonite was characterized by S-type isotherms (Weber, 1980; Weber et al., 1986). Quinoline adsorption on Na-montmorillonite has been described by both S- and H- (accentuated L-type) isotherms (Doehler and Young, 1961; Helmy et al., 1983; Ainsworth et al., 1987). However, Ainsworth et al. (1987) examined quinoline adsorption at relatively low total concentrations (4×10^{-7} to 5×10^{-4} mol L^{-1}), whereas, Helmy et al. (1983) observed quinoline adsorption at higher quinoline loadings. Both studies examined different regions of an expanded quinoline adsorption isotherm whose overall curve could be described as S-type.

Adsorption of aromatic N-heterocycles is generally controlled by a combination of electrostatic, London-van der Waals, and entrophic forces. The relative contribution of each to the adsorptive process is dependent upon the given adsorbate, adsorbent, and solvent (Banwart and Hassett, 1982; Zachara et al., 1986; Ainsworth et al., 1987; Zachara et al., 1987; 1988; , 1989). In systems containing low organic carbon levels, adsorption of aromatic N-heterocycles occurs primarily as a result of cation exchange reactions of the cationic species at negatively charged sites on the solid surface. Adsorption of quinoline on Na-montmorillonite was dominated by the quinolinium ion at dilute solution concentrations (Ainsworth et al., 1987; Traina and Onken, 1991). Enhanced adsorption of the quinolinium at solution pH values above its pK_a are believed to be the result of the apparent surface acidity of clay minerals (Mortland and Rahman, 1968; Frenkel, 1974; Karickhoff and Bailey, 1976). Thus at low total quinoline concentrations, quinoline adsorption in aqueous clay mineral suspensions occurs via the following reactions:

$$Q + H^+ = QH^+$$
$$QH^+ + MX = M^+ + QHX$$

where Q = quinoline, QH^+ = quinolinium ion, M = any inorganic cation and X^- = a unit mole of negative charge on the clay mineral surface. Though detailed mechanistic studies of the aqueous phase adsorption of alkyl-substituted pyridines on clay mineral surfaces are lacking, it is likely that adsorption within the range of concentrations examined in this study (0-200 $\mu mol\ L^{-1}$) occurs predominantly as a result of cation exchange via the 2-methylpyridinium ion.

The percent of 2-MP adsorbed on the given solids were examined. In the montmorillonite, hectorite, and Dowex$^-$ suspensions 80-99 percent of the total 2-MP was associated with the solids. However in suspensions of illite, kaolinite, and vermiculite, up to 95 percent of the total 2-MP was present in the solution phase. CEC values for the clay minerals and Dowex$^-$ are given in Table 2. In general, the degree of adsorption of 2-MP on the adsorbents was in positively correlated with their CECs; suggesting that adsorption occurs through a cation exchange reaction involving the 2-methylpyridinium ion. However, the vermiculite and Dowex$^-$ were notable exceptions. Reference vermiculites generally have measured CECs in the range of 100 - 150 cmol kg^{-1}, however the vermiculite used in this study was only 68 cmol kg^{-1}. Since the clay suspensions were prepared in mineral salts medium containing 0.84 mmol L^{-1} KCl, K^+ may have adsorbed to sites in the interlayer region, causing a localized collapse of the layers. This would inhibit the exchange of K^+ by other cations, accounting for the decrease in measured CEC. Steric factors (restricted diffusion of 2-MP into the interlayer region) and reduced CEC likely account for the limited adsorption of 2-MP on vermiculite. Steric hindrance may also have restricted diffusion of 2-MP into the matrix of the Dowex$^-$ copolymer matrix. The Dowex$^-$ used in this study had a high degree of cross-linkage, which may have limited adsorption to sites on the surface of the bead.

Biodegradation of Adsorbed 2-Methylpyridine

Due to the time constraint imposed by sample preparation during the course of the biodegradation experiments, it was necessary to divide the treatments into two series. Series one examined 2-MP biodegradation in vermiculite, hectorite, Dowex$^-$, and no clay suspensions, while series two consisted of kaolinite, , illite, montmorillonite, Dowex$^-$, and no clay treatments. No clay and Dowex$^-$ treatments were examined in both series as a means of comparison between the two experimental runs. The cell densities in series one and two were 3.4×10^9 and 2.93×10^9 cells mL^{-1}. During the degradation experiments, the concentration of both total and solution phase 2-MP were monitored. The reported total concentrations of 2-MP in each of the suspensions was corrected for the relative extraction efficiencies of the extracting solution (NH_4OAc -saturated methanol). For all clays but kaolinite, there was decreased recovery at 10 mmol L^{-1} 2-MP.

In the time frame of 0 to _ 50 min, the presence or absence of adsorbents had little effect on the degradation rate of 2-MP. In the region of 50 to approximately 150 min some differentiation in the degradation rates became apparent. The final region of the degradation curves indicate a change in the relative effects of the adsorbents on biodegradation. In each of the clay and Dowex$^-$ treatments degradation was attenuated relative to the no clay treatment. The time to complete

degradation in the absence of clay was 162 min, while in the vermiculite suspensions degradation was not complete until 192 min. The most pronounced reduction in biodegradation was observed in the Dowex- and hectorite suspensions, with 0.78 and 6.91 μmol of 2-MP L^{-1} (respectively) remaining in solution at the termination of the experiment at 272 min (110 min after complete degradation in the absence of clay). Two-methylpyridine degradation in the second series was nearly complete after 191 min in treatments without clay. In contrast, degradation was attenuated in illite and montmorillonite suspensions. With the exception of the montmorillonite system, the total concentration of 2-MP was less than the limit of detection in all treatments by 212 min. Detectable 2-MP (0.94 μmol L^{-1}) was present in the montmorillonite suspensions at the last sample time (272 min). In contrast, 2-MP degradation was enhanced in the presence of kaolinite relative to clay-free cultures. Degradation was nearly complete at 169 min in this system. A similar enhancement was observed by Weber and Coble (1968). Microbial degradation of diquat was 50-100 percent greater in kaolinite suspensions than in no clay treatments, though greatly reduced in montmorillonite suspensions.

The degradation curves for both the no clay and Dowex- treatments were generally paralleled in each experiment. In both series, complete degradation occurred earlier in the absence of adsorbent. However an additional 29 min were required for complete loss of 2-MP in the second experiment. The final 2-MP concentration in the Dowex- treatments was approx. 0.78 μmol L^{-1} for both series; however again this level was reached 45 min earlier in the first experiment one. The longer degradation times seen in the series two cultures were likely the result of differences in the cell densities of the two inocula or in the physiological status of the cultures from which the cells were collected (cells were collected during late log to early stationary phase).

Estimation of Biodegradation Rates

Although an estimation of the degradation rate could be attempted the differences between the treatments were most accentuated in the region where degradation was most rapid. The calculated degradation rates are presented in Table 3. With the notable exception of kaolinite, attenuation of the degradation rates relative to the no clay treatment was correlated within each series to the degree of 2-MP adsorption indicated by the adsorption isotherms. Overall, the degradation rate was highest on kaolinite suspensions, followed by no clay> illite> vermiculite> Dowex-> montmorillonite/hectorite. Given the high cell density relative to the substrate concentration, one may assume that degradation occurred with a static (no growth) microbial population (Simpkins and Alexander, 1984). Under these conditions the degradation rate should be a function of the concentration of the substrate. Assuming that the clay itself does not affect the physiological response of the bacteria, the degradation rates should be similar for all treatments if the total 2-MP is available for degradation. Clearly this was not the case in the present study. The general decrease in degradation rate with increasing CEC indicates that adsorbed 2-MP was less available for bacterial transformation. It is evident that the degradation rates were a function of the solution phase concentrations.

Summary

Organisms capable of degrading pyridine and alkylpyridines were present in the sediments.

"*In situ*" degradation of pyridine and alkylpyridines in the sediments was observed with aerobic incubation at 25 °C. Further rresearch is needed to assess the degradative potential at lower temperatures.

A bacterium, identified as a *Rhodococcus sp.*, was capable of growth utilizing quinoline as the sole carbon, nitrogen, and energy source. A metabolite was isolated and identified as 2-hydroxyquinoline on the basis of exact matches of UV, fluoresence emission mass spectra with authentic 2-hydroxyquinoline. Ring nitrogen was released as ammonium.

An *Arthrobacter sp.*, isolated from subsurface sediments, was able to utilize 2-MP as the sole carbon, nitrogen, and energy source. Though no metabolites were identified, accumulation of NH_4 in the growth medium suggests complete mineralization.

The degradation of 2-MP was evaluated in the presence and absence of reference phyllosilicate clay minerals and synthetic cation exchange resin. With the exception of kaolinite, degradation was attenuated in clay suspensions: kaolinite > no clay > illite > vermiculite > Dowex$^-$ > montmorillonite/hectorite. In general, adsorbed 2-MP was not available for degradation.

Principal Findings and Significance:

To isolate from sediments those organisms capable of degrading selected heterocyclic compounds, preferably with pH optima close to the pKa of the substrates they are isolated on,; and, to characterize the kinetics of biodegradation (as well as induction kinetics), and the threshold concentration of substrate necessary for induction of biodegradation pathways.

To determine adsorption parameters of the sediment, to facilitate subsequent experiments with rigorously described total concentrations and aqueous activities of pyridines.

To determine the effects of total/solution concentration of substrates on the bioavailability as evedenced by kinetics of biodegradation.

Presetations/Manuscripts/Theses

The following presentations were or will be made from the work described above:

O'Loughlin, E. J., S. R. Kehrmeyer, and G. K. Sims. 1989. Isolation characterization and substrate utilization of a quinoline degrading microorganism. Agron. Abstr. 81: 224.

Sims, G. K., C. Staron, and M. R. Brill. 1990. Degradation of pyridine and alkylpyridines by bacteria isolated from a contaminated aquifer. American Society for Microbiology Annual Meeting. May 13-18, 1990, Anaheim, Ca.

Sims, G.K., M.R. Brill, C.J. Staron, E.J. O'Loughlin, and M.L. Prichard. 1990. Biodegradation of N-heterocycles in contaminated subsurface sediments. Agron. Abstr. 82:258.

O'Loughlin, E.J., S.J. Traina, and G.K.Sims. 1991. Effects of adsorption on the biodegradation of 2-methylpyridine. Agron. Abstr. (in press).

The following manuscript includes in part work from this project.

O'Loughlin, E. J., S. R. Kehrmeyer, and G. K. Sims. 1991. Isolation characterization and substrate utilization of a quinoline degrading microorganism. Appl. Environ. Micro. (In review)

Training - Master's theses:

Kehrmeyer, S.R. 1991. The Effect of Alkyl Ammonium Surfactant-Clay Complexes on the Bioavailability of Naphthalene To Pseusomonas putida: Bacterial Growth and Substrate Degradation Kinetics. The Ohio State University.

O'Loughlin, E. J. 1991. Adsorption and Biodegradation of 2-Methyl-pyridine in Aqueous Suspensions of Specimen Clay Minerals. The Ohio State University.

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Table List

Table 1. Compounds screened for utilization by isolate R1.

Alkylpyridines and PANHs

Pyridine
2-Methylpyridine
3-Methylpyridine
4-Methylpyridine
2,3-Dimethylpyridine
2,4-Dimethylpyridine
2,5-Dimethylpyridine
2,6-Dimethylpyridine
3,4-Dimethylpyridine
3,5-Dimethylpyridine
2,4,6-Trimethylpyridine
2-Ethylpyridine
3-Ethylpyridine
Quinoline
Isoquinoline
Acridine

Benzoates

Benzoic acid
2-Hydroxybenzoate
3-Hydroxybenzoate
4-Hydroxybenzoate
2,3-Dihydroxybenzoate
2,4-Dihydroxybenzoate
2,5-Dihydroxybenzoate
2,6-Dihydroxybenzoate
3,4-Dihydroxybenzoate
3,5-Dihydroxybenzoate
2-Aminobenzoate
4-Aminobenzoate
4-Methoxybenzoate
Phenol
Catechol
Phthalic aicd

Substituted Aromatic N-Heterocycles

2-Hydroxypyridine
3-Hydroxypyridine
4-Hydroxypyridine
Picolonic acid
3-Hydroxypicolinic acid
3-Pyridinecarboxylic acid
4-Pyridinecarboxylic acid
3,4-Pyridinedicarboxylic acid
3,5-Pyridinedicarboxylic acid
2-pyridinecarboxaldehyde
3-pyridinecarboxaldehyde
2-Hydroxyquinoline
4-Hydroxyquinoline
5-Hydroxyquinoline
2,4-Dihydroxyquinoline

Low Molecular Weight Organic Acids

Acetic acid
Formic acid
Fumaric acid
Maleic acid
Pyruvic acid
Succinic acid
Tartaric acid
Maleamic acid
Propionic acid
Citric acid
Aspartic acid
Glutamic acid
Arginine
Glutamine

Table 2. Cation-exchange capacities of the clay minerals and synthetic cation-exchange resin used in this study.

Sorbent	CEC[a] cmol kg^{-1}
KGa-2 Kaolinite	3.5
IMt-1 Illite	17.0
SHCa-1 Hectorite	89.2
SWy-1 Montmorillonite	94.5
Vermiculite	68.0
Dowex 50W-8X	418.0

[a] CEC: Cation-exchange capacity

Table 3. Degradation rates calculated from regressions in Figures 36 - 38.

		rate $\mu mol\ L^{-1}\ min^{-1}$	Δrate $\mu mol\ L^{-1}\ min^{-1}$	r^2
Series #1				
	No clay	-1.099	0.000	0.994
	Vermiculite	-0.923	0.176	0.992
	Hectorite	-0.702	0.397	0.998
	Dowex	-0.772	0.326	0.999
Series #1				
	No clay	-0.894	0.000	0.998
	Kaolinite	-0.925	-0.031	0.998
	Illite	-0.848	0.046	0.996
	Dowex	-0.721	0.174	0.999
	Montmorillonite	-0.545	0.349	0.998

SYNOPSIS

Project No.: 04

Start: 7-01-1989 (actual)
End: 6-30-1991 (actual)

Title: The Role of Soil Humic Substance-Mineral Complexes in the Adsorption and Transport of Anthropogenic Organic Solutes in Natural Waters

Investigators: Traina, Samuel J. and Logan, Terry J., The Ohio State University, Columbus

COWRR category: 05B **Congressional District:** Fifteenth

Descriptors: Adsorption and exchange, contaminant transport, groundwater quality, pesticides, solute transport

Problem and research objectives:

The last decade has seen much concern by the general public, the scientific community, and public agencies over contamination of the nation's groundwater by anthropogenic organic solutes (AOS) in the form of pesticides and industrial contaminants. Widespread contamination of underground water supplies by AOS has been reported in recent surveys conducted by the USEPA (1 and 2) and the Environmental Assessment Council (3). Admittedly some of this contamination has resulted from point sources, but numerous cases of nonpoint-source groundwater contamination from agricultural pesticides have been identified (4). Recently, 11 different pesticides have been detected in agricultural tile-drainage in northwest Ohio (5), providing direct evidence of the transport of AOS from surface to subsurface waters. Clearly there is great need for accurate contaminant transport models which predict the leaching of AOS into subsurface environments.

At present, most existing models have been successful in predicting the leaching of water and nonadsorbing solutes through soils. These models have been less successful in describing the transport of strongly adsorbing substances, such as AOS. These poor predictions are due in part to the overly simplistic descriptions of AOS adsorption that are common to most transport models. In particular, describing AOS adsorption by soil with a linear partition coefficient normalized to the organic matter content (K_{oc}) is inadequate, yet just such an approach has been taken by numerous investigators (6-9). Whereas, a large body of literature indicates that the humic substances (HS) in soil organic matter represent the principal adsorption sites for AOS in soils, recent studies have shown that the binding of AOS to HS can not be adequately described by single-valued, linear partition coefficients (10-17). This latter body of research has shown that the chemical composition of the soil solution, the chemical composition of the soil HS, the distribution of HS between the solution and solid phases of the soil, and the interactions of HS

with clay minerals to form HS-mineral complexes, are just as important in controlling AOS adsorption, as are the total quantities of HS in the soil. Thus the value of K_{oc} is not a constant that can be readily inserted into a transport model. This research examined the effects of organic solute sorption by organo-mineral complexes in single and binary solute systems, as a function of C content and mineral type.

Anthropogenic organic solutes adsorb to soil HS mainly through "hydrophobic" bonds which form between nonpolar regions of AOS molecules and similar structures present in HS. In a previous Water Resources study (17) we found that the binding of AOS to **dissolved** HS was controlled by the "aromaticity" of the HS and by the type of cations in the background solution. The former property was a measure of the number of "hydrophobic AOS binding sites" present on the **dissolved** HS polymers. Variations in the type of cation present in the background solution resulted in changes in the colloidal configurations of the **aqueous** HS polymers (from linear in monovalent electrolytes, to coiled in di- and trivalent electrolytes), altering the accessibility of the "AOS binding sites". Hence the K_{oc} for **soluble** HS-AOS complex formation was dependent on the configurations of the **dissolved** HS polymers. Similar phenomena may be responsible for much of the variations in K_{oc} observed during the adsorption of AOS to **undissolved** HS in soils and sediments. We anticipate that the extent of binding of organic pollutants to HS adsorbed onto clay particles will be directly proportional to the degree of "aromaticity" of the HS polymers, and to the accessibility of AOS binding sites. The accessibility of the AOS binding sites will likely be greater when the HS molecules are adsorbed on the mineral surfaces in linear configurations than in coiled configurations. Since the molecular configurations of the adsorbed polymers should be coiled when adsorbed by clay particles in the presence of di- and trivalent electrolytes, and linear when adsorbed onto the Na-saturated clays, we expect that the propensity of a specific AOS molecule to bind to a soil particle will be dependent upon the dominant exchangeable cations present in the system (prior to HS adsorption). Finally, the different chemical properties of each of the mineral surfaces used in this study should result in differences in the molecular configurations of the adsorbed HS polymers, thus affecting the availability of AOS adsorption sites.

Methodology:

Adsorbents

Reference Na-montmorillonite (SWy-1) was obtained from the Source Clays Repository of the Clay Mineral Society, while fine, crystalline reagent grade $CaCO_3$ was obtained from Mallinckrodt®. Fifty g samples of the clay were washed with 200 mL of 1 mol L^{-1} NaCl, followed by 200 mL of pH 5, 0.05 mol L^{-1} NaOAc-HOAc buffer, to insure Na saturation and to remove contaminating carbonates. The clay was then washed 3 times with 0.05 mol L^{-1} NaCl (in HPLC-grade H_2O) followed by 3 washings with HPLC-grade H_2O. The $\leq$ 1-μm fraction of the clay was removed by sedimentation. This clay fraction was then washed 3 times with 0.1 mol L^{-1} $CaCl_2$ (in HPLC-grade H_2O) and then washed 3 times with HPLC-grade H_2O. The $CaCO_3$ was used as prepared by Mallinckrodt®. A specimen Icelandic Spar calcite was also used in these studies. The specimen calcite was disk milled and the fraction passing through a 100 mesh sieve was accepted for use in experiments.

Twenty g samples of Aldrich® humic acid (HA) were washed 3 times with 0.1 N HCl and then centrifuged. The resulting supernatant was discarded and the solid was solubilized in 0.1 N NaOH, and centrifuged. The resulting supernatant was decanted and the remaining solid was discarded. The solution was reduced to pH 2 to precipitate the HA. The HA was then washed 3 times with 0.05 N HCl, 0.05 N HF and centrifuged to remove inorganic solids. The remaining solid HA was resuspended in HPLC-grade H_2O with sufficient NaOH to cause solubilization. The resulting suspension was dialyzed in 10,000 d dialysis tubing against deionized H_2O until the dialyzing H_2O was determined to be free of Cl ions. This suspension was analyzed for C content and stored at 280° K in amber glass bottles.

The characterized materials, either SWy-1, $CaCO_3$, or specimen calcite were reacted for 24 h with a 725 mg L^{-1} suspension of the purified HA. The resulting material was washed three times with HPLC-grade H_2O to remove any free HA present in the material. The organo-mineral complex was then freeze-dried and stored for use in isotherm experiments.

Organic C determinations on the mineral-HA complexes were made on a Dohrmann® DC-80 Carbon Analyzer. The induction furnace unit of the Dohrmann Analyzer at a temperature of 1073° K was used to determine the organic C content of the HA-SWy-1 complexes. HA-calcite and HA-$CaCO_3$ complexes were pretreated with HNO_3 to remove inorganic C. The subsequent solutions were then analyzed for organic C using the $K_2S_2O_8$ oxidation module of the Dohrmann Analyzer.

Isotherm Procedure

All experiments were performed in 25 mL Corex® glass centrifuge tubes with methanol-washed metal foil interposed between solutions and the Teflon® lined screw caps. Suspensions of organo-

mineral complexes were made from freeze-dried materials as needed. Aliquats of an organo-mineral suspension were added to appropriate quantities of pyrene, anthracene, $CaCl_2$ (for constant ionic background), and H_2O (to maintain constant volume) in centrifuge tubes. Total liquid volume in each centrifuge tube was 24 mL, leaving approximately 1 mL of head space. The centrifuge tubes were then sealed and placed in an incubator at 298° K and agitated on a reciprocal shaker for 18 h.

Analysis

After the 18 h reaction time, the centrifuge tubes were centrifuged at 5900 RCF for 30 m. The supernatant solutions were then analyzed for pyrene and anthracene by UV absorption with an Altex- high-pressure liquid chromatograph, equipped with a 100 µL injection loop, a 25-cm Alltech- Econosphere C-18 column, in a mobile phase of 85% methanol, 15% H2O, at l = 238.5 nm and 251nm respectively.

Results

The sorption of the nonionic organic solutes (NOS) pyrene and anthracene, to the organo-mineral complexes in these studies can be described by a linear function. Nonlinear isotherms are contradictive of partitioning. Whereas linear sorption isotherms do not prove partitioning is occurring, they are consistent with partitioning processes. The present data indicate that pyrene sorption at all f_{oc} levels (f_{oc} = grams C/total grams of sample) studied was by partitioning and that as the f_{oc} of the sorbent increased the sorption of pyrene increased. It should be noted that no NOS sorption occurred to the mineral surface in the absence of HA. For Aldrich HA, NOS sorption increased with increasing f_{oc} in all cases, however, the strength of this reaction varied with the total C content. This is indicated by the K_{oc}, the distribution coefficient normalized to the solid-phase organic C content or:

$$K_{oc} = K_d / f_{oc}$$

The K_{oc} for pyrene sorption on Aldrich HA decreased as the f_{oc} of Aldrich HA increased on the mineral surface. Clearly pyrene sorption by Aldrich HA-coated minerals can not be described by a single-valued K_{oc}, but rather K_{oc} is itself dependent upon the amount of HA present on the mineral surface. By definition K_{oc} should be independent of changes in f_{oc}. Whereas it is not yet readily apparent as to why K_{oc} is dependent on f_{oc}, this data set is consistent with published data from other organo-mineral systems (Traina and Onken, 1991, and Murphey et al., 1990). It should also be noted that for a given solid-phase organic C content, the value of K_{oc} was dependent upon the specific underlying mineral surface. This is readily apparent when one compares the values of K_{oc} on specimen calcite and reagent-grade $CaCO_3$, at f_{oc}'s of 19×10^5 and 14×10^5, respectively. Clearly it is not appropriate to use a single valued K_{oc} to predict NOS sorption in low C environments such as aquifer materials. This brings into question the utility of simple octanol-water partition coefficient based predictions of K_{oc} for many deep subsurface environments.

The binary NOS sorption isotherms showed no evidence of competitive sorption. Thus, the sorption of pyrene was not reduced by dissolved anthracene, and vice-versa. Rather, pyrene sorption by HA-reagent-grade $CaCO_3$, pyrene sorption was enhanced by the presence of anthracene as a cosolute. This may at first seem to contradict a simple partitioning mechanism for pyrene retention, since it has been generally thought that the partitioning of a given solute "A" into solid-phase organic C is independent of the presence or absence of other solutes. However it is possible that very low levels of f_{oc} the sorption of anthracene by Ha-coated surfaces may sufficiently increase the solid-phase C content, to effectively increase the amount of *partioning-medium* present. This would result in greater pyrene sorption. This experiments were repeated several times, at multiple anthracene concentrations, and in all cases, the pyrene sorption was greater in the presence of anthracene. Similar results have been observed by Brusseau (University of Arizona, personal communication).

In contrast, analogous results were not obtained when the total concentration of pyrene was held constant and the anthracene concentration was varied. In essence the presence or absence dissolved pyrene had no effect on anthracene sorption. A compilation of K_{oc} values can be found in Table 1. All results are consistent with a partitioning phenomena.

Table 1. The effect of anthracene on the sorption of pyrene to organo-mineral complexes.

Specimen: Calcite			Reagent: CaCO3			SWy-1		
Anthracene (nmol L-1)	f_{oc} (X105)	$LogK_{oc}$	Anthracene (nmol L-1)	f_{oc} (X105)	$LogK_{oc}$	Anthracene (nmol L-1)	f_{oc}	$LogK_{oc}$
0	3	5.70	0	0.3	6.95	0	0.017	4.03
0	19	5.18	14	0.3	7.16	14	0.017	4.04
0	25	5.04	49	0.3	7.30	49	0.017	4.08
			0	3.0	6.03	0	0.025	3.92
						14	0.025	3.93
						49	0.025	3.93

Principal Findings and Significance:

The results of these studies provide the following conclusions:

1) The sorption of the NOS pyrene and anthracene, to the organo-mineralcomplexes in these studies can be described by a linear function.

2) For Aldrich HA, NOS sorption increases with increasing f_{oc} and varies with the mineral surface to which the HA is associated.

3) The K_{oc} for pyrene on Aldrich HA decreases as the f_{oc} of Aldrich HA increases on the mineral surface.

4) There is no evidence of competitive adsorption in binary mixtures of NOS.

5) Pyrene sorption was enhanced by anthracene as a cosorbate on Aldrich HA modified $CaCO_3$.

6) All results are consistent with partitioning phenomena.

The lack of competitive effects between NOS in binary sorption experiments, even at very low f_{oc} values ($1x10^{-5}$), indicated adsorption reactions were not involved in the interactions of NOS with these organo-mineral complexes. For Aldrich humic acid modified $CaCO_3$, NOS sorption was greater in binary solute systems than in single solute systems suggesting the occurrence of synergistic partitioning phenomena.

Research Products

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SYNOPSIS

Project No.: 05

Start: 07-89 (actual)
End: 06-91 (actual)

Title: Development of a Buried Valley Aquifer Management Model

Investigators: Ward, Andy and Bair, E. Scott, The Ohio State University, Columbus, Ohio

COWRR Category: 04B **Congressional District:** Fifteenth

Descriptors: Groundwater modeling, groundwater quality, groundwater management, aquifer characteristics, flood plain management

Problem and Research Objectives:

Forty percent of Ohio's population relies on ground water for its industrial, agricultural, and domestic needs. Several major cities, including Dayton, Canton, Springfield, Hamilton, and Columbus, dependsolely, or in part, on ground water from buried valley aquifers. To supply this population with water, more than a billion gallons of ground water are pumped daily from at least 500 municipal and 700,000 domestic wells. There is also about 10 million gallons a day is used for irrigation.

Seventy percent of Ohio's ground water supplies are obtained from glacial-outwash and alluvial deposits. The most common geologic setting to find these deposits is in buried bedrock valleys. Many of the buried valley aquifers in Ohio receive a significant part of their recharge from induced stream infiltration. This infiltration can be increased by extensive ground water abstraction, and the water quality in these aquifers may deteriorate from infiltration of poorer quality surface waters.

The Scioto Valley Buried Valley Aquifers are located below highly productive agricultural bottomlands. This location and land use could promote deterioration of the ground water quality by agricultural chemicals.

The purpose of this research is to develop an alluvial valley aquifer management model (AAMM) for the study area. This AAMM involves using published and field-collected data to assess surface-water and ground-water factors that might influence the quality and availability of water within the system. The primary objectives of this investigation are to evaluate the null hypotheses listed:

1) Wellfields like those near Piketon derive their water from a combination of three sources: the alluvial aquifer, the river, and the bedrock.

2) The rate of induced river infiltration near the wellfields near Piketon is a function of:

   a) the proximity of the wells to the river,
   b) the hydraulic conductivity of the aquifer surrounding the wells,
   c) the hydraulic conductivity of the streambed sediments,
   d) the abstraction rate of the wells, and
   e) the difference in head between the river surface and the water table at the wells.

3) For the wellfields, over time:

   a) there will be significant changes in the quality of ground water in the vicinity of the abstraction wells; and
   b) the quality of the abstracted ground water near the wellfields will approach the quality of the river water.

Methodology:

To meet the objectives of the study the following approach was used:

1) developed a database from published and unpublished data, and field data collected as part of the study;

2) based on the data base develop a conceptual model of the geologic and hydrogeologic system within the study area;

3) constructed a numerical (finite-difference) model to represent the river/aquifer system:

4) calibrated the numerical model with aquifer data from field study activities and historic data;

5) conducted sensitivity analyses and tested the hypotheses;

6) used hydrochemical data, graphical methods, and model results to estimate how bedrock water, alluvial aquifer water, and river water influence well water;

7) suggested additional research, where necessary, to support the above hypotheses.

Principal Findings and Significance:

A management model was developed for the Scioto River alluvial valley aquifer near Piketon, Ohio. Data from wellfields in the valley enabled evaluation of ground-water quantity and quality to develop management concepts for future use of alluvial aquifers. A finite-difference flow model and chemical mass-balance principles indicate that about 75 percent and 65 percent, respectively, of the wellfield-produced water is derived from induced river infiltration. The model shows the remaining water sources to be areal recharge in the main and tributary valleys, tributary stream leakage, and bedrock underflow. A particle-tracking program was used to track hypothetical particles from the wellfields to recharge locations.

It was determined that induced aquifer recharge with river water:

a) decreases as the distance of the wells from the river increases;
b) decreases as the horizontal hydraulic conductivity of the alluvial aquifer material increases;
c) increases as the vertical hydraulic conductivity of the alluvial aquifer material increases;

d) increases as the hydraulic conductivity of the Scioto River increases;
e) decreases as the hydraulic conductivity of a nearby tributary stream (i.e., Big Beaver Creek) increases;
f) decreases as areal recharge increases;
g) increases as the abstraction rate of the wells increases;
h) increases as the difference in head between the river surface and the water table at the wells increases;
i) decreases as the head at Lake White is increased.

Two groups of water samples, a 1963-65 group and a 1990 group, from the Scioto River and from wells at DOE and surrounding residenceswere evaluated to characterize water quality at the wellfieldsand throughout the study area. Trilinear and mixing-line plots were used to characterize water quality as related to: wellfieldabstraction over time, relative residence time of the waterin the aquifer, geologic origin of the water, and mixing processes. Water samples from the DOE wellfield and Scioto River are characterized as calcium-magnesium-bicarbonate type; and waters from Lake White, a main recharge source, are a calcium-magnesium-sulfate-bicarbonate type water. ANOVA results indicate that significant changes in ground water quality at the DOE wellfield has occurred between the 1963-65 and the 1990 data groups. Na+, Cl-, and SO42- show a significant increase in average concentration; Ca2+, Mg2+ showed a significant decrease in concentration; and HCO3- showed an insignificant decrease in concentration. Analysis of the water chemistry data indicated that 50 to 88 percent of well water is derived from induced infiltration. The analysis with Na+ and Cl- gave an estimate of 75 percent which is consistent with the MODFLOW results and results reported in the literature.

The flow-model and water quality analysis results indicate that the dissolved ions concentrations of ground water at the DOE wellfield is dominated more by the quality of river water than the "unmixed" ground water from the alluvium. For waters from the alluvial aquifer to dominate the water chemistry induced recharge of river water would need to be reduced to less than 20 percent. For this to occur, it would be necessary to reduce the density of wells by installing additional wells along the river or to reduce abstraction rates. The flow model would be a useful tool to evaluate various alternative pumping scenarios.

In addition to the site specific results valuable products of this study include: (a) demonstration of an approach which uses modelling techniques to develop management scenarios in terms of wellfielddesign,abstractionpractices,andwaterquality differences in the system; (b) a compilation of ideas and techniques for flow modelling, water quality characterization, and aquifer management; and (c) recommendations on what additional studies and modifications should be conducted.

Training

M.S. thesis: An Alluvial Valley Aquifer Management Model at Piketon, Ohio. 1991. Nortz, Patrick E. Agricultural Engineering Department, The Ohio State University, Columbus, Ohio.

Publications and Professional Presentations:

Nortz, Bair, and Ward. 1992. Modelling interactions between the Scioto River and a buried valley aquifer wellfield at Piketon, Ohio. To be submitted for publication in the Water Resources Bulletin.

INFORMATION TRANSFER ACTIVITIES

The Water Resources Center is in the Agricultural Engineering Building on The Ohio State University campus. This location provides daily opportunities to work and share ideas with researchers in the College of Agriculture as well as the College of Engineering. It also provides a close working relationship with the OSU Agricultural Engineering Cooperative Extension Service.

A series of tasks were continued or initiated to transfer and disseminate information developed by researchers affiliated with the Water Resources Center to a wide range of State, Federal, County and Municipal agencies; to the private sector; to the academic community and to private citizens throughout Ohio.

Water Luncheon Seminars

The Water Resources Center continued to co-sponsor a bi-monthly Water Luncheon Seminar Program for the water resources community in Central Ohio. This program was developed cooperatively with The Ohio Department of Natural Resources (ODNR), the Ohio Environmental Protection Agency (OEPA), the Soil Conservation Service (SCS), the District Office of the United States Geological Survey (USGS), and the Agricultural Engineering Cooperative Extension Service of The Ohio State University. More than eighty water resources professionals from Federal, State, County and Municipal Agencies, the private sector and the academic community attend each meeting to discuss current state, federal and local water policy issues, problems, programs and research results.

In addition to the growing attendance to these meetings, the newsworthiness of the information transferred at these meetings is being recognized by the local media and luncheon program information is being transfered to the general public via the press.

The Center not only provides administrative support for the seminar series and mailing costs, but on occasion also provides financial support for rental of various media effects and meals.

The moderators, speakers, and topics that were presented during the 1990/91 program year follow.

WATER LUNCHEON SEMINAR, FY 1990

Topic	Speaker/*(Sponsoring Agency)*
Ohio's Canals 9-25-90 OSU - Fawcett Center	Mr. Barnett L. Golding, Trustee Canal Society of Ohio Mr. Joel Reed, Administrator Dam Permits & Hydraulics - ODNR Blaine Gerdes, Administrator Canal Lands - ODNR *(Robert Goettemoeller, Chief Division of Water, ODNR)*
The Dimension of Water Management 11-15-90 OSU - Kottman Hall	Dr. Warren H. "Bud" Viessman, Assoc. Dean Research/Graduate Study, University of Florida *(This Water Luncheon Seminar was presented in conjunction with the Wayne Nichols Lecture Series with the OSU School of Natural Resources)*
OSU'S Water Quality Program 1-8-91 OSU - Fawcett Center	Dr. Kirklyn Kerr, OARDC Dr. Bobby Moser, OCES Dr. Karen Mancl, Rural Drinking Water Dr. Andrew Ward, Ohio's MESA Project *(Moderator: Dr. Larry Brown, OSU Cooperative Extension Program)*
National Water Quality Assessment Program 3-12-91 OSU - Fawcett Center	Mr. Mark Ayers, U.S. Geological Survey, Reston, Virginia *(Moderator: Mr. Steven Hindall, Chief, USGS District Office* *Co-Sponsor: Dr. Charles King, BiOhio, The Ohio Biological Survey)*
On The Water Front - (Lake Sedimentation Study) 5-14-91 OSU - Fawcett Center	Mr. Jim Wade & Mr. Bill Hofacker, Soil Conservation Service *(Moderator: Mr. Robert Burris, Soil Conservation Service)*

Water Management Association of Ohio (WMAO)

The Water Resources Center has been the administrative office for the WMAO since 1989. This not-for-profit, 300 member, state-wide organization promotes and supports the development, conservation, control, protection and utilization of the water resources of Ohio for all beneficial purposes. This is the only Ohio organization that is solely concerned with managing Ohio's water. The Center provides staff support, office space and equipment to WMAO as a portion of its information transfer program.

The WMAO holds an 2 day- Annual Meeting in the fall at which nearly 200 people attend. The 1990 topic was "1990: Wetlands". Researchers, professional water managers, environmentalists, and government officials each presented aspects of this topic. Laws, implications, applications, developments and compromises from each group's perspective were illustrated and balanced. The Water Resources staff provides administrative support and coordination before, during and following the meeting.

Information Dissemination Activities

The Center continues to meet with the leading water resources officials in the state to share information on current water management and policy issues; to seek continued support for the waterresearchprogramand to disseminate the information and technology developed throughout the state and region.

A newsletter, WATER, was developed and publication started in FY 1988. It focuses on Ohio's water research, technology, issues, legislation in process, education and Center activities. It has a wide circulation which includes water professionals, researchers and general public throughout Ohio and the nation. The newsletter has been well received. The editor is Mrs. Carol Moody, who is also the secretary for the Center.

Consultation and Collaboration Activities

The Center's Director has continued to meet with the leading water resources officials in the state for the purposes of consultation and collaboration to identify the major water problems and the research needs of the state and region; to share information on current water management and policy issues; to seek continued supportforour water research program and to disseminate the informationandtechnology developed through this program and others at the universities throughout the State and Region.

The Director has been appointed by the Governor of Ohio to serve onthe Ohio Water Advisory Council, a statutory commission that advisestheWaterDivision of the Ohio Department of Natural Resources.

The Director serves on the Board of Directors to the Ohio River Basin Research and Education Consortium. He is the Lead Delegate to the Universities Council on Water Resources (UCOWR) and is a past member of the Board of Directors; he serves on the Water Programs Public Advisory Group to the Ohio Environmental Protection Agency and is a member of the Toxics Technical Advisory Committee; and he is a member of the Ohio Inter-Agency Water-Use Data Coordinating Committee for the Ohio District of the U. S. Geological Survey.

Presently the Director is cooperating with six other Water Resources Institutes on a proposal for the Department of Energy on educational programs for water remediation.

COOPERATIVE ARRANGEMENTS

Program Development

All of the research projects that were initiated in the FY 1989 State Water Resources Research Program were continued in the FY 1990 Program. A letter was mailed in November 1989 which indicated that the present projects would be continued for a second year. This letter was mailed to more than forty public and private colleges throughout Ohio. Central State University, the only historically black university in the State qualified to participate in the program, also received this letter. A copy was sent to the following faculty and administrators at Central State University: Dr. Thyrsa Svager, Vice President & Provost; Dr. Henry Smith, Director, International Center for Water Resources Management.

The process used to initiate proposals for the 1989 program was: a call for pre-proposals for the Fiscal Year 1989 State Water Resources Research Program was sent to research administrators and qualified faculty investigators at over 40 private and public colleges and universities throughout Ohio on November 15, 1988. This announcement, contained the research priorities identified for the major water problems in the Great Lakes, Upper Mississippi and Ohio River Basins by the Water Resources Research Institutes in the Region.

The announcement also required interested researchers to request a copy of the Preliminary Proposal Application Form which was to be completed and returned to the Water Resources Center in mid-January, 1989. More than 250 names were included on the distribution list.

Pre-Proposals/Federal Guidelines

In 1989 Preliminary Proposal Application Forms were requested by and sent to twenty-eight investigators and research administrators at seventeen colleges and universities in Ohio. A copy of this Application Form was requested by Central State University and they submitted a pre-proposal.A copy of the federal guidelines for the Program was enclosed with the Form.

Evaluation/Selection Procedures

Nineteenpre-proposalsfromtenuniversitiesandcolleges throughout the state were submitted for evaluation and consideration. These pre-proposals were subjected to a review by all of the members of the Water Resources Center's Advisory Committee. In addition, the nineteen pre-proposals were distributed to the various divisions within the three principal state and federal water-related agencies in the State by the representatives of these agencies who serve on the Advisory Committee, requesting that the divisions review the proposals. The three agencies included in this evaluation were the Ohio Department of Natural Resources, the Ohio Environmental Protection Agency, and the District Office of the United States Geological Survey.

The results of these reviews were presented at a meeting of the Advisory Committee where this panel selected nine of the pre-proposals and instructed the Center's Director to request fully developed proposals from the investigators for the Committee's further consideration.

All nine of the selected pre-proposals were developed more fully and were re-submitted for consideration. The proposals were subjected to a technical review by at least three qualified evaluators selected by individual members of the Water Resources Center's Advisory Committee. Many of these evaluators were from state and federal agencies and from universities other than The Ohio State University.

The results of these reviews were presented at a meeting of the Advisory Committee and this panel ranked the leading five proposals in the order they felt would best meet the needs and objectives of the Water Resources Center's program. The Advisory Committee then instructed the Center's Director to incorporate as many of these projects as Federal funds would permit into the FY 1989 Program and to develop a project for information transfer for the Center. There was only enough Federal monies to support four projects.

The membership of the Water Resources Center's Advisory Committee, which includes representatives from five colleges and eleven departments of The Ohio State University and the three representatives of the principal water-related state and federal agencies, is included in this report.

Regional Cooperative Initiatives

The four projects selected for this program had been compared in the FY 1989 Program synopses of the projects included in the programs of the other Water Resources Institutes in the Great Lakes, Upper Mississippi and Ohio River Basin to ensure that there was no duplication of efforts in the Region's research programs.

The Ohio State University has agreed to continue as a Charter Member of the Ohio River Basin Research and Education Consortium, and the Director of the Water Resources Center continued serving as one of the University's three representatives to the Consortium.

Program Management

At least once each quarter, the Director contacts the Principal Investigator on each research and information transfer project to discuss progress made during the quarter and to discuss the next quarter's plan of activities. At this same meeting budget details are reviewed and discussed, and necessary operating and reporting procedures to the Water Resources Center and to The Ohio State University Research Foundation's business office are described.

Progress Reports or Completion Reports were prepared for each Project by the Principal Investigators and were used by the Program Director to prepare the Program Final Report.

All of the investigators are urged to publish the results of their findings in the technical literature of their major disciplines and in other journals that are appropriate to the topic of their research. They are also encouraged and invited to present their findings at the Water Luncheon Seminar that is a part of the technology transfer activities of the Center.

The manuscripts that constitute the project completion reports are first reviewed by the Director of the Water Resources Center. As needed, the Director seeks the advice and council of appropriate state, federal and university scientists for methods of enhancing the value of the technical completion reports to the water-related community in the state and in the region.

WATER RESOURCES CENTER ADVISORY COMMITTEE

COLLEGE OF ENGINEERING

1. Dr. Vincent T. Ricca
   Civil Engineering

2. Professor L-S Fan
   Chemical Engineering

3. Dr. Robert C. Stiefel
   Director, Water Resources Center

School of Architecture

4. Dr. Steven I. Gordon
   City and Regional Planning

5. Professor J. W. Simpson
   Landscape Architecture

COLLEGE OF BIOLOGICAL SCIENCES

6. Dr. Robert M. Pfister
   Microbiology

7. Dr. Jeffrey Reutter
   Lake Erie Programs

8. Dr. David Culver
   Zoology

9. Dr. Bruce Vondrachek
   Ohio Cooperative
   Fisheries Unit

COLLEGE OF MATHEMATICAL AND PHYSICAL SCIENCES

10. Dr. E. Scott Bair
    Geology and Mineralogy

COLLEGE OF AGRICULTURE

11. Dr. Terry J. Logan
    Agronomy

School of Natural Resources

12. Dr. Robert L. Vertrees
    Resources Management

ADMINISTRATIVE MEMBER

13. Ms. Theresa Heitzman
    Ohio State University
    Research Foundation

OHIO ENVIRONMENTAL PROTECTION AGENCY

14. Dr. John F. Estenik

OHIO DEPARTMENT OF NATURAL RESOURCES

15. Dr. William Mattox

UNITED STATES GEOLOGICAL SURVEY

16. Mr. Steve Hindall
    District Chief

UNITED STATES DEPARTMENT OF AGRICULTURE

17. Dr. Norman Fausey
    Agricultural Research
    Service

TRAINING ACCOMPLISHMENTS

The following tabulation shows, by fields of study and training levels indicated, the numbers of individuals participating in projects that were financed in part with this grant.

Training Category	Training Level				
	Undergraduate*	Master's Degree	Ph.D. Degree	Post - Ph.D.*	Total
College of Agriculture					
—Agricultural Engineering		1			1
—Agronomy		2			2
Engineering					
—Chemical				1	1
TOTALS		3		1	4