

# **Pacific Northwest Laboratory Annual Report for 1985 to the DOE Office of Energy Research**

**Part 2 Ecological Sciences  
February 1986**



**Prepared for the U.S. Department of Energy  
under Contract DE-AC06-76RLO 1830**

**Pacific Northwest Laboratory  
Operated for the U.S. Department of Energy  
by Battelle Memorial Institute**





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**Pacific Northwest Laboratory  
Annual Report for 1985 to the  
DOE Office of Energy Research**

**Part 2 Environmental Sciences**

R. E. Wildung and Staff Members  
of Pacific Northwest Laboratory

March 1986

Prepared for  
the U.S. Department of Energy  
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory  
Richland, Washington 99352



## PREFACE

This 1985 annual report from Pacific Northwest Laboratory (PNL) to the Department of Energy (DOE) describes research in environment, health, and safety conducted during fiscal year 1985. The report again consists of five parts, each in a separate volume.

The five parts of the report are oriented to particular segments of our program. Parts 1 to 4 report on research performed for the DOE Office of Health and Environmental Research in the Office of Energy Research. Part 5 reports progress on all research performed for the Assistant Secretary for Environment, Safety and Health. In some instances, the volumes report on research funded by other DOE components or by other governmental entities under interagency agreements. Each part consists of project reports authored by scientists from several PNL research departments, reflecting the multidisciplinary nature of the research effort.

The parts of the 1985 Annual Report are:

Part 1: Biomedical Sciences

Program Manager - J. F. Park

D. L. Felton, Report Coordinator  
and Editor

Part 2: Environmental Sciences

Program Manager - R. E. Wildung

C. M. Novich, Report Coordinator  
and Editor

Part 3: Atmospheric Sciences

Program Manager - C. E. Elderkin

C. E. Elderkin, Report Coordinator  
E. L. Owczarski, Editor

Part 4: Physical Sciences

Program Manager - L. H. Toburen

L. H. Toburen, Report Coordinator  
J. E. Danko, Editor

Part 5: Overview and Assessment

Program Manager - L. G. Faust

L. G. Faust, Report Coordinator  
R. W. Baalman, Editor

Activities of the scientists whose work is described in this annual report are broader in scope than the articles indicate. PNL staff have responded to numerous requests from DOE during the year for planning, for service on various task groups, and for special assistance.

Credit for this annual report goes to many scientists who performed the research and wrote the individual project reports, to the program managers who directed the research and coordinated the technical progress reports, to the editors who edited the individual project reports and assembled the five parts, and to Ray Baalman, editor in chief, who directed the total effort.

A highlight this past year was the appointment of a Scientific Advisory Committee. Honoring us by accepting our invitation to serve on the committee are:

Dr. Franklin I. Badgley	University of Washington
Dr. Leo K. Bustad	Washington State University
Dr. Franklin Hutchinson	Yale University
Dr. Albert W. Johnson	San Diego State University
Dr. J. Newell Stannard	University of Rochester
	University of California, San Diego

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S. Marks, Associate Manager  
Environment, Health and Safety  
Research Program

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1983	PNL-5000, Pt. 1-5
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## FOREWORD

In May 1985, Dr. Burton E. Vaughan, who served as the Subprogram Manager for Ecological Programs from 1973 to 1985, accepted a position as PNL Assistant Director for Research. Dr. Vaughan's dedication to science over this period resulted in an exceptional environmental program and a fundamental basis for continued growth and new initiatives. A team of PNL scientists was subsequently formed to aid in future planning and guidance of the program. The team and their areas of responsibility included:

L. E. Rogers	Arid Land Sciences
J. A. Strand	Marine Sciences
R. G. Riley	Biogeochemical Phenomena in Indirect Exposure Pathways
J. M. Zachara	Subsurface Transport
J. M. Thomas	Theoretical (Quantitative) Ecology

An overall plan is being developed to focus PNL's environmental research efforts on helping to establish a basic understanding of key processes for predicting the long-term fate and biological effects of fugitive chemicals and other stresses that result from energy development.

Using remote sensing, biogeochemistry, hydrodynamics, and quantitative and systems ecology, major new initiatives will focus on arid land ecosystems, subsurface transport, biotechnology, and the effects of stress at physiological and biochemical levels.

New emphasis is being placed on expanding university liaisons and technology transfer by encouraging university staff and student use of the National Environmental Research Park at Hanford, the Marine Research Laboratory at Sequim, the new Intermediate-Scale Subsurface Transport Facility, and other unique PNL facilities. These facilities provide the opportunity to bridge the gap between the laboratory and field sciences. They have been particularly valuable in studies to validate predictive models and to distinguish random fluctuations caused by natural perturbations from long-term changes caused by technology development.

The organization of the annual report this year reflects some of the initial planning effort and focus on new initiatives. We welcome your comments and suggestions for improvement.

Raymond E. Wildung  
Subprogram Manager  
Environmental Sciences





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## Arid Land Sciences

The Hanford National Environmental Research Park (see "University Relations and Technology Transfer") provides a focus for environmental field studies of arid land ecosystems. During the first ten years, research conducted under its auspices provided data needed to define the ways in which energy production and development activities disturbed natural systems and to determine whether, and to what extent, energy-related contaminants become incorporated, transported, or accumulated in biological pathways.

It is clear from this earlier work that the biological, hydrologic, and biogeochemical processes operating within arid systems interact and that those interactions are the primary factors influencing the type and extent of the environmental response. For example, we know that plants can profoundly affect local energy and chemistry budgets. Some effects are indirect, as when plants reflect sunlight and modify wind patterns, thus influencing evaporation or temperature flux patterns. Other effects are more direct, as when plant roots extract chemicals from the soil and make them available to consumer pathways or concentrate various chemicals near the soil surface.

Recent analyses suggest that, at times, nearly all of the annual precipitation within an arid region may be returned to the atmosphere by evapotranspiration. Evapotranspiration, therefore, may largely control the amount of soil water and any leaching of minerals or chemical contaminants to the subsurface system. An understanding of how the major processes interact is necessary before predictive capabilities and effective management strategies can be developed.

### DYNAMICS OF ARID LAND ECOSYSTEMS

L. E. Rogers

PNL is studying the effect of stress resulting from fire, on the water balance of bunchgrass-dominated communities and comparing water use between communities with and without a shrub component. Comparisons of changes in the soil water profile will be used to determine the effect of the fire on soil water accretion and depletion patterns. Results of the research will be compared to data sets from other arid ecosystems to identify commonalities and differences between a cold and warm desert ecosystems.

#### Wildfire Influences Soil Water Dynamics

Plants play a major role in controlling the water balance of arid ecosystems. Living plants transpire water stored in the soil and minimize evaporation by providing shade for the soil surface. In addition, plant material, both living and dead, enhances water infiltration into the soil, thereby reducing the amount of water that runs off the surface. Because soil water dynamics are intimately affected by plant processes, researchers hypothesize that any perturbations of vegetation over large areas will affect the water balance of that area. A

large wildfire on the Arid Lands Ecology (ALE) Reserve in the summer of 1984 permitted PNL to test this hypothesis.

In September and October of 1984, three study plots were established, one in each of the following vegetation types: an area dominated by sagebrush and bunchgrass, an area dominated by bunchgrass that burned in 1981, and an area dominated by bunchgrass that burned in 1984. The study sites are within 100 meters of each other and are located within the Ritzville soil type, a deep, well-drained silt-loam. Hydroprobe ports were installed to monitor soil moisture, and portable meteorological stations were placed on each site. Soil moisture data were collected every two weeks, meteorological data were collected hourly, and leaf area and biomass of the vegetation by species were collected at the time of peak standing crop.

Preliminary analysis of vegetation and soil water data indicate measurable differences in soil moisture, biomass, and leaf area among the three treatments. Total biomass and leaf area were greatest on the unburned plot and lowest on the most recently burned plot (Table 1).

Sagebrush (*Artemisia tridentata*) had a leaf area index of  $0.2307 \pm 0.0843$  (ratio of leaf

**TABLE 1.** Estimates of leaf area and biomass for three different study plots (mean  $\pm$  standard error).

Plot	Leaf Area (m <sup>2</sup> leaf/m <sup>2</sup> ground)	Biomass (g/m <sup>2</sup> )
Sagebrush-bunchgrass (Unburned)	0.33 $\pm$ 0.01	332.2 $\pm$ 116.3
Bunchgrass (1981 burn)	0.18 $\pm$ 0.02	52.1 $\pm$ 7.8
Bunchgrass (1984 burn)	0.13 $\pm$ 0.02	41.3 $\pm$ 5.9

area to ground area) and a biomass of 302.70 g/m<sup>2</sup>  $\pm$  112.20. Sagebrush was absent from the burned plots. Bluebunch wheatgrass (*Agropyron spicatum*) had the greatest biomass and leaf area on the plot burned in 1984. Intermediate values were recorded for the plot burned in 1981. Sandberg's bluegrass (*Poa sandbergii*) was greatest on the plot burned in 1981, followed by the plot burned in 1984. Phlox (*Phlox longifolia*) and fleabane (*Erigeron filifolius*) were greatest on the 1981 burn and lowest on the 1984 burn site.

During the fall of 1984, soil water content was highest for the plot that burned in the summer of 1984. The increased water content on the 1984 burn plot may be attributed to late summer rainfall that infiltrated the soil and was not removed by evapotranspiration because the 1984 fire burned off the above-ground plant parts. Both the sagebrush-bunchgrass and the bunchgrass (1981 burn) plots had plants with green leaves during the fall of 1984, suggesting active transpiration. The sagebrush-bunchgrass plot had the driest soil profile, suggesting that these plants used water to a greater depth than did the plants on the bunchgrass plots.

Recharge of moisture during the winter months was similar for all three plots. There was no evidence of surface runoff from any of the plots at any time during the test period. By early August 1985, the three plots showed drying patterns reflecting their plant cover status. The 1981 burn plot was driest in the upper portion of the soil profile, and the 1984 burn plot was wettest in the lower profile.

Results of this experiment demonstrate that changes in leaf area and biomass of a plant community affect water storage within the soil profile. The relationships between these variables will be further investigated and incorporated into models for predicting the effects of perturbation on soil water dynamics.

#### ECOLOGICAL STUDIES AT THE NATIONAL ENVIRONMENTAL RESEARCH PARK

W. H. Rickard

At Hanford, the National Environmental Research Park represents a restricted access land area of approximately 570 square miles. It includes the watershed region bounded on one side by Rattlesnake Hills, the Arid Land Ecology Reserve (120 square miles), a significant stretch of the Columbia River, and pond locations on either side of the river. Historically, the site has provided a major focal point for university research at PNL, and it has provided relatively undisturbed habitats for short- and long-term studies on semiarid ecosystems of the western United States.

Experimental and observational studies are conducted at the Environmental Park to develop the scientific bases for 1) predicting impacts of energy development activities on biological resources of the site, and 2) recommending appropriate management strategies for animal life that affects either the technological operations at the site or nearby communities. During the past year, approximately 12 university faculty and graduate students participated in PNL's ecological sciences program.

The Hanford Environmental Park Serves as a Focal Point for University Research

Graduate students are using the Hanford Environmental Park to study resident wildlife populations of western Canada goose (*Branta canadensis moffitti*), American elk (*Cervus elaphus*), coyote (*Canis latrans*), and Great blue heron (*Ardea herodias*). Studies were also begun on plant populations of *Lupinus leucophyllous* and *L. sulfureus*. These plants are of importance because they are symbiotic nitrogen fixers and provide protein-rich forage for herbivorous animals.



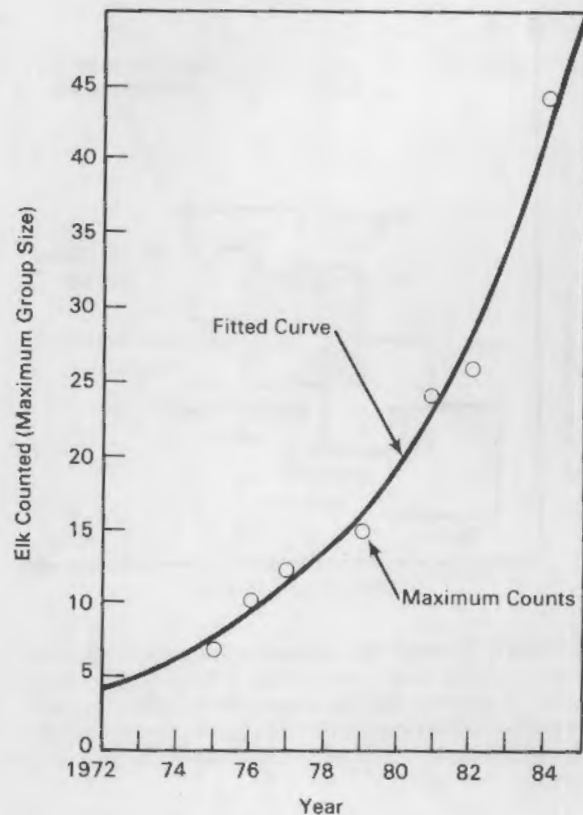
Three students completed Master of Science degrees and graduated. Six students currently are on site, representing Washington State University, University of Washington, Oregon State University, University of Idaho and Western Washington University.

Brood ecology studies of the western Canada goose were conducted over two consecutive years. Small radiotransmitters attached to parent geese were used to monitor their movements along a 40-kilometer section of the Columbia River. The data show that predation losses to goslings was severe. Of 23 monitored broods, nine were destroyed completely. Other broods had at least some mortality. Information on riverine habitat use and other habitat requirements of broods was also obtained.

A study of the American elk herd living on the ALE Reserve is continuing. Eleven young animals were added to the herd in 1983, 13 in 1984, and 15 in 1985 (Figure 1). The total population in 1985 was 72 animals. The reasons for the success of the elk herd over the past 10 years is believed to be freedom from threatening encounters with people and the absence of livestock grazing. In the few instances when individual elk did leave the ALE Reserve, their absence was temporary and their travel direction was to the northwest.

The elk living on the ALE Site represent the only known elk population to voluntarily inhabit the shrub-steppe region in 200 years of recorded history (Figure 2). Factors influencing how elk adapt to forage paucity, lack of concealment cover, excessive temperatures, and lack of shade-producing trees are subjects currently under study.

Five elk were captured and fitted with electronic devices that continuously radio-transmit signals of body and subcutaneous temperatures. Physiological responses of individual elk to intense summer drought, desiccated forages, and the absence of concealing vegetative cover are recorded remotely for later analysis. The data show that even on a hot July day, the deep body temperature of an elk remains physiologically stable (Figure 3). These data indicate that wild elk can cope with temperature extremes without benefit of shading from direct sunlight.

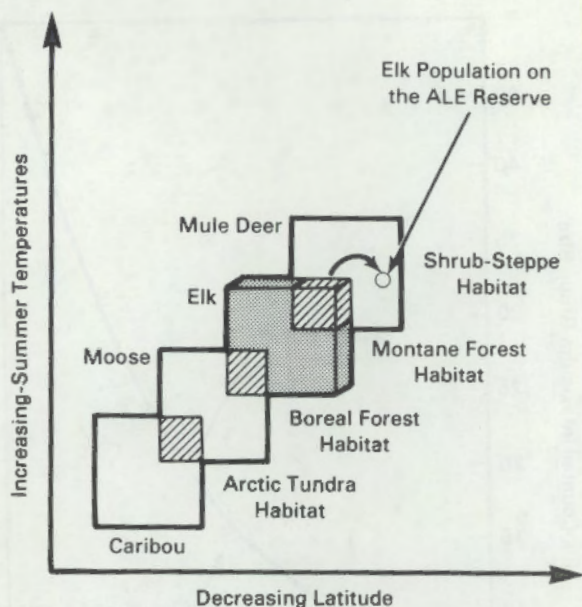


**FIGURE 1.** The population of wild elk on the Arid Land Ecology (ALE) Reserve increased rapidly from 1975 to 1984. The rapid increase is attributed to low predation by other animals such as coyotes and to Federal regulations that prohibit hunting on the reserve.

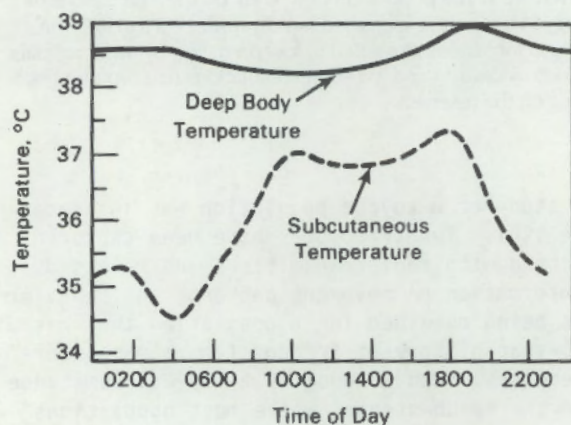
A study of a coyote population was initiated in 1984. Twenty coyotes have been captured, fitted with radiotransmitters and released. Information on movement patterns and behavior is being obtained for a population that has a 30-year history of freedom from human interference. Such freedom is a rare circumstance in the shrub-steppe, where most populations are exploited by fur and predator trappers and by sports hunting.

The great blue heron is a large fish-eating bird that nests in tree colonies on the Hanford Site. Graduate student research involves studying the feasibility of using heron colonies as biological indicators of organic and trace metal contaminants, especially PCBs, in the environment. Data indicate that Hanford Site herons have lower PCB





**FIGURE 2.** This illustration shows the distribution of western North American populations of deer (Cervids) in relation to their characteristic habitats, summer air temperatures, and latitude. The self-establishment of a resident elk population in the semi-arid shrub-steppe habitat in Washington is a singular event.



**FIGURE 3.** Elk on the Arid Land Ecology Reserve were able to maintain deep body temperatures at a steady state even on a hot July day when shade provided by trees or other structures was not available.

concentrations than herons in industrial areas of western Washington.

Study plots were established in the spring of 1984 to record the response of wild lupines

to changing microclimates and artificial irrigation. Density counts were made, individual plants were permanently marked for future identification, and seeds of individual plants were collected for laboratory experimentation.

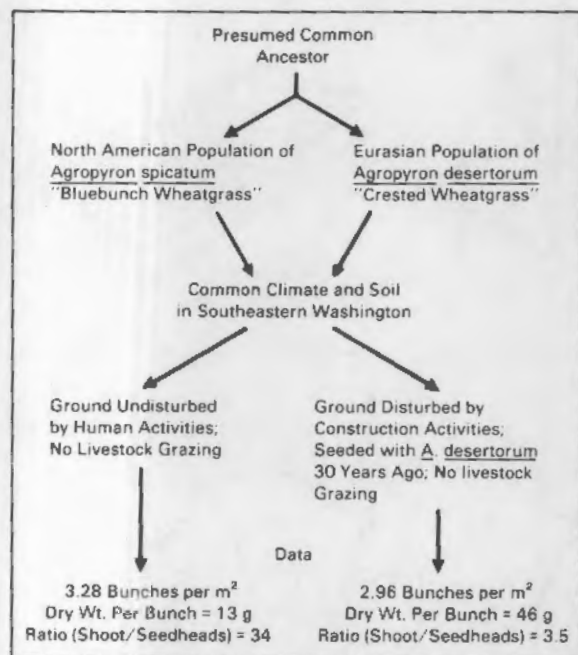
#### Long-Term Ecological Research

One of the important purposes of the Hanford National Environmental Research Park is to provide a setting relatively free from human-induced perturbations so that fluctuations of wild plant and animal populations can be investigated under a "control" or "baseline" condition over extended periods of time. A special part of the Environmental Park that provides this important function is the ALE Reserve.

Crested wheatgrass, *Agropyron desertorum*, was introduced to the United States from Russia at the turn of the century. Since then, it has been widely planted throughout the shrub-steppe region to control erosion and provide forage for livestock on rangeland that formerly supported a native grass, bluebunch wheatgrass, *Agropyron spicatum*. Because crested wheatgrass is genetically and physiologically closely related to bluebunch wheatgrass, we hypothesized that given sufficient time after artificial seeding, the spacing of crested wheatgrass would become much like that of the native bunchgrass. The diagram in Figure 4 supports this hypothesis. Crested wheatgrass bunches are, however, larger than those of bluebunch wheatgrass, and they produce more seed heads with a greater proportion of the shoot weight allocated to seed heads.

Experience on the ALE Reserve over the past two decades indicates that most shrubby taxa, particularly sagebrush, bitterbrush and spiny hopsage, are highly vulnerable to summer burning. During the past 10 years repetitive wildfires have eradicated or depleted 80% to 90% of the stands of these shrubs on the reserve. Rock outcrops normally do not support sagebrush, bitterbrush or spiny hopsage. Fuel buildup is so light that wildfires seldom burn across them. The characteristic shrub that grows on these rock crops is *Eriogonum sphaerocephalum*, a small shrub 2 to 3 dm in diameter and less than 2 dm tall.





**FIGURE 4.** Thirty years after planting, populations of crested wheatgrass attained bunch densities much like those of native bluebunch wheatgrass.

Like sagebrush and bitterbrush, the buds are positioned on the twigs and are elevated above ground level, where they are vulnerable to wildfire. We predicted that *E. sphaerocephalum* would also be vulnerable to burning, but opportunities to observe its fire response have seldom been available. A wildfire in the summer of 1984 burned entirely across a rock outcrop on the ALE reserve. This particular community was studied prior to burning, and by re-examining four study plots we were able to describe the shrub response to burning. Almost all the shrub population was damaged, but 34% of the population survived. This percentage of survival was higher than for other desert shrubs.

The geographic distribution of greasewood shrubs, *Sarcobatus vermiculatus*, is confined to a few hectares near Rattlesnake Springs, where roots have access to ground water as deep as 15 meters. Greasewood shrubs are believed to be very old (several centuries) and most of the shrubs sprout after summer burning. Greasewood accumulates large quantities of sodium in its leaves each year, and the release of that sodium from dead leaves

year after year adds to the sodium content of the soil beneath the canopy spread. Data collected from a study this year clearly show that after long periods of time, the presence of a greasewood shrub has a pronounced effect on the water-soluble anions and cations of the soil profile, and that the changes are most evident near the soil surface (Figure 5). Should the shrubs be destroyed, the chemical composition of the soil is expected to become much like that of the adjacent grassy area, since the soluble ions are leached downward into the soil by rainfall percolation. The amount of time required for the soluble inorganic constituents to revert to background concentrations under prevailing climatological conditions has not yet been determined.

## BIOTIC TRANSPORT RESEARCH

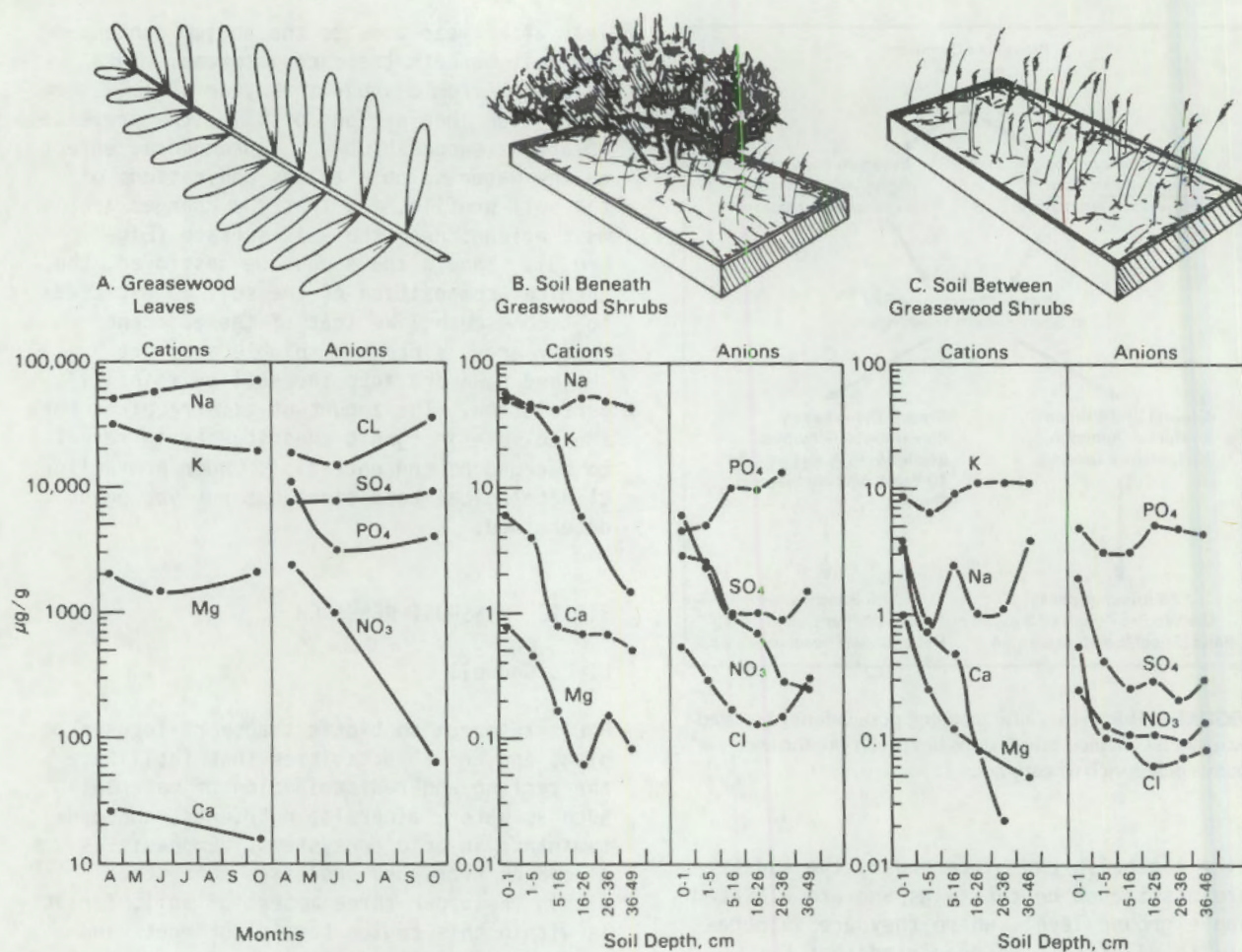
L. L. Cadwell

PNL's research on biotic transport focuses on plant and animal activities that facilitate the cycling and redistribution of materials such as water, minerals, nutrients, and contaminants in arid ecosystems. Emphasis is placed on transport processes that occur within the upper three meters of soil, for it is within this region that plant roots and burrowing animals initiate the transport of buried waste products, control the flow and partitioning of surface water, and extract and cycle minerals necessary for growth.

### Actinide Element Redistribution in Soils

Samples of soil cores were analyzed radiochemically as part of a larger study to examine the uptake and mobility of actinide elements in soil and plant systems. Containers initially spiked with selected actinide elements have been maintained for the past 10 years in field enclosures under conditions that approximate arid land agricultural and range conditions. The purpose of this study is to determine the effects of natural environmental processes (e.g., freezing/thawing, weathering, microbial decomposition) and range or agricultural cropping practices (e.g., tilling, fertilizing, irrigation) on the uptake of radionuclides by plants and the distribution of the radionuclides in the soil profile over extended periods of time.





**FIGURE 5.** Leaf chemistry and leaf fall has a long-term effect on the concentrations of major anions and cations in the soil beneath greasewood shrubs. Greasewood is the only shrub on the Arid Land Ecology Reserve that has the capacity to accumulate sodium in its leaves from sodium-rich soils.

Some of the outdoor containers, called lysimeters, are sacrificed periodically to examine radionuclide redistribution within the soil profile over time. In the original spiking configuration, one-meter-long soil columns were spiked with a 0.6-cm layer of contaminated soil at the 10-cm depth. Care was taken not to overwater the crops, since the bottoms of the containers were sealed and excess water could not drain. Annual tillage operations were restricted to the upper few centimeters of the lysimeter surface to avoid mechanically mixing the thin layer of contaminated soil.

Vertical redistribution of selected actinide elements in the soil column was observed after four growing seasons. Radionuclides

were found to have migrated upward and downward in both irrigated (crops) and nonirrigated (rangeland) lysimeters. All moisture available to the soil plant/system entered the soil from the surface either as irrigation water or as natural precipitation.

Sampling for radionuclide redistribution in the irrigated and nonirrigated soil column after an additional five growing seasons revealed no further radionuclide migration in most of the soil columns. The originally mobile actinide elements appeared to be bound within the soil column and, in general, were not available for appreciable redistribution. However, one of the irrigated soil columns containing plutonium-238 showed detectable surface contamination the year



before the soil cores were analyzed. Observations revealed the presence of small burrowing bees (*Agapostemon texanus*) occupying several of the lysimeters.

Measurements made with portable alpha survey instruments indicated that plutonium-238 was present in soil that had been cast to the surface during the burrowing activity. These observations were supported by the results of subsequent biotic transport modeling for these experimental conditions.

The concentration of plutonium-238 in the surface soil of the disturbed lysimeter was approximately 470 times greater than that of the undisturbed lysimeters. Lysimeters containing other actinide elements that were not disturbed by bee burrowing activity showed typical concentrations of radionuclides in the surface soil.

Invertebrate burrowing resulted in significantly more plutonium-238 movement to the soil surface than had occurred as a cumulative result of other functional processes operating over several previous years. The other processes, which may include plant translocation and unsaturated flow through soil solution, appear to have been more effective early in the experiment.

These results clearly demonstrate the potential importance of animal intrusion pathways and imply that radiological impact assessments should consider these pathways (for example, resuspension/ingestion or resuspension/inhalation) in assessing dose to humans.

#### Uptake of Neptunium in Freshwater Organisms

Several short-term (2- to 27-day) bioconcentration experiments were performed with freshwater organisms to develop concentration ratios (CR) for neptunium. This information provides valuable insight to the potential environmental hazards of releases of neptunium to the environment.

Neptunium is a decay product of americium-241, which has a half-life of 432 years. As americium-241 decays, increasing amounts of neptunium-237 will accumulate in radwaste facilities. Neptunium has an unknown potential for accumulation in aquatic organisms

and increasing the radiological dose to humans who consume aquatic organisms that have come in contact with the material.

Preliminary results on tissue burdens of rainbow trout exposed to two levels of neptunium for 27 days indicate that little of the observed levels of the actinide were found in fillets. The majority of Np was associated with the carcass. Levels associated with the skin were equivalent to, or higher than, levels found in muscle tissue. The predicted dose to the human consumer would be considerably less, and more realistic, if CR values were based on fillet tissue burdens and not on whole body burdens.

Bioconcentration data have also been collected for two freshwater invertebrates, two species of green algae and one species of blue-green algae. Collectively, this information will be used to assess the distribution of neptunium and estimate the potential for the material to move through aquatic food chains.

#### FUTURE RESEARCH

Future research in the arid land ecology program will focus on investigations designed to provide a basic understanding of how abiotic and biotic interactions influence the response of terrestrial ecosystems to energy-related perturbations. An understanding of how major processes interact within arid systems will be used to develop predictive capabilities and to aid in the design of effective management strategies.

An ability to predict impacts associated with energy development, energy production, and land disposal of waste materials will require information about the hydrologic, atmospheric, ecologic, and biogeochemical processes that govern site stability. As these understandings develop, the investigations will need to be "scaled up" to evaluate response characteristics at higher levels. Critical to the attainment of these long-term objectives is the integration of remotely sensed data with other advanced information bases in order to help resolve previously intractable problems at landscape and regional levels.



## Marine Sciences

PNL research in the marine sciences is focused on establishing a basic understanding of the mechanisms of stress and tolerance in marine organisms exposed to contaminants. Several environmental stressors had been investigated in earlier energy-related research. In a landmark study, for example, PNL had established that the severity of fish disease caused by the common infectious agent, *Flexobacter columnaris*, was seriously aggravated by thermal enhancement and certain ecological factors. Subsequent studies demonstrated that the primary immune response in fish, challenged by columnaris, could be permanently suppressed by comparatively low tritium exposures. The research has suggested that a potential exists for a significant biological impact when an aquatic stressor is added to an ambient background of other stressors, which may include heat, heavy metal ions, radiation, or infectious microorganisms.

More recently, PNL investigators have shown that in response to heavy metal contaminants, animals synthesize specific proteins (metallothioneins), which bind and sequester metals in the animals, thus decreasing metal mobility and effects. Companion studies with host-specific intracellular pathogens are being used to investigate the effects of heavy metals on the synthesis of immune proteins, which mitigate disease processes. The results of these studies aid in predicting the ecological effects of energy-related contaminants on valued fin and shellfish species.

### MARINE CHEMISTRY OF ENERGY-RELATED CONTAMINANTS

E. A. Crecelius

In previous studies, PNL quantified the concentrations and sources of soluble and particulate metals in Puget Sound waters. This information serves as the foundation for studies to identify the geochemical processes that control the concentrations and biological availability of metals to aquatic organisms. Results of the research will be used to determine the upper limits of contaminant exposure that organisms and ecosystems can safely tolerate.

#### Crabs Accumulate Silver Primarily from Food

PNL studies have shown that Dungeness crabs, *Cancer magister*, caught in urban bays contain higher concentrations of silver than crabs caught in non-urban bays. This year laboratory experiments were conducted to determine whether the primary route of uptake was through water or diet. An important natural food of Dungeness crab, the littleneck clam, *Protothaca staminea*, was selected as the food item.

Juvenile crabs were exposed to three concentrations of silver in flowing seawater over a three-month period. The crabs were fed littleneck clams that had been exposed for a month to one of three concentrations of

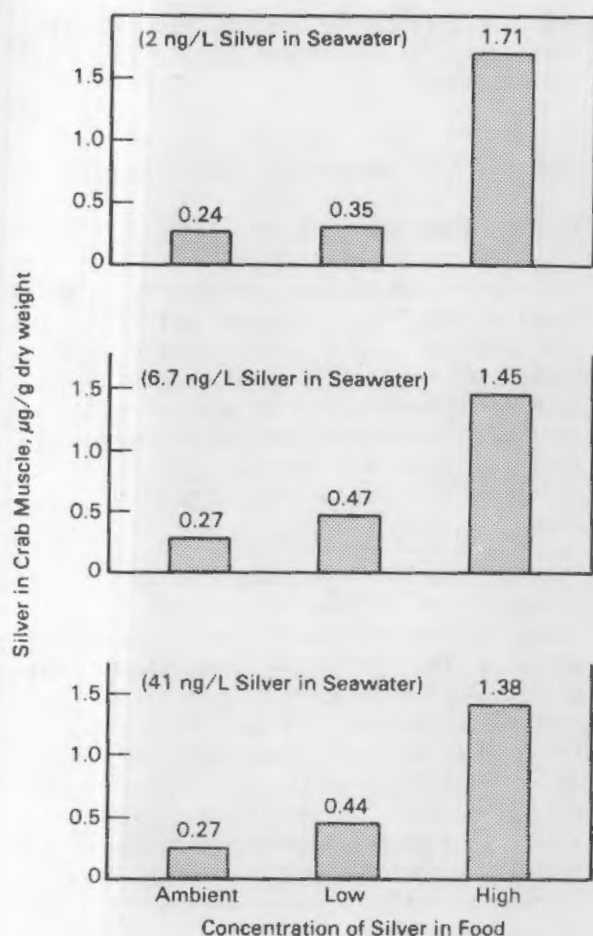
silver. During the course of the exposure and at termination, seawater and clam muscle tissue were analyzed for silver. The average growth rate of the crabs over the three-month exposure was also determined.

The concentrations of silver in the exposure tanks averaged 2 ng/L in the ambient seawater tank, 6.7 ng/L in the low-concentration tank, and 41 ng/L in the high-concentration tank. Crabs were fed clam tissue containing silver concentrations of 0.11 µg/g dry weight for clams exposed to ambient seawater, 0.35 µg/g for clams held in seawater containing 56 ng/L, or 1.61 µg/g for clams held in seawater containing 314 ng/L.

The concentrations of silver in crab muscle tissue after three months of exposure are shown in Figure 1. The upper bar graph displays the concentrations of silver in three groups of crab exposed to ambient seawater but fed clams with different body burdens of silver. The concentration of silver in crab tissue is similar to that in the clams fed to the crabs. The middle and lower bar graphs are identical to the top graph even though the crabs in these tanks were exposed to elevated concentrations of silver in seawater.

The results of this experiment indicate crabs do not significantly accumulate silver from seawater under the conditions of this exposure. However, crabs do accumulate silver when fed silver-contaminated clams. The





**FIGURE 1.** Dungeness crabs accumulated silver after eating silver-contaminated clams. Uptake of silver from seawater was minimal, which indicates that ingestion is the more important pathway of exposure.

concentration of silver in the crab muscle tissue was approximately the same concentration as in the clam tissue.

During the three months of exposure, the crabs grew from approximately 25 gm to 55 gm and went through one molt-cycle. The growth rate of the crabs was not affected by the concentration of silver in water or food.

#### ENVIRONMENTAL STRESS AND DISEASE IN MARINE ANIMALS

R. A. Elston

The objective of this PNL research is to identify the effects of environmental con-

tamination on the immune systems of marine animals. Another equally important objective is to determine the interactive effects of contaminants and host-specific pathogens within whole organisms and at cellular and subcellular levels.

#### Environmental Stress Affects Disease Susceptibility

The expression of infectious diseases in animals is a dynamic process determined by the effectiveness of the immune response, virulence of the pathogens and, importantly, the condition of the host animal. An organism's health is strongly influenced by environmental stress. Most animals typically carry or are exposed to specific pathogens, which, depending on environmental factors, may or may not be expressed as disease. Study of the components of these processes requires appropriate animal models and *in vitro* systems so that specific biological components can be examined.

This year, PNL developed several components of model systems for studying the interactive effects of environmental stress and species- and tissue-specific pathogens. The effects of cadmium on an *in vitro* cell system were characterized, a tissue-specific pathogen was isolated, and the *in vitro* infection dynamics of the pathogen were characterized. Results of these studies will ultimately be used to study disease processes in marine invertebrates. The initial *in vitro* studies to investigate basic biological mechanisms are being conducted with marine fish species, because suitable marine invertebrate models are not available.

#### Standard Methods Developed for Studying Cadmium on an *In Vitro* System

Standard methods for testing and identifying a specific cell line were developed. The effects of cadmium on an epithelioid cell line derived from chinook salmon embryos (CHSE/214) were characterized under various dosages and methods of administration. In a 60-hour bioassay, total cadmium in cell culture medium suppressed cell growth at concentrations of 1.0, 10.0 and 20.0 µM. The cadmium concentration was lethal at levels of 40.0 µM and greater. Cells grown in the presence of 10 µM cadmium over eight generations did not appear to show enhanced



resistance to the higher levels of cadmium but did show slightly depressed growth levels both under the 10- $\mu$ M exposure regime and when cadmium was removed from the culture medium. A series of cell clones was also produced by selecting survivors of a short-term exposure to a high level of total cadmium (40  $\mu$ M).

Studies will continue in fiscal year 1986 to cytologically localize and quantify cadmium in the cells. Selected cell clones (cadmium exposed and non-exposed) will be used to study the infectious process described in the following paragraphs.

#### Tissue-Specific Pathogen Isolated and Adapted for Experimental Studies

Availability of an *in vitro* system in which both cadmium effects and pathogen effects could be studied was a key element in these studies. A highly species- and tissue-specific obligate intracellular pathogen of the chinook salmon was isolated and adapted to culture in the CHSE/214 cell line. This pathogen is an obligate intracellular eukaryotic organism of presently undefined taxonomic identity (but descriptively termed "rosette"). Over a period of six months the organism was cultivated (having been derived from infected fish) at low levels in the cell line. The pathogen has undergone 22 generations of *in vitro* subculture and can now be cultivated at sufficient numbers to perform experimental studies.

#### Infection Dynamics of In Vitro Pathogen Characterized

The characteristics of rosette organisms in the CHSE/214 cell system were studied with respect to temperature, initial inoculation levels, structure of vegetative life stages, nutritional aspects, and *in vitro* host susceptibility. Organisms replicated at an increasing rate from 5° to 20°C. Multiplicity of infection must be 1.0 or greater to produce infection within the normal culture generation period. Up to 50% of host cells became infected within 30 days at a multiplicity of infection of 4.0. Vegetative pre-capsular stages of the organism were identified by fluorescent antibody methods and by electron microscopy in infected cultures. The rosette organisms demonstrated

host-specificity for the CHSE/214 cell line when tested in cell lines derived from other salmon species.

#### TRACE METAL METABOLISM IN MARINE ANIMALS

G. Roesijadi\* and J. E. Morris\*\*

The emphasis of current research is to study metallothioneins, a class of sulfhydryl-rich low molecular weight, metal-binding proteins recognized for their central role in metabolism and detoxification of various trace metals. The occurrence of these proteins has been confirmed in a variety of organisms, including most marine animals studied to date.

Previous studies have shown that metallothioneins are induced by exposures to low concentrations of copper, zinc, cadmium, or mercury. This induction is associated with an increase in the tolerance of marine organisms to toxic concentrations of metals. Enhanced tolerance is presumably the result of an increased capability for cellular metal detoxification. An understanding of the limits of such compensatory responses will be useful in predicting and evaluating potential detrimental effects of metal exposure on marine animals.

Procedures to detect and quantify the proteins in biological samples are not generally available. Therefore, current work has focused on isolation of an invertebrate metallothionein and the development of an immunological assay for its detection and quantification. These efforts will facilitate continued research on the function and biological significance of the proteins.

The mercury-induced metal-binding proteins of the marine mussel, *Mytilus edulis*, separate into three protein peaks when subjected to DEAE anion exchange chromatography. One of these peaks was investigated in further detail and shown to be similar to cadmium-binding proteins isolated from the mussel.

\*Department of Biology, The Pennsylvania State University

\*\*Biology and Chemistry Department, Pacific Northwest Laboratory.



The protein appears to exist as a dimer with a relative molecular weight ( $M_r$ ) of about 20 kD and unequal subunits. Amino acid analysis of the dimer indicated a high content of cysteine (26%) and glycine (16%) and very low (<1%) levels of tyrosine, phenylalanine, histidine, and leucine. The composition is consistent with that previously described for other molluscan variants of metallothionein. This protein, referred to hereafter as mussel metallothionein, is being used as the antigen in efforts to develop antibodies and immunoassay for its detection and measurement.

Both monoclonal and polyclonal antibodies directed against the mussel metallothionein have been developed within the past year. Monoclonal antibodies obtained using hybridoma technology are currently being characterized prior to further development and application. Polyclonal antibodies were prepared through injection of goats and extraction of the IgB fraction from goat sera. These antibodies have been shown to possess cross-reactivity with the two other metal-binding protein variants mentioned for mussels, but not with commercially available rabbit metallothionein. These findings are consistent with earlier results, which showed that antibodies against rat metallothionein do not react with mussel metallothionein. These findings support the conclusion that proteins from mammals and those from mussels exhibit dissimilar antigenicity.

Considerable progress has been made on the development of an enzyme immunoassay (ELISA) for use with the polyclonal antibodies against a mussel metallothionein. Initially the operating parameters for the ELISA were evaluated with respect to antigen, antibody, and other reagents in a direct assay. The results indicated a sigmoid relationship between the amounts of antigen and  $\log_{10}$  antibody and a linear relationship when the antigen levels were transformed using Logit

Y. These findings justified continued efforts, and we are now modifying the assay into an indirect, competitive assay, which will improve sensitivity and reduce background interferences. This research is expected to result in the development of a specific and sensitive assay for metallothioneins from a marine invertebrate species of widespread geographical distribution and one that is frequently used in marine pollution and monitoring studies.

#### FUTURE RESEARCH

Future PNL research in marine sciences will focus on the development of monoclonal antibody and other genetic engineering techniques to purify, synthesize, and examine, in detail, specific proteins that account for enhanced tolerance to energy-related pollutants. Research to understand gene synthesis and expression have application in developing contaminant and disease-resistant strains of fin and shellfish, and in identifying and producing proteins with high biomedical potential. Tissue culture techniques would be developed for marine invertebrates. These do not currently exist and would allow detailed evaluation of the mechanisms of stress and tolerance in the laboratory, reducing the need for expensive, time-consuming whole animal and population studies in extrapolation of the results to the environment.

To make maximum use of knowledge developed on the existing programs, PNL will build on the current interdisciplinary approach by adding staff in the fields of molecular biology, physiology, chemistry, and genetics. Specialized equipment is urgently needed for isolation of DNA/RNA, synthesis of genes, and application of recombinant DNA techniques. The combined additional support would markedly accelerate progress in protection, management, and pharmaceutical use of valuable marine species.



## Biogeochemical Phenomena in Indirect Exposure Pathways

Complex relationships exist between chemical and biological phenomena that markedly affect the transfer and form of inorganic and organic contaminants in human ingestion pathways. These pathways are often far more significant than inhalation exposure but are also more difficult to understand and evaluate. PNL has pioneered research on processes that control the transfer of radionuclides and energy-related metals and organic compounds in soil-plant-animal and water-fish-animal pathways. The common denominator is development of an understanding of how these processes influence chemical form, which governs transfer and toxicity and provides an integrated view across contaminant types and exposure pathways.

This program is being reoriented from research emphasizing specific radioelements toward biogeochemical phenomena controlling the behavior of representative classes of inorganic elements and organic compounds important in human ingestion pathways. In addition, basic concepts developed in the laboratory are being validated in controlled field studies. This information is being used to add kinetic and metabolic dimensions to food ingestion models, which previously lacked long-term predictive capability and the ability to incorporate metabolic products of organic chemicals.

### SOIL AND PLANT PROCESSES CONTROLLING CATION BEHAVIOR IN TERRESTRIAL SYSTEMS

R. E. Wildung, D. A. Cataldo, and  
T. R. Garland

Previous research at PNL has shown that ligands produced by plant roots and by microbial decomposition processes have significant ability to mobilize trace elements in soil. The research implicated low molecular weight organic acids, amino acids, and peptides as major complexing ligands produced by plant roots. However, complexation of elements by ligands from plant roots may be limited because microorganisms are excellent scavengers of these compounds in the rhizosphere. Rhizosphere organisms, in turn, release metabolic products, which PNL research has shown may also act as specific complexing agents. The combined role that root exudates and subsequent microbial metabolites have in complexation of inorganic elements, such as radionuclides and trace metals, is little understood. The objective of this project is to determine the fundamental processes governing the production and fate of these ligands and their interactions with cations in soils. This information serves as a basis for prediction of the cycling and mobility of resulting mobile anionic species in soils and subsoils.

Research has focused on determining the importance of organic complexation processes in soil-plant-animal systems under laboratory conditions. In FY 1985, efforts were directed toward 1) characterizing the organic

components in plant exudates produced in lysimeter systems, 2) determining the role of isolated rhizosphere microflora as models for complexation of selected elements, and 3) determining the net capacity of the combined plant-microbial metabolic products to complex and influence the soil mobility of key elements in the rhizosphere of plants growing in the field.

The major biomass/metabolic component of soils consists of the rhizosphere and associated microorganisms. Belowground plant biomass is considered to be 1.5 to 2 times greater than the standing, aboveground biomass. Combined, above- and belowground biomass provide a large source of soluble organic carbon. Root exudations to soil has been estimated at 12% to 25% of the photosynthetic carbon fixed in cereals. The composition of these exudates is relatively complex and consists of polysaccharides, sugars, amino acids, organic acids, and a range of nucleotides and enzymes, which undoubtedly vary with plant type.

Laboratory and field approaches are being used in PNL studies to determine the role of organic complexation in mobilization of cationic contaminant elements. Detailed examination of the chemical form of complexes is not possible in the field without some preliminary knowledge as to the complexes involved. Therefore, the laboratory-based studies employ manipulated microbial culture and plant lysimeter systems to define the production and chemical form of organic

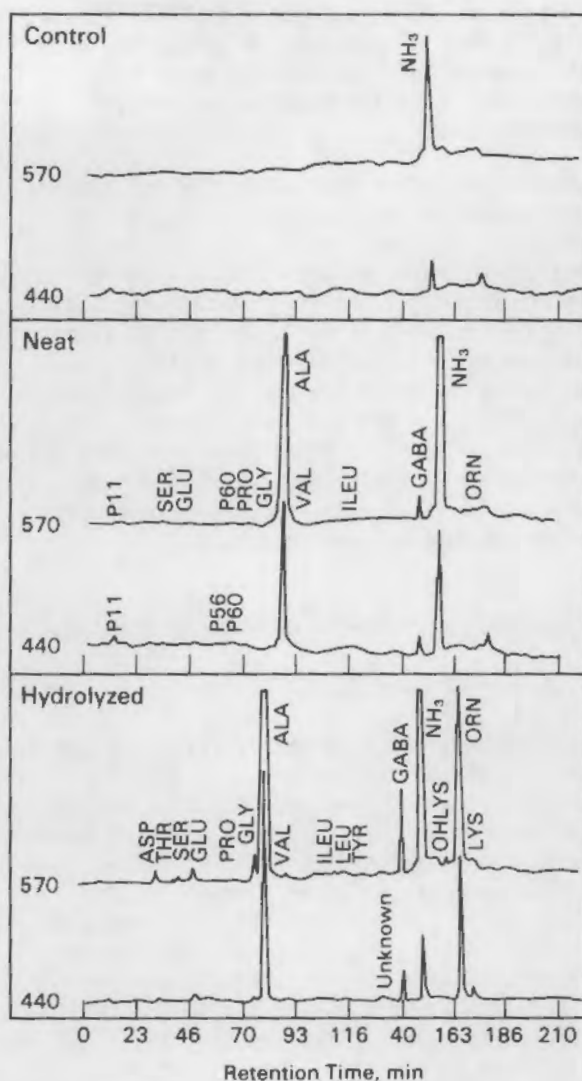


ligands involved in complexing cations. The latter studies use a number of tree, western shrub, and grass species.

Chemical fractionation of rhizosphere leachates, followed by back reaction with radionuclides, shows a preferential association of the radionuclides with the amino acid fraction. Further analysis of the complexing fraction (i.e., comparing the amino acid composition of neat and hydrolyzed subsamples) demonstrates the presence and persistence of a substantial quantity of small peptides and polypeptides that contain a small number of aspartic acid, threonine, glutamine, proline, glycine, leucine, tyrosine, and lysine residues, and a substantial quantity containing alanine, valine, gamma amino butyric acid and ornithine residues (Figure 1). The presence of these simple, low molecular weight components within the rhizosphere can, depending on complexation capacity and stability constants, represent an important mechanism responsible for the mobilization and cycling of elements.

Bacteria of the genus *Pseudomonas* are ubiquitous in soil and are excellent competitors in the root-soil environment, where they have been shown to reach numbers as high as  $10^7$  per gram of soil. They are also capable of broad metabolic activities and produce a wide range of secondary metabolites, including compounds such as siderophores, which efficiently complex iron to the point of making it unavailable to other microorganisms.

*Pseudomonads* are being isolated from plant roots to be used as models for characterization of microbial metabolites produced during growth on root exudates. One such isolated organism produces several fluorescent and nonfluorescent low molecular weight compounds in a minimal-salts media. These compounds are hypothesized to be polypeptides, similar to some of the complexing ligands in plants. Axenic and sterile plant root systems will be used in conjunction with a previously employed ligand characterization protocol to identify complexing ligands in the presence of plants alone (sterile system) and with the model bacteria (axenic system). The results will be used to identify complexing ligands produced by the root-associated bacteria resulting from the growth on root exudate. This information will provide the foundation for related studies with other



**FIGURE 1.** The composition of amino acid/peptide fractions from black walnut lysimeter leachates was examined. Sand lysimeter controls, black walnut rhizosphere leachates (neat, showing both amino acids and peptides), and hydrolysates of black walnut leachates (hydrolyzed) were analyzed on an amino acid analyzer, and their constituents were compared on the basis of uv absorbance at 570 and 440 nm.

classes of soil microorganisms, such as fungi and actinomycetes, to determine the role of soil microbial metabolites in transport of solutes in the soil-plant root continuum.

The field studies are interactive with laboratory studies and involve evaluation of rhizosphere processes occurring in arid environments. For these studies, the chemical environments associated with a range of



rhizosphere conditions are analyzed. Of particular interest, within the rhizosphere, are the spatial patterns of Eh and pH, and the temporal and spatial fluxes of both endogenous soluble elements and soluble carbon.

The field studies have shown the rhizosphere environment to be chemically reducing and dynamic; i.e., subject to seasonal pulses of soluble organic carbon. Cation-anion balances in the rhizosphere indicate that soluble cations are, in part, associated with soluble organic carbon which differ in their influence on trace element and radionuclide solubility and mobility. The dynamic nature of these effects is being examined using geochemical modeling methods which describe changes in solid phase solubility controls based on chemical speciation.

#### ENVIRONMENTAL BEHAVIOR OF INORGANIC ANIONS

T. R. Garland and D. A. Cataldo

Land disposal of potentially toxic inorganic wastes, whether of nuclear or industrial origin, requires some degree of engineered containment for safe disposal. The requirements and associated costs for selected containment scenarios will depend on our understanding of the potential for specific components of the waste to migrate from the disposal site. Anionic contaminants, or contaminants which can easily form anionic species through chemical or biological modifications (technetium, iodine, ruthenium, selenium, arsenic, molybdenum, chromium, and vanadium) are emphasized in our studies and present the greatest potential for migration in soil and accumulation in biota.

The basic hypothesis driving these studies is that the toxicity and high biological availability of the stated anionic chemical species result from their interaction with the sulfur, nitrogen, and phosphorus cycles. All three of these cycles involve anion reduction, and are present in some form in all biological systems.

This fiscal year, our efforts emphasized two areas: 1) evaluation of seasonal changes in anionic species in perennial spring and surface stream water draining arid lands, and 2) compositional differences in water soluble anionic species in native plant tissues.

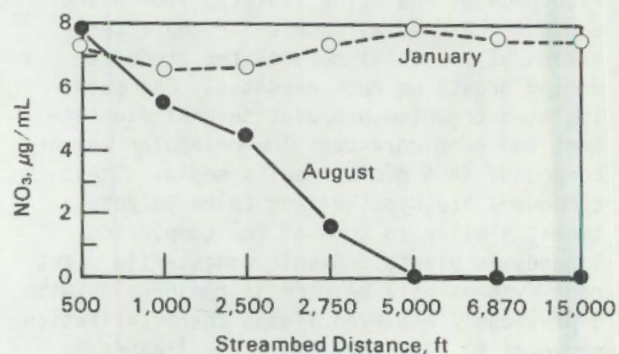
#### Seasonal Behavior of Nitrate in Snively Stream

Snively Canyon, on the Arid Lands Ecology Reserve, contains a small perennial surface stream and a riparian habitat. The location and characteristics of this canyon make it ideally suited to the evaluation of nutrient and non-nutrient ion dynamics and transport.

The anionic composition of stream water varied significantly on a seasonal basis, namely when transpiring vegetative biomass growing in or near the flowing stream was at a maximum (August) or at a minimum (January). Changes in the nitrate ion in August (Figure 2) indicate that the riparian vegetation and other associated biota were using the nitrate ion as water flowed downstream from the discharge point. Because this phenomenon occurred during hot and cold seasons, we can rule out the possibility that water highly concentrated with nitrate was recharging the low-nitrate water in the streambed. Although the nitrate ion exhibited the greatest change, a similar trend was also noted for phosphate. Sulfate and chloride, not generally limiting in plant systems, showed no such seasonal behavior.

#### Differences in Composition of Water-Soluble Anions in Native Plant Tissues

Plant litter, particularly in an arid environment, can represent a major seasonal



**FIGURE 2.** Riparian vegetation was responsible for removing nitrate ions from the surface water of Snively Stream with distance from its headwater. Removal was greatest in August, when plants were actively growing, and lowest in January when active plant growth is lowest.



source of soluble organic matter and accumulated ions to the rhizosphere. Before we can understand the impact of these soluble inorganic and organic species on contaminant solubility and transport, we need to understand the dynamics of the system. To address this need, we have begun a preliminary characterization of the water-extractable anionic and organic components of greasewood, sagebrush, cheatgrass, tumble mustard, tarweed, and chrysanthemum tissue.

Results of this investigation showed the presence of a wide range of soluble constituents whose composition and concentration varied as a function of plant species and season. The major water extractable component associated with plant tissues is organic in nature and consists of organic acids, amino acids, small peptides, and sugars. These components constitute approximately 8 to 17 millimoles of carbon per gram of tissue. Concentrations of inorganic anions were lower in comparison to the total carbon, and ranged from 20 to 130 micromoles/gram of tissue for sulfate, chloride, and phosphate. The concentration of extractable inorganic nitrogen was similar in magnitude and range to other nutrient anions (22 to 100 micromoles/gram of tissue). However, the concentrations of  $\text{NO}_3$  and  $\text{NO}_2$  were generally low and ranged from detection limits (less than 0.03) to 1 micromole/gram of tissue. On the basis of these data, it would appear that the presence of nitrate in the rhizosphere beneath the canopy of the native plants results from a microbial conversion of the organic nitrogen and the ammonium ion present in litter fall.

#### BIOACCUMULATION AND BIOCHEMICAL BEHAVIOR OF XENOBIOTIC ORGANIC COMPOUNDS IN TERRESTRIAL SYSTEMS

D. A. Cataldo and R. M. Bean

A significant fraction of organic contaminants released to the environment eventually accumulate in soils. The quantity of the contaminants that reach humans after soil deposition depends on a range of factors, including the contaminant's environmental persistence and its likelihood to be accumulated, transformed, or transported via components of the food chain.

In-depth studies of the environmental and biological behavior of xenobiotics have been limited to a few polycyclic aromatic compounds. The objective of this PNL project is to develop an overall understanding of the behavior and fate of a range of xenobiotic organic residues in soils and plants.

Specific organic compounds such as phenols, amines, and n-heterocycles were selected for study because of their presence in energy-related materials and their toxicity to some plants and animals. Each compound is evaluated for its soil persistence and solubility, for its biological availability to plants, and for its chemical fate within plants. Chemical fate within plants was determined by measuring the extent to which organic constituents are oxidatively decomposed ( $\text{CO}_2$ ) and chemically modified by plant metabolic processes. We are also studying the rate at which parent compounds and modified products are re-emitted to the atmosphere.

Previous studies have shown that phenol is readily decomposed in soil. Plants, for example, absorb an average of 180  $\mu\text{g}$  phenol/g fresh weight root per day from solutions containing 50  $\mu\text{g}/\text{ml}$ . The phenol was found to be uniformly distributed between roots and leaves. Ninety percent of the residual phenol was associated with a broad range of metabolic products. Thus, phenol represents an organic contaminant which does not persist in plants and is readily metabolized and decomposed.

Aniline, which is more persistent than phenol in soil, has a root absorption rate of 13  $\mu\text{g}/\text{g}$  fresh weight root/day from solutions containing 10  $\mu\text{g}/\text{ml}$ . Aniline is less plant mobile than phenol; approximately 90% of the accumulated aniline is retained by the root.

Quinoline was more persistent in soils than either phenol or aniline. In addition, the overall behavior of quinoline in plants was substantially different from that of phenol or aniline. The most striking difference was that approximately 60% of the absorbed quinoline was re-emitted to the atmosphere through volatilization from the leaves, while less than 1% of the absorbed quinoline was decomposed and released as  $\text{CO}_2$ .

Of the quinoline accumulated in roots and leaves, greater than 90% of the activity was



found to be associated with extractable quinoline, indicating an absence of significant plant metabolism. Eight percent of the remaining radioactivity was accounted for in four intermediate metabolic products (Figure 3). The major component, accounting for 5% of the total activity in leaves, was hydroxy-quinoline, which was present in conjugated form and released by acid hydrolysis. In addition, lesser amounts of methylated and ring-hydrogenated quinoline were identified, along with approximately 0.5% of partially degraded residues. It should be noted that each of these processes--hydroxylation/conjugation, methylation, hydrogenation and ring opening--are mechanisms commonly used by plants to detoxify herbicides, and may represent an important aspect in our understanding of the fate of fossil fuel-related xenobiotics in the food chain.

Initial results of these studies clearly show that plants accumulate organic xenobiotics. Some compounds can be almost completely degraded in plants (e.g., phenol), making them innocuous, while others are sequestered as conjugates (e.g., aniline, quinoline), and still others are accumulated unmodified

(e.g., quinoline). In each case, plant metabolic processes alter the amount of a compound that may reach humans via food chain transport.

#### AQUATIC FOOD CHAIN TRANSFER

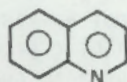
D. D. Dauble

Researchers at PNL are studying the processes that influence the bioaccumulation of organic compounds by fish and other aquatic organisms. Specifically, the studies are designed to identify the mechanisms by which organisms regulate the body burden of contaminants. Such knowledge will eventually be used to determine potential human health effects.

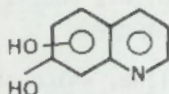
Recent studies have examined uptake, distribution, elimination, and metabolism of the basic nitrogen heterocycle, quinoline, by rainbow trout. We have established that the route of exposure largely determines the site of absorption and ultimately the fate and disposition of quinoline within the body tissues of trout. The kinetics of uptake and elimination and relative body burden also differ between diet and water exposure routes. Further research is planned to clarify relationships among exposure time, compound form, and concentration. An understanding of the physiological processes that limit uptake at the site of absorption will aid our ability to predict body burden based on contaminant concentration in various environmental compartments.

Aquatic organisms have the capacity to metabolically transform many types of chemicals. These modifications may result in detoxification of a toxic constituent or possibly conversion to either a more biologically active or a more persistent form. Thus, knowledge of the factors that influence biotransformation of organic compounds by fish are essential to provide an understanding of potential for food chain transfer. Previous studies have shown that algae, zooplankton, decapod crustacea and fish all metabolized several one- and two-ring organic compounds after adsorption. Up to 80% of the original radiolabeled parent compound was stored as metabolites during chemical exposures. This indicates that the bioaccumulation potential

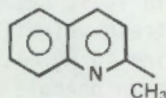
Parent Compound, Quinoline



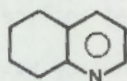
Hydroxylation



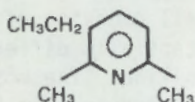
Methylation



Hydrogenation



Ring Opening

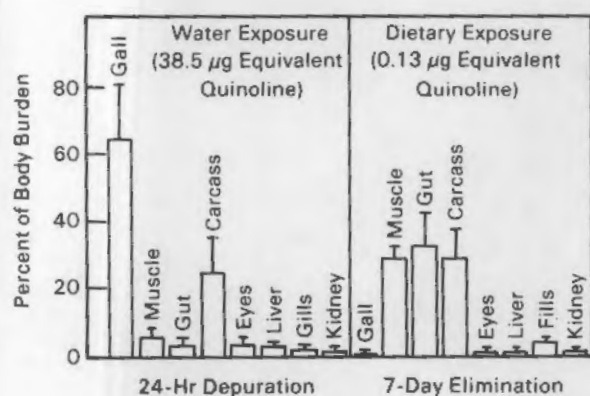


**FIGURE 3.** Structure of quinoline supplied to plant roots, and the structures of major metabolites isolated from leaves of soybean plants. The chemical processes noted are common degradation steps for a range of compounds, which include herbicides.



of these compounds will be greatly overestimated if metabolism is not taken into account. Identification of metabolic products is also important for determining the chemical form of a stored material and the specific metabolic pathways that led to its formation. Although specific biotransformation pathways differ among phyla, similarities exist with respect to extent and type of biotransformation, suggesting that metabolic processes largely control the physiological fate of most organic materials.

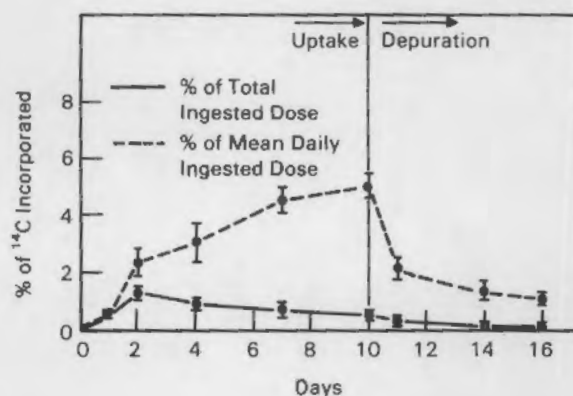
In FY 1985, we continued to characterize the chemical fate of quinoline after it had been taken up by trout and stored in body tissues. We identified a sulfur-conjugated metabolite as the primary transformation product of quinoline in water exposures, but we did not find this product in fish that had been exposed to quinoline in the diet. Storage of quinoline was also related to physiological processes of storage and elimination. For example, starved trout in water exposures retained high levels of quinoline plus metabolites in the bile during depuration (i.e., elimination). In contrast, trout that absorbed dietary quinoline excreted the metabolized quinoline into the gut where most was eliminated from the body (Figure 4). We plan to continue feeding studies in FY 1986 and to examine the role of biliary excretion as a rate-limiting step in regulation of body



**FIGURE 4.** Relative tissue distribution of quinoline plus metabolites was compared for dietary and aqueous routes of exposure after depuration. Digestive processes associated with feeding clearly influence the deposition of a contaminant.

burden of organic xenobiotics. A promising approach would be to link hepatic clearance with metabolic activity, using procedures currently being developed by Dr. H. Richard Frevold at the University of Montana for detection of quinoline hydroxylase activity. Induction of mixed function oxidase systems by xenobiotics may increase the rate of oxidative metabolism, thus decreasing the residence time of a toxicant in the organism. Knowledge of specific enzyme activity levels would provide a measure of factors regulating uptake rates and total body burden during long-term exposures to a xenobiotic (Figure 5).

We expanded our studies of aquatic animals to include exposures of the freshwater crayfish to quinoline and p-amino biphenyl. The major storage tissues for both compounds were located in the digestive system after 24-hr exposure to 1 mg/L in the water. Hepatopancreas levels of quinoline plus metabolites were only about three times higher than water levels. P-amino biphenyl levels in the gut and green gland (an organ analogous to the vertebrate kidney) of crayfish were about 23 times those detected in the water. Elimination of total body burden was minimal for a 24 depuration period. Related experiments examined the effects of pre-exposure on the bioaccumulation and metabolism of quinoline in hepatopancreas, but, and muscle tissue of



**FIGURE 5.** Quinoline and its metabolites were incorporated into rainbow trout tissues at 0.5% to 1.2% of the total ingested dose. Rates based on mean daily ingested dose, however, declined after the second feeding, which suggests that fish were able to regulate their body burden of the contaminant after initial exposure.

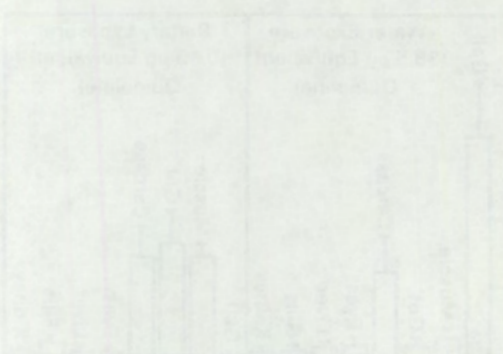
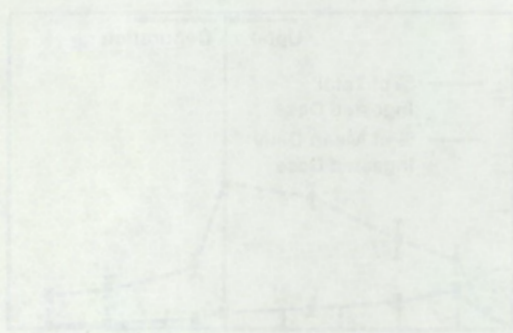


crayfish. We found an apparent uptake plateau of quinoline in crayfish that had been exposed to 5 mg/L for one week. Crayfish that had been pre-exposed to quinoline maintained lower body burden levels of quinoline plus metabolites following a single exposure to radiolabeled quinoline than naive crayfish. However, no differences were evident in the ability of pre-exposed crayfish to metabolize the parent material. Further studies are planned to characterize the temporal patterns of quinoline bioaccumulation by crayfish.

Studies to date will provide the basic framework for current studies that focus on interactive effects of complex mixture exposures on the biotransformation and fate of single compounds. Endpoints selected for study are related to bioactivation and detoxification/elimination mechanisms in fish. This approach increases the applicability of these studies to other organism systems.

#### FUTURE RESEARCH

The fundamental approach taken in the program to define the phenomena controlling soil microbial and plant metabolite interactions with specific inorganic elements is now providing the basis for explanation of the behavior of major classes of elements. Future efforts will be focused on evaluation of concepts developed to describe more general phenomena and to define the soil microbial-plant root environment as an integrated system. The understanding developed on the major enzymatic systems involved in transformation of low-molecular-weight compounds in plant and fish systems is now being used to develop methods for prediction of the behavior of classes of organic compounds previously shown to represent a range in behaviors. Thus, the biogeochemical studies have evolved to the point where major contributions to our general understanding of key processes controlling contaminant behavior can now be realized.





## Subsurface Transport

A major focus of PNL research over the past decade has been to define the mechanisms controlling the release of solutes from energy-derived wastes over the long term. Another equally important objective has been to identify the factors controlling the movement and persistence of organic chemical mixtures in ground water. The research is fundamental in emphasis and has provided new concepts and advanced numerical models of waste leaching and contaminant transport phenomena. Complex interactions were shown to occur between microbial and geochemical processes and between organic chemicals in contaminant mixtures. These findings have profound implications for the rate of solute movement in ground water. The interactions have been probed at the mechanistic level and described with thermodynamic models. The significance of these accomplishments is two-fold. First, improved predictions can now be made of the migration of inorganic and organic solute mixtures, an often baffling situation common to hazardous waste sites. Second, innovative amelioration strategies based upon the manipulation and enhancement of chemical and microbial interactions in water and subsurface regimes are evolving to assist in the cleansing of contaminated ground-water zones.

### SUBSURFACE BEHAVIOR OF ORGANIC COMPOUNDS AND MIXTURES

J. M. Zachara and C. C. Ainsworth

Accurate predictions of organic transport in the subsurface environment must be based on a sound understanding of aquifer fluid dynamics, microbiology, and interfacial chemistry. To aid in developing this fundamental knowledge, PNL is investigating specific interfacial chemical reactions of aromatic molecules exhibiting both hydrophobic and ionizable behavior. Research is focused on the physics and chemistry of sorption of nitrogen-, sulfur-, and oxygen-containing aromatic compounds and their mixtures on unconsolidated materials and surrogate adsorbents. Results of this research are expected to provide a firm mechanistic understanding of the reactions and controlling variables that limit the movement of organic compounds in unsaturated and saturated subsurface water systems.

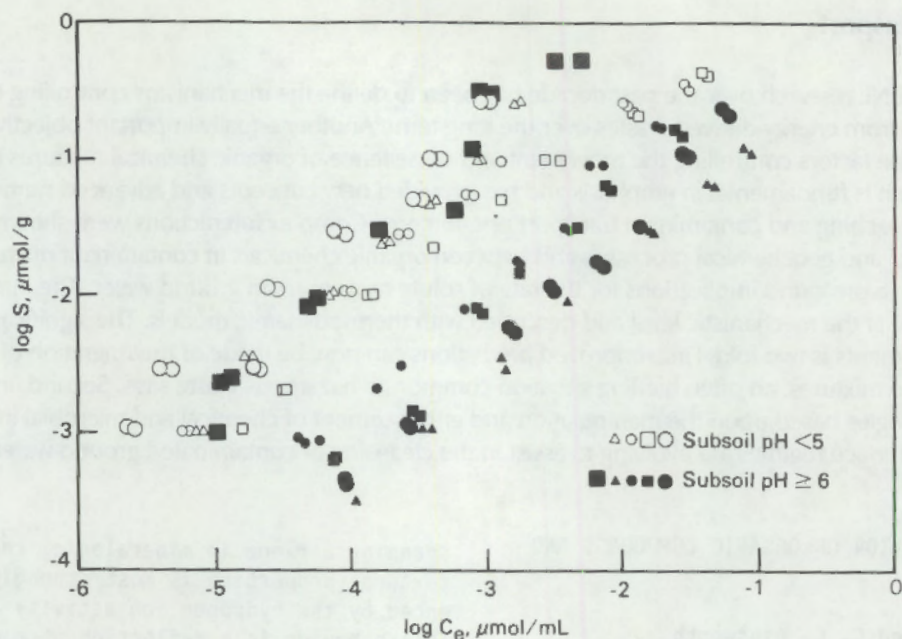
Experimental studies over the past year have revealed new insights into the subsurface behavior of nitrogen-containing (N-containing) compounds. Three phenomena were specifically studied: sorption of acridine, pyridine, and aminonaphthalene on subsurface materials, multiple sorbate interactions of N-heterocycles and aromatic amines on subsurface materials, and quinoline exchange selectivity and adsorption on Na-montmorillonite.

The sorption of both aminonaphthalene (Figure 1) and acridine on subsurface materials

spanning a range in mineralogic, chemical and textural properties is most strongly influenced by the hydrogen ion activity (pH). This behavior is a reflection of compound ionization to the protonated or cationic form via the reaction  $BH^+ \rightleftharpoons B + H^+$ . The acid dissolution constant ( $pK_a$ ) for these reactions is 4.42 for aminonaphthalene and 5.58 for acridine. Our earlier work with quinoline has demonstrated the controlling role of compound ionization in sorption on subsurface materials with low organic carbon. With quinoline, the cationic form is invariably preferred because of the preponderance of fixed negative charges on common aquifer materials and the higher energetics of electrostatic as compared to solvophobic forces. These new findings with aminonaphthalene and acridine extend the conceptual model developed for quinoline to the aromatic amines and N-heterocycles with greater ring number, lower solubility, and higher  $K_{ow}$ . Like quinoline, these results suggest that ionization and surface selectivity must be considered to interpret and estimate retention by subsurface material. The relative importance of coulombic interactions, however, decrease with decreasing solubility, reflecting a larger molecular surface area in the aqueous phase and stronger solvophobic force driving the solute to the solid/solution interface.

Organic contaminants are usually released to the subsurface environment as heterogeneous solute mixtures containing organic compounds and inorganic electrolytes of often diverse properties. Multisolute sorption behavior is being investigated to determine if solute





**FIGURE 1.** In this isotherm that relates solution ( $C_e$ ) to sorbed concentration ( $S$ ) at equilibrium, acidic subsurface materials are shown to consistently sorb more aminonaphthalene than basic materials. This reflects stronger binding of the organic cation.

interactions influence transport. Last year it was reported that quinoline sorption was reduced in the presence of pyridine and acridine, suggesting competition for surface sites. This phenomenon was only observed when the sorbing compounds were dominantly ionized. Additional binary solute sorption studies performed this year have substantiated these findings and shown that while pyridine and quinoline mutually compete for high-energy surface sites at low aqueous concentration, acridine, while inducing the greatest depression in quinoline sorption, sorbs independently (Figure 2). This behavior is consistent with the binding strength of the individual solutes on subsurface materials. Similar results have also been obtained for binary mixtures of aniline and aminonaphthalene.

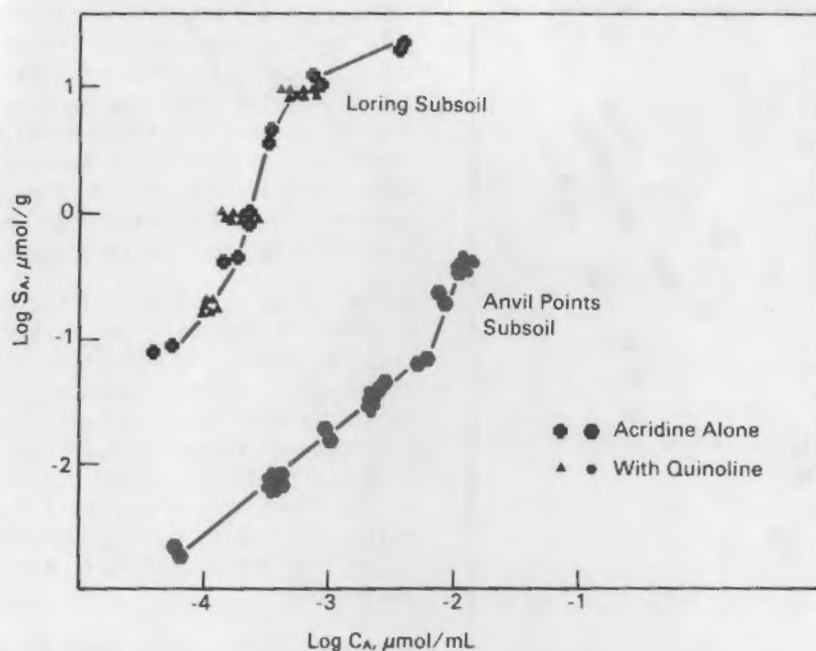
Although qualitative success has been achieved in simulating the results of the competition experiments with N-heterocycles using a binary solute extension of the Freundlich equation and a simplified numerical model of ideal adsorbed solution theory (IAST), a rigorous mechanistic interpretation is complicated by the heterogeneous nature of the subsurface materials. Additional experi-

ments are planned to further elucidate the nature and mechanism of these solute interactions using a pure phase adsorbent (amorphous silica) with well-defined surface charging properties.

All studies performed with N-heterocycles and aromatic amines in subsurface materials suggest that site-specific sorption or surface complexation of the ionized organic molecule control subsurface movement. To provide an enhanced understanding of this phenomenon, PNL investigated the adsorption of quinoline as a function of pH and solute concentration on a well-characterized 2:1 lattice silicate (montmorillonite).

The pH dependence of N-heterocycles observed in subsurface materials was apparent for quinoline sorption on Na-montmorillonite (Figure 3). Even at pH values two units above the  $pK_a$  (4.92), the adsorption of the cationic form appeared to dominate. Using a redefined reference state (0.5 mole fraction surface coverage) that is necessary for organic solutes, a thermodynamically valid equilibrium constant was determined for exchange of the protonated species for Na at pH 5. However, as the initial concentration





**FIGURE 2.** Acridine is a hydrophobic ionizable organic compound that reduces quinoline and pyridine retention in solute mixtures while sorbing independently. The isotherm demonstrates that acridine sorption is similar in both the presence and absence of quinoline.

of quinoline and pH increased, it appeared that a critical surface density was reached at which sorption of the neutral compound became increasingly important. Retention of the neutral molecule reflects a shift in the sorption driving force from electrostatic to hydrophobic. Prior to this change in driving force, montmorillonite demonstrated considerable preference for the ionized quinoline species over the saturation cation, which reflected ring stabilization of the surface organic complex (Vanselow selectivity coefficient,  $K_v$ , of 350). This high selectivity explains, in part, the strong adsorption of quinoline noted in acidic subsurface materials.

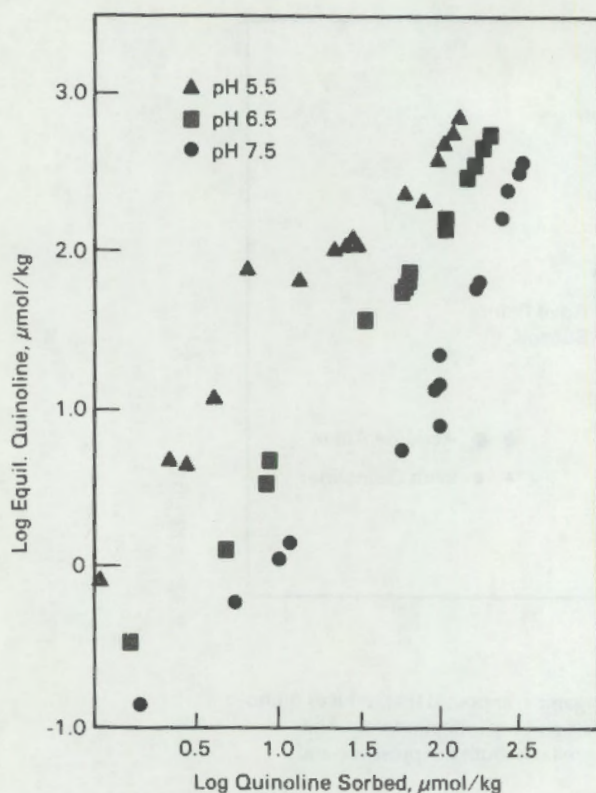
Experimentation over the next fiscal year will probe surface interactions of other organic compounds that vary in  $pK_a$ , molecular surface area, and solubility to elucidate the relative importance of coulombic versus solvophobic forces in the retention of hydrophobic ionizable compounds on both surrogate subsurface adsorbents and subsurface materials.

#### HYDROGEOCHEMICAL PHENOMENA CONTROLLING SOLUTE BEHAVIOR

R. E. Wildung

This program is currently in transition. Investigations are being completed on the behavior of solutes mobilized from energy-derived wastes and transported in the subsurface. This has entailed use of earlier laboratory and field studies to establish and model solubility and hydrologic controls on the behavior and toxicity of solutes in high salt, alkaline environments typical of many waste disposal sites. New research emphasis is on defining the behavior of energy-derived organic compounds in the subsurface environment. Efforts in this area have been directed toward identification and selection of appropriate organic compounds for study, a state-of-the-art review of organic subsurface transport models, and preparation of a national research plan for examination of the chemistry, microbiology, hydrology, and modeling of the multiphase behavior of organic





**FIGURE 3.** On montmorillonite, quinoline adsorption exhibits strong pH dependency, reflecting compound ionization and preferential adsorption of the organic cation. The results substantiate behavior noted in subsurface materials and imply that sorption of the neutral compound occurs only at higher aqueous concentrations.

constituents in the subsurface. The planning effort involved multidisciplinary collaboration within PNL and with universities and industry and focused on key aspects of the subsurface environment, including anaerobic microbiology, computer modeling, and design of field studies.

#### Inorganic Solubility Controls

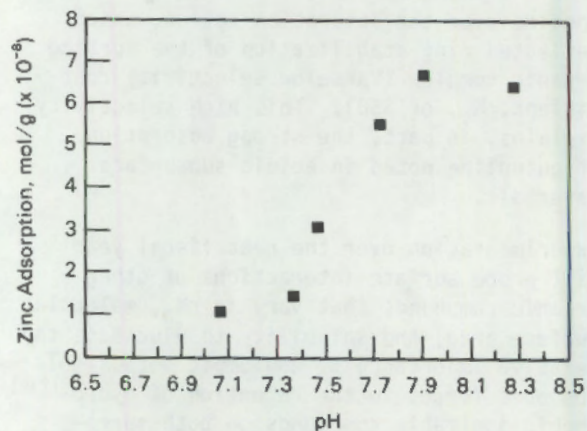
J. M. Zachara

Research on solubility phenomena in wastes has focused on recarbonization reactions, which occur in alkaline wastes disposed for long periods of time. Previous studies demonstrated the important role of carbon dioxide absorption from the atmosphere on leachate chemistry through precipitation of anhydrous and hydrated carbonate minerals.

Studies were therefore initiated to investigate the carbonate phases that precipitated during recarbonization and their capacity to remove soluble constituents from the leachates by co-precipitation, solid solution formation, occlusion, or exchange reactions with carbonate coatings on other waste particles. Initial emphasis has been on  $\text{CaCO}_3$ , a common component of these systems.

While the release and mobility of metallic elements in the waste may be strongly influenced by interfacial and bulk phase interactions with calcium carbonate, little quantitative information exists for predicting the magnitude of these reactions over the long term. Accordingly, detailed investigations were performed on the chemistry of zinc-calcium carbonate partitioning to advance the scientific understanding of this important phenomenon.

Time-course equilibrations, pH, adsorption edges, and adsorption isotherms were determined for  $\text{Zn}^{+2}$  on two calcites with different surface areas in aqueous solutions in equilibrium with calcite ( $p\text{CO}_2 = 10^{-2.5}$  atm). Zinc exhibits a time-dependent uptake on the  $\text{CaCO}_3$  surface, reflecting initial adsorption/surface exchange (complete in 4 hours) and slow incorporation into the solid interior with recrystallization that continues for long time periods (measured to 350 hours). Significant molar adsorptivity of zinc was observed over the pH range 7.0 to 8.5 (Figure 4) despite a net negative charge on the



**FIGURE 4.** As pH increases in an equilibrium  $\text{CaCO}_3$  suspension,  $\text{Ca}^{2+}(\text{aq})$  decreases and allows more  $\text{Zn}^{2+}$  adsorption via exchange with  $\text{Ca}^{2+}$  on lattice positions on the calcite surface.



surface. The increase in adsorption with pH likely arises, not from enhanced stability of hydrolyzed surface complexes of Zn, but rather from decreasing aqueous concentrations of Ca as the amount of dissolved bicarbonate/carbonate increases. Dissolved  $\text{Ca}^{+2}$  is viewed as an ion that competes with  $\text{Zn}^{+2}$  for the calcite surface, and the surface reaction involves exchange of  $\text{Zn}^{+2}$  with hydrated Ca at the interface.



Evaluating the Zn adsorption isotherm at pH 7.25 yields an estimated maxima of  $\sim 1.27 \times 10^{-7}$  mole of Zn/m<sup>2</sup> of calcite, which represents the reaction of Zn with 1% of the exchangeable Ca on the surface. Additional experiments have been performed to evaluate Zn adsorption at variable pH and constant Ca activity, and as a function of calcite surface charge to allow calculation of a thermodynamic exchange constant.

#### Modeling Inorganic Solubility Controls

E. A. Jenne and C. E. Cowan

Speciation and solubility calculations were used to identify those solid phases that limited and/or controlled dissolved constituents in leachates from laboratory columns containing alkaline wastes. This represents an important environment typical of most energy wastes. The chemistry of these systems is poorly understood. The wastes employed were derived from Colorado oil shale that had been retorted using the Tosco, Paraho, and Lurgi processes. Previous calculations indicated that gypsum limited the concentrations of calcium and sulfate in the leachates from wastes derived from the Paraho process. Tosco retorting (lowest temperature) resulted in the least alteration in the raw shale of the three processes compared; therefore, the solubility-controlling phases tend to be the same as the solid phases present in the raw shale. For example, calcite and dolomite, the predominant solid phases in raw shale, are calculated to be in equilibrium with the solutions at most sampling times. Other solid phases that were in apparent equilibrium at various time-periods are fluoride, barite, allophane, ferrihydrite, and magnesite.

The high temperature (Lurgi) process markedly altered the composition of the raw shale because of the high retorting temperature. The temporal changes in the leachate chemistry were quite complex. The concentrations of the constituents in the leaching solutions at different times exhibited six different temporal patterns (see Figure 5). The largest group of dissolved constituents, including pH, exhibited an increase with time to a maximum value and subsequently decreased. The exact shape of the curve and the time of the peak concentrations varies for each constituent. Silica and iron exhibited a variation in this pattern in that their concentration reached a minimum and then increased sharply at the longer reaction times. The second largest group of constituents exhibited a characteristic increase in concentration over the course of the experiment. Five elements decreased in concentration, except that at the latter times, the aluminum and carbonate concentrations increased. The solid phases identified as permissible solubility controls for the various constituents are listed in Table 1. These solid phases were in apparent equilibrium with the solution at one or more sampling times, although they were not necessarily in equilibrium throughout the experiment.

The set of permissible solubility-limiting solids identified using the speciation and solubility calculations permit the probable reaction pathways to be established. For example, in the case of Lurgi retorted shale, the initial increase in the pH and dissolved calcium concentration is probably caused by the dissolution of matrix oxides, such as CaO and MgO, which are formed by decarbonization of calcite and dolomite during the retorting process. After 12 to 48 hours, the pH and the calcium concentration decreased when a carbonate solid phase, as yet unidentified, precipitated from solution. The decrease in strontium concentration appeared to be caused by the formation of celestite, and the decrease in magnesium concentration appeared to be caused, initially at least, by the formation of sepiolite. The fluoride concentration was likely limited by the formation of fluorite after approximately 96 hours. Thus, the speciation and solubility modeling has permitted solubility controls to be identified for the majority



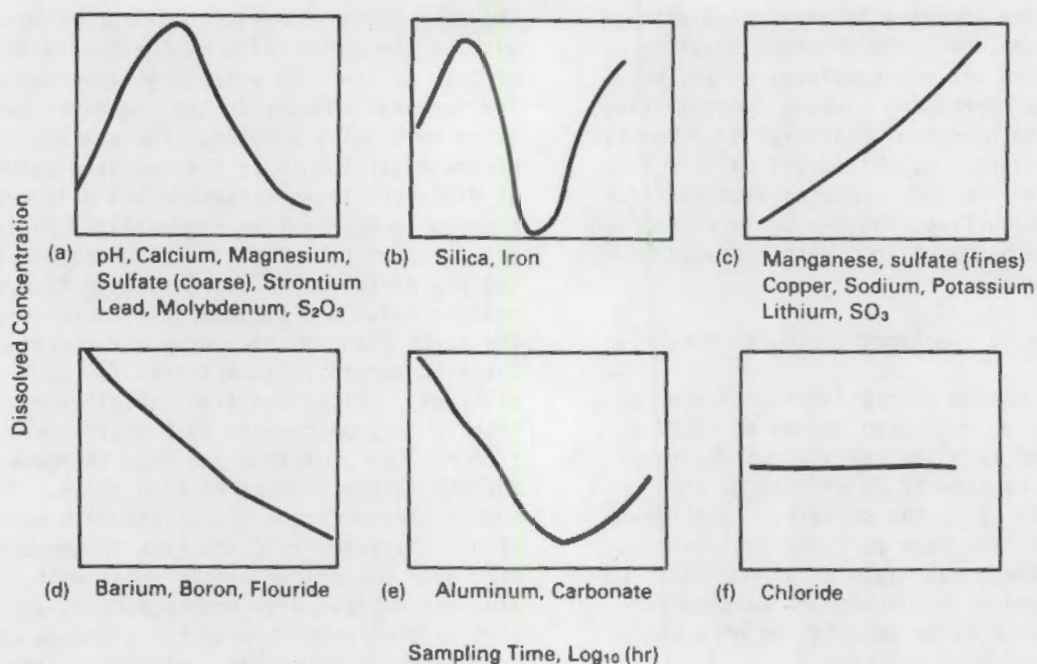


FIGURE 5. Concentration of solutes in alkaline waste leachate with time.

TABLE 1. Permissible solubility controls on alkaline waste leachate.

	Calcium <sup>1</sup>	Magnesium <sup>4</sup>	Iron <sup>6</sup>	Strontium <sup>2</sup>	Manganese <sup>7</sup>	Barium <sup>5</sup>	Aluminum <sup>3</sup>
OH	Portlandite [Ca(OH) <sub>2</sub> ]		Fe <sub>3</sub> (OH) <sub>8</sub>		Pyrochroite [Mn(OH) <sub>2</sub> ]		(A100H)
SO <sub>4</sub>	Anhydrite (CaSO <sub>4</sub> )			Celestite (SrSO <sub>4</sub> )		Barite (BaSO <sub>4</sub> )	
SiO <sub>4</sub>		Sepiolite (Mg <sub>2</sub> Si <sub>3</sub> O <sub>7.5</sub> OH·3H <sub>2</sub> O)					Leucite (KAlSi <sub>2</sub> O <sub>6</sub> ) Analcime (NaAlSi <sub>2</sub> O <sub>6</sub> )
F	Fluorite	Clinoenstatite (Mg <sub>2</sub> Si <sub>2</sub> O <sub>6</sub> )					

of the dissolved constituents. This information, coupled with the mechanistic studies of solid phase-solute interventions described previously provides a sound basis for interpreting and predicting variations in the concentrations of these constituents in waste leachates with time in the environment.

#### Field Evaluation of Laboratory Studies

T. R. Garland

The final task in field investigations of the mobility of solutes in disposed oil shale wastes involved coring of the (Anvil Points,

Colorado) lysimeters containing Paraho retorted oil shale. These lysimeters have been intensively studied since the site was constructed. Leachates were collected from two sets of lysimeters over eight years of weathering. One set had received supplemental irrigation water to approximate rainfall conditions in the Piceance Creek Basin, Colorado. A total of 1.8 column volumes of water percolated through the lysimeter. Over this period, the composition of leachates changed markedly as the waste weathered from a sterile, thermodynamically reduced material to one more typical of oxidized surface soil. Ultimately, the waste supported a heterotrophic microbial population and a plant community. The second set of lysimeters from which cores were collected did not receive supplemental irrigation water after the first year. Drainage (0.01 column volume) from this lysimeter occurred twice over the entire weathering period, both times in early spring.

The 2-m cores were collected in May, as soon after snowmelt as possible, using Shelby tubes (7.5 cm). The cores were capped and stored upright in an insulated container for transport to the laboratory. A total of four cores were obtained from each of the two lysimeters. The cores were broken down under cold-room conditions in 10- to 15-cm increments, sieved (2 mm), and subsampled by quartering. Each subsample was analyzed for water content and then extracted with additional water. The water extracts were analyzed for pH, Eh, conductivity, dissolved organic carbon, inorganic carbon, ammonium, anions, cations, and trace elements.

In general, the composition of the waste with depth reflected phenomena predicted to have occurred in the waste from observations made on the leachate composition over the 8-year weathering period. On the basis of previous field and laboratory studies, it was postulated that leachate composition was influenced principally by the length of time that the retorted shale was exposed to environmental weathering processes, rather than by the total quantity of water that passed through the retorted shale. This was confirmed by the core analyses and is illustrated by the fact that leachate pH decreased to equivalent levels in the lysimeters, which differed by several orders of magnitude in quantity of water that was leached through

the waste due to temperature over the weathering period (previous report).

The integrated effects of key phenomena on leachate composition were also observed. For example, the combined effects of microbial activity in specific layers and the infiltration of pulses of water from successive snows and irrigation events markedly influenced the dynamics of the nitrogen in the waste (Figure 6). The ammonium extracted from the waste in the laboratory likely represents the total ammonium associated with the solid phase, and the concentration changes little with depth. The nitrate concentration, however, varies markedly with depth. It appears that nitrification (microbial conversion of ammonium to nitrate) occurs in distinct horizons and that the highly soluble nitrate is moved with the water from the site of conversion. The concentration of nitrate is therefore related to the time and magnitude of nitrification relative to the time and magnitude of the previous water movement event. The final leachate analyses in 1984 indicated peak nitrate concentrations of approximately 200  $\mu\text{g}/\text{ml}$ , the maximum soluble nitrate observed in the upper reaches of the waste.

The completion of this research and the compilation, interpretation, and publication of information on all the soluble chemical species observed in the leachates and cores should be of major value in the design of

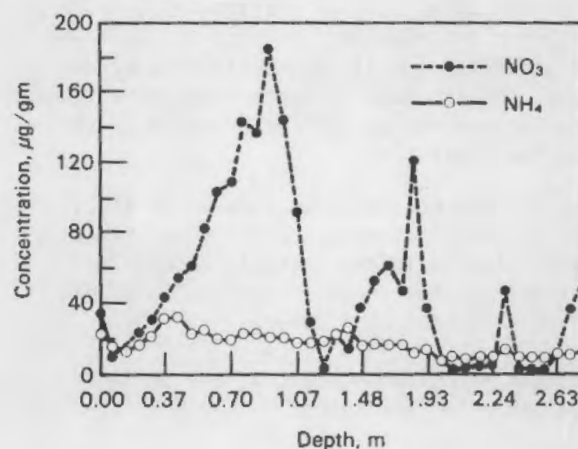


FIGURE 6. Concentration of soluble  $\text{NO}_3$  and  $\text{NH}_4$  with depth in lysimeters containing weathered (8 years) retorted shale.



field research and disposal facilities for retorted oil shale and other wastes that are not in equilibrium with the environs of the disposal site.

#### Relationship Between Aqueous Speciation of Solutes and Solute Biological Effects

C. E. Cowan

Methodology was developed for quantifying the relationship between the chemical speciation of a solute and the toxicity of that solute. First, the aqueous speciation of an element was computed using the geochemical model, MINTEQ, which contains a thoroughly reviewed and partially-validated thermodynamic data base for the element. Then, advanced statistical methods, which were determined to be stable when used with collinear data and underdetermined systems, were used to relate the thermodynamic activities of the aqueous species of the metal and measures of toxicity.

This methodology was applied to four toxicity data sets from the literature wherein the toxicity of copper to fish was studied, and for which the primary molecular species had been tentatively identified by the original authors. The results indicated that the toxic chemical species were primarily the free copper ion and the hydroxide species; the carbonate species were nontoxic.

Because the relationship between chemical speciation and toxicity can now be quantified, it is possible 1) to predict toxicity from the water quality characteristics, 2) to evaluate the relative toxicity of the chemical species, and 3) to quantify the effect of other factors such as concentration of other solutes, pH and age of the organism on the observed toxicity.

The methodology can also be used to assist in the design of bioassay experiments that will permit the toxic and nontoxic effects of various aqueous chemical species to be distinguished from each other.

#### Effects of Environmental Factors on Water Available for Transport

G. Gee

Investigations of the climatic, waste, and vegetational factors governing water balance

in waste and soil systems were previously a part of this program. During the past year, these studies were transitioned into the Arid Land Sciences Program. This entailed 1) synthesizing previous information on arid land water balance into a chapter for publication in the PNL Shrub-Steppe Synthesis Volume, and 2) participation in OHER workshops and joint research planning with other national laboratories, other federal agencies, and the universities for the Arid Land Ecology, REFLEX, and Subsurface Transport Programs.

#### Subsurface Transport of Organic Compounds

C. S. Simmons and G. P. Streile

The initial literature search, planning activities, and design of laboratory and intermediate scale experiments for an integrated organic subsurface transport program were conducted principally on this project.

A literature review of organic transport models was undertaken by S. Simmons and G. Streile to provide guidance for future research and model development required for a new generation of more rigorous transport models.

The modeling review focused on major physical and chemical phenomena responsible for organic chemical dissemination and persistence in the subsurface environment. Technical issues pertaining to hydrologic, chemical, and microbiologic processes were identified. Theoretical aspects of the individual processes as well as mathematical and numerical formulations as related to current code technology were investigated to determine limitations in understanding that must be overcome to advance the capability of transport codes.

It was concluded that fundamental research into the mathematical formulation of constitutive relationships between physical quantities and phenomenological parameters for fluid flow and dispersive transport should be continued beyond its present status and supported by focused experimentation. The appropriate mathematical formulations describing processes such as solubility, sorption, molecular association, and biodegradation should be established through experimentation at the fundamental level for important organic compound classes and mixtures. Interactions of processes on a common



microscopic scale should be examined to enable a coherent, unified theoretical extension of constitutive relationships to macroscopic scales, which are meaningful for modeling of the field scale. And, finally, there is a need to develop more advanced transport models using new generation computing capabilities (super-computers, parallel processors) and merging state-of-science multiphase transport codes with new theoretical advances for modeling basic processes relevant to organic substances.

#### FUTURE RESEARCH

Future research in the subsurface transport program area will focus on the behavior of organic chemicals and mixtures in subsurface environments. The studies will provide the much needed theoretical underpinnings for a new generation of contaminant transport models by elucidating the controlling chemical, microbiological, and hydrologic processes at the fundamental level. Two major scientific questions will be addressed:

- Can organic chemical adsorption and degradation in ground water be predicted from first principles?
- Can the flow of organic liquids in ground water be accurately described at the field scale?

PNL research will use an advanced-concept, intermediate-scale experimental facility where key variables can be carefully regulated to examine processes governing transport at a scale relevant to the natural ground-water system. Construction and capital equipment funds are urgently needed for this facility. New staff, with strong background in the basic sciences (nonaqueous fluid dynamics, organic physical chemistry, molecular biology) and complex environmental problems, are needed to advance understanding of subsurface processes. Only a focused, concerted, scientific effort at the fundamental level can elevate the state of knowledge to the point necessary to resolve the endemic national health problems of ground-water contamination by organic chemicals.



## Theoretical (Quantitative) Ecology

Standard experimental designs, demographic theory, and methods of statistical estimation are generally not applicable for estimating the influence of natural and man-induced stresses on wildlife and fish populations. The PNL theoretical ecology program is focused on developing new concepts and more appropriate methods to meet environmental needs. Such needs are particularly persistent at the large DOE sites in the west. The program has resulted in several major scientific advances: 1) research that indicates many of the currently accepted principles, and thus the theoretical models, used to describe the relationships of habitat to wildlife population dynamics are incorrect; 2) studies which suggest that unbiased estimates of animal survival rates are possible, opening the way for the use of demographic theory in predicting animal population dynamics and providing a basis for understanding population regulation, one of the most important scientific issues in management of large mammals and international fisheries; and 3) the development of new, highly innovative approaches to sampling that will allow precise estimates of pollutant inventories, patterns of dispersal, and depth of penetration as well as the success of remedial measures.

### DYNAMICS OF WILD POPULATIONS

L. L. Eberhardt

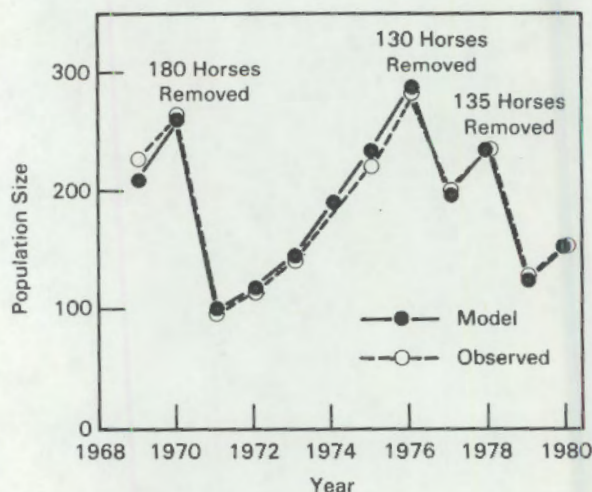
Much of what is presently called "theoretical ecology" lacks the kind of confirmation feasible in, for example, experimental physics. Models familiar to physicists, such as those described in the book Theoretical Ecology, edited by R. M. May (Sinauer Associates, 1976, 1981), are being examined by PNL researchers for their relevancy to data obtained for wild animal populations.

One ecological area in which distinct, well tested "laws" exist, akin to those of particle physics, is that of population dynamics. The existing theory does not, however, take into account populations at or near equilibrium, nor can it, because scientists lack a detailed understanding of regulatory mechanisms. The objective of our long-term research on dynamics of large-mammal populations is to fill this gap in modern theory.

A major difficulty is that virtually all living populations of wild animals have been affected by human activities. One of the more evident impacts occurs when animals are periodically removed from a population. Their removal makes it difficult to estimate essential parameters such as population growth rate. In a variety of cases, it appears that simple models may be used to explore these effects. For example, a simple model derived from our earlier study of the dynamics of feral horses appears to reflect

the actual course of the population, despite the impact of three substantial removals (Figure 1).

This year we completed a study of ways to approximate the basic equations of population dynamics by simple, functional models. These functional representations are not intended to replace actual data on survivorship and reproduction when those data are available in adequate detail. Rather, the purpose is to provide a serviceable approximation in the



**FIGURE 1.** A population model developed by PNL accurately predicted the numbers of feral horses in an Oregon area, even though many of the animals had been removed from the population.



many situations where detailed information is lacking. An important example is survivorship of older age classes. In ecological studies, rarely is it possible to obtain enough observations to accurately assess survivorship at ages when senescence begins to be important. Moreover, there is a widespread impression that senescence may not be a matter for concern, since the few older animals contribute relatively little to dynamics of the population as a whole. However, by using the approximate equations, we have been able to demonstrate that senescence biases one of the methods most widely used in estimating overall survivorship.

A major paper was published on population dynamics of the Yellowstone grizzly bear, and two others were submitted for publication. This work has helped further define the prospects that grizzlies will ultimately be extirpated in the contiguous United States because so few fully adult female bears exist. It is of considerable theoretical interest that survivorship of younger age classes may not be substantially different than that under undisturbed, primeval conditions. The results of the grizzly bear study may thus contribute to scientific knowledge of population regulation and provide an explicit statement of the practical prospects for preventing loss of an important component of western wilderness areas.

Research on incorporation of age structure data in population analyses and investigation of population indices were conducted, using a large set of data on the Pribilof fur seal.

#### QUANTIFYING ENERGY-RELATED IMPACTS ON MOBILE SPECIES

J. R. Skalski, M. A. Simmons, and D. S. Robson\*

Typical analyses of environmental data have as their focus the mean response of biological systems. However, natural systems are also characterized by varying degrees of environmental patchiness or grain. The attributes of this environmental variability

have usually been viewed as a nuisance rather than as a possible opportunity for conveying information on environmental health. The purpose of PNL's research on Mobile Species is to investigate the role of environmental heterogeneity in the design and analysis of field studies.

In biological systems, changes in mean response levels are likely to create changes in spatial heterogeneity as well. For this reason, a statistical analysis of environmental data that detects a significant change in environmental grain or patchiness through repeated observations of an ecosystem would strongly suggest a corresponding change in mean response levels. For instance, changes in the patchiness of Northeast forests could indicate corresponding changes in forest biomass or timber production as a result of acid precipitation.

From a series of multiplot small-mammal investigations, we observed that while the between-plot variance in animal abundance declined with lower mean abundance values, the relative variance or coefficient of variation (C.V.) increased sharply with lower animal abundance (Figure 2). Similar mean-to-variance relationships may be expected with other environmental components that undergo stress as a result of regional impact. Theoretical relationships we have

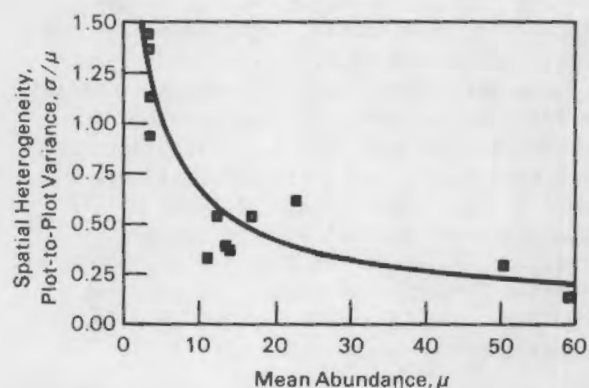


FIGURE 2. Spatial heterogeneity (plot-to-plot variance component) decreases as the mean abundance of small-mammal populations increases. Data sets represent nine different small-mammal species and studies from four different shrub-steppe habitats in Washington and Colorado.

\*Biometric Unit, Cornell University, Ithaca, New York.



investigated lead to an expression for this mean-to-variance phenomenon wherein

$$\sigma_N^2 = \mu_N + \mu_N^2 C.V. [E(N|\lambda)] \quad (1)$$

where  $\lambda$  is associated with the local (micro-habitat) conditions of the plot. Expression (1) is simply a more general case of the variance function

$$\sigma_N^2 = \mu_N + \frac{\mu_N^2}{R} \quad (2)$$

typical of the negative binomial frequency distribution, which has been observed to describe a spectrum of biological communities. The C.V. in Equation (1) may be relatively invariant to different levels of overall abundance ( $\mu_N$ ) and thus not dependent on the more specific negative binomial model. Such invariance has been widely observed or attested to in biological systems. As such, this invariance further strengthens the contention of a strong interrelationship between the detection of change in spatial patchiness and changes in abundance or response levels in ecological systems.

The mean-to-variance relationship observed in small-mammal populations (Figure 2) and predicted by Equation (1) may provide a measure of the spatial fabric of natural systems. Apparently, the spatial organization of natural communities remains relatively intact (i.e., invariant) despite local and seasonal effects on the absolute abundance of an organism. In other words, undisturbed communities may follow a trajectory (i.e., the mean to  $\sigma/\mu$  curve, Figure 2) along which abundance and spatial aggregation are related. As such, the fidelity of this spatial structure of natural populations could be used to assess effects on wild populations.

For instance, permits for surface mining include the requirement to return sites to preoperational conditions. Historically, validation of reclamation success has consisted of comparing preoperational abundance values with sample surveys conducted some years after surface restoration. Time and location effects make such straightforward

comparisons statistically indefensible and ecologically meaningless. An alternative to this traditional approach to reclamation validation would be to use the mean-to-variance relationship as part of the statistical decision process.

Local and climatic conditions may alter the mean response levels of post-reclamation surveys, but the invariance of the mean-to-variance relationship could be used to determine whether such survey results fall along a trajectory identified from preoperational sampling. Should the post-reclamation results not fall along the preoperational trajectory, it would be concluded that the community structure has not yet recovered from the surface disturbance of the energy exploration and extraction. The anticipated result of our approach would be a more holistic and realistic assessment of wild populations that accounts for the biology and statistical behavior of natural systems.

#### DESIGNS FOR ENVIRONMENTAL FIELD STUDIES

J. M. Thomas and L. L. Eberhardt

A shortcoming of current field studies in ecology and environmental science is that some objectives of the studies cannot be satisfied experimentally. This shortcoming can be corrected through the development of sampling designs suitable for the objective of the field work. Suitable designs for several classes of sampling have been developed in non-ecology fields and are thus largely unknown to ecologists and environmental scientists. Hence, an important part of this project is devoted to adapting results from these other fields (for example, geostatistics and industrial research) for use in ecological studies. An essential feature of the research is to assemble a sizable number of sets of data from environmental and ecological studies to provide a suitable basis for adapting and implementing appropriate field designs.

Initial research in this project will focus on sampling for pattern and for modeling. An overview paper will define the problems involved in field sampling and analysis and describe the research approach for the scientific community.



Initial efforts on sampling for pattern will have three components. One component will address the basic assumptions of current methodology (kriging), developed in geostatistics and how well the assumptions are substantiated in those environmental and ecological circumstances where appropriate measurements are available. Data from PNL studies of radionuclides, heavy metals, and pesticides in soils and biota will be utilized to determine how well typical field data serve to estimate both the form and parameter values of the major structural component of the kriging process (the variogram, or correlation structure model). In the few instances thus far examined, the basic assumption does not appear to be substantiated in practice. The more difficult second component, the effect of measurement errors on kriging results, will likely be addressed only in a limited way the first year. It is questionable whether the usual kriging model adequately addresses the well-established existence of major variability attributed to this source.

A third component of the work on sampling for pattern will probably be explored only in the first year, but it will be an important part of subsequent efforts. This component is the use of data that are collected by unconventional measurement techniques, such as "distance sampling" and line transects. It is anticipated that it will be possible to state the issues and describe potential routes for further development in an initial review. The review will be published in an effort to stimulate further attention by mathematical statisticians who may be able to contribute to further theoretical development. These methods may markedly increase sampling efficiency when the main emphasis is on pattern.

The first-year objective in sampling for modeling will be to compile a catalog of appropriate models for ecological and environmental work, with estimates of optimum sampling points. Results from studies in other contexts require reasonably straightforward revision, because the published versions are based on normal, additive error terms, whereas our experience indicates that

a logarithmic transformation is required. This difference changes the form of the model and the corresponding optimal points. More complex models can only be assessed numerically and require the use of a computer. Existing computer search routines will be supplemented with more efficient methods, possibly implemented on faster computers. Although this change is likely to be time consuming, it will follow a reasonably well-defined path.

An essential extension of sampling for modeling (to start in only a limited way during the first year) will be a thorough assessment of the existing methodology, using extensive sets of ecological and environmental data. The present theory and practice have been thoroughly developed in the context of industrial research. Specific examples from that field, primarily models for chemical kinetics, have direct applications in environmental studies and have been applied in a few cases. While these examples provide suitable evidence of the potential value and applicability of the approach, it will be necessary to study more data before a suitably well-grounded prescription for general use in environmental scenarios can be devised. A number of further demonstrations will probably be needed over the long term before the approach becomes widely known and accepted.

#### FUTURE RESEARCH

Future research in these projects will focus on: 1) developing a scientific basis for field-testing current theoretical ecological models; 2) devising approximate methods for estimating population survival rate from age data, so that demographic theory can eventually be used to understand population regulation, and 3) developing new, highly innovative sampling approaches to allow precise estimates of contaminant inventories, patterns of dispersal, and an evaluation of the success of remedial measures. The basic research currently under way as well as that projected for the near term will lay the foundation for the attainment of these longer-term goals.



## Other Research

Research for other agencies offers the opportunity to develop new technology in the environmental sciences and to apply existing talent in solving practical problems faced by the federal government. The research topics identified in this section complement those discussed in other sections of this report but do not duplicate our work for DOE's Office of Energy Research.

For additional information on any of these projects, contact the principal investigator listed. Projects marked with an asterisk (\*) were completed in fiscal year 1985.

### BONNEVILLE POWER ADMINISTRATION

Provide Technical Assistance to Fish and Wildlife Division in Support of BPA Habitat Improvement and Fish Passage Work Plan (D. H. Fickeisen, TD2068\*)

Facilitate Annual Review Meeting for Fish and Wildlife Division Contractors (D. H. Fickeisen, TD2092\*)

Evaluate Fish Passage and Protection Facilities in the Yakima Basin (D. A. Neitzel, TD2107)

Assist in Development of a Comprehensive Research Plan for White Sturgeon in the Columbia River Basin (D. H. Fickeisen, TD2125)

### DEPARTMENT OF INTERIOR

Develop Assessment Procedures for Physical and Chemical Injury to Soil under Comprehensive Environmental Response, Compensation, and Liability Act (P. Van Voris, TD2191)

### ENVIRONMENTAL PROTECTION AGENCY

Acute Effects of New Coal-Conversion Technologies on Aquatic Ecosystems (D. H. Fickeisen, TD1123\*)

Demonstrate Utility of Honey Bees As A Monitor for Chemical Contamination Over Wide Areas (J. M. Thomas, TD1589)

Evaluate Quantitative Reliability of Bioassay Data Extrapolated to Actual Field Data (J. M. Thomas, TD1598)

Provide Technical Support to NEA and IAEA Related to Sea Disposal of Radioactive Waste (W. L. Templeton, TD1776)

Technical and Management Analysis to Support the Puget Sound Water Quality Management Program (J. A. Strand, TD1789\*)

Prepare Field Guides for Biological Testing at Hazardous-Waste Sites (D. A. Neitzel, TD1907)

Provide Support for the Acid Deposition Planning Staff in Support of Task Group E (Aquatic Effects) and F (Terrestrial Effects) (W. E. Fallon, TD1929)

Compile A Set of Screening Models for Environmental Radiological Assessment (W. L. Templeton, TD1994)

Manage Elements of EPA's Cold Region Research Program in Alaska (J. B. States, TD1668)

### NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION

Conduct Workshops to Identify Priorities for Preparation of the National Marine Pollution Program and Prepare Regional Reports on Marine Pollution (D. H. Fickeisen, TD1887\*)

Perform Research on a New Disease Affecting Chinook Salmon (R. A. Elston, TD1921)

Investigate Sea Surface Contaminants in Puget Sound (J. T. Hardy, TD2076)



#### NUCLEAR REGULATORY COMMISSION

Relevance of Biotic Pathways to the Regulation of Nuclear Waste Disposal (D. H. McKenzie, TD1403)

Determine the Biological Availability of Speciated Radionuclides from Ground-Water Seepage (W. H. Rickard, TD1620\*)

Develop Statistical Rationale for Implementation of 10 CFR 61 Regulations on Site Suitability, Characterization, Monitoring, and Closure (J. M. Thomas, TD1634\*)

Safety Implications of Auxiliary System Biofouling (T. L. Page, TD1640\*)

Soil-Biotic-Hydrologic Processes Governing Mobility and Biological Availability of Radionuclides in Commercial Low-level Waste Disposal Sites (R. E. Wildung, TD1913)

Provide Technical Assistance to Study Mud, Silt, and Corrosion-product Fouling of Nuclear Power Plant Open-cycle Water System (T. L. Page, TD2230)

#### PACIFIC MARINE FISHERIES

Water Budget Council (D. H. Fickeisen, TD1980\*)

#### UNC NUCLEAR INDUSTRIES, INC.

Conduct Fish Studies to Evaluate Compliance with NEPA Statutes (T. L. Page)

Provide Technical Input to 316(a) Demonstration for Evaluating Compliance with NEPA Statutes (D. A. Neitzel)

#### U.S. ARMY

Analyze Sediment, Tissues, and Water for Polynuclear Aromatic Hydrocarbons and Metals (J. W. Anderson, TD1722\*)

Analyze Sediments from Everett Harbor to Determine Contents of Organic and Metal Pollutants (J. W. Anderson, TD1819\*)

Assess the Acute Toxicity of Army Smoke Screen Materials to Aquatic Organisms (T. M. Poston, TD1840\*)

Evaluate and Characterize Mechanisms Controlling Transport, Fate, and Effects of Army Smokes in PNL Wind Tunnel (P. Van Voris, TD1915)

Ocean Disposal Studies for Grays Harbor Navigation Improvement Project (W. H. Pearson, TD1948)

Provide Aid in Collecting Cores and Sediment Samples from the Duwamish River (J. W. Anderson, TD1011\*)

Investigate Immunoassay Development for Field Detection of Saxotoxin and Gonyautoxins (R. A. Elston, TD2022)

Determine the Fate of Metal Powder in a Laboratory Aquatic Microcosm (E. A. Crecelius, TD2039\*)

Demonstrate Production of Monoclonal Antibodies in Fish and Determine Their Suitability for Assaying Environmental Media (T. M. Poston, TD2070)

Evaluate Environmental Fate of Selected Agent Simulants on Water and Study Their Acute Effects (P. Van Voris, TD2089)

Biological and Chemical Characterization of Sediments from Oak Harbor, Washington (J. W. Anderson, TD2091\*)

Biological and Chemical Characterization of Port Gardner Disposal Sites and Marine Sediments from Everett Harbor, East Waterway (J. W. Anderson, TD2098)

Assess Effects of Dredged Material Disposal on Dungeness Crab Chemoreception and Behavior (W. H. Pearson, TD2114)

Collect and Analyze Snake River Sediments from Clarkston in Support of Dredging Activities (E. A. Crecelius, TD2165)

Chemical Surety Research (P. Van Voris, TD2148)

Handle, Prepare and Document Sediment Samples  
for the Duwamish River Navigation Improvement  
Project (J. S. Young, TD2202)

Sample and Analyze Snake River Sediments from  
Lewiston, Washington (E. A. Crecelius,  
TD2246)

Juvenile Ling Cod (W. H. Pearson, TD2257)

Fish Mortality/Manganese (C. E. Cowan,  
TD2285)

PS Dredged Disposal Analysis (J. T. Hardy,  
TD2297)

U.S. NAVY

Evaluate the Environmental Impact of Site  
Preparation, Construction, and Operation of  
the Southeast Alaska Acoustic Measurement  
Facility (SEAFAC) at Behm Canal, Alaska  
(J. A. Strand, TD2201)





University  
Interactions and  
Technology Transfer

## University Relations and Technology Transfer

Activities that bring together Laboratory staff and members of universities and outside organizations are effective in enhancing public awareness and use of the results of research conducted at PNL. Toward that end, PNL continues a planned program of external interactions with local, state and federal governments; industry; educational institutions; and the general public. Formal programs include activities to transfer federally developed technologies to industries and to state and local governments.

### UNIVERSITY INTERACTIONS

For many years PNL has maintained a program of active collaboration with visiting research scientists and the university community. There is active collaboration under way on every project conducted for the Office of Energy Research. There are, however, several primary traditional and new avenues to achieving these important interactions. The National Environmental Research Park and its antecedent Arid Land Ecology program have provided a doorway for university participants interested in environmental research at the Hanford Site since 1968. Several hundred faculty and students have participated in research under this program, profiting by their access to the facilities of PNL and contributing to the many DOE research projects in environmental sciences.

During the past decade, the program has sponsored over 70 master's theses and doctoral dissertation studies by joint arrangement with universities in 25 states. In FY 1985, 129 assignments allowed students and faculty from all parts of the United States to participate in research projects. It is noteworthy that many individuals continue to publish collaboratively with PNL many years after their initial contacts.

The National Environmental Research Park program has always been planned cooperatively with the Northwest Organization for Colleges and Universities for Science (NORCUS) operating under the auspices of the Tri-Cities University Center, and more recently with PNL's Office of University Relations. Funds from both the University/Laboratory Cooperative Program, administered by NORCUS, and from PNL programmatic sources are used in support of participants.

### Subsurface Transport Program

The PNL Subsurface Transport Program has strong ties with the university community. Highly qualified university scientists are functioning as technical advisors to the program by reviewing research plans for laboratory and intermediate-scale experiments and suggesting alternative and possibly more fruitful approaches where necessary. Several university research groups possessing unique expertise are collaborators on the PNL program as subcontractors to strengthen fundamental research in specific scientific areas germane to subsurface transport. Collaborative research is also under way with other university scientists who are funded separately by the DOE/OHER Subsurface Transport Program. Joint research with the university sector will undoubtedly expand as the PNL program progresses from basic scientific research in the laboratory to larger-scale experiments in the intermediate-scale facility and the field. Currently, the collaborative relationships exist with the following universities: Purdue, Princeton, University of Florida, California Institute of Technology, Stanford University, University of Notre Dame, Massachusetts Institute of Technology, Pennsylvania State University, and University of Illinois.

### Environmental Center

During the past year, Hanford-related ecological studies have been coordinated with other environmental studies through an Environmental Center established under the auspices of PNL's Assistant Director of Research. This includes selected radiological, hydrological and biological projects, which will be reported on at the end of the current fiscal year. An Environmental



Information Center and efforts related to coordination of activities on the Arid Land Ecology Reserve, and the larger areas of the Hanford Site collectively referred to as the National Environmental Research Park, comprise the current Center.

The Center affords opportunities to university faculty and students for laboratory-oriented research, field research, and validation/verification studies. These opportunities are funded through the NORCUS/PNL cooperative arrangements discussed above. The Center provides a single point of access to an extensive long-term and systematic data collection covering: climatology; micrometeorology; radiological, hydrological, wildlife and fisheries baselines; vegetative dynamics; soil characterizations; and other environmental topics. For many of these examples, the data bases extend to 30 years or more.

#### Other Interactions

PNL staff members hold affiliate appointments at a number of universities and contribute directly to these educational programs by teaching at the Tri-Cities University Center in Richland, Washington. Scientists and engineers at PNL comprise more than two-thirds of the teaching staff at this center. In so doing, they serve as faculty members of the University of Washington, Washington State University, and Oregon State University, the institutions that operate the center.

#### TECHNOLOGY TRANSFER

When appropriate, federally owned or originated technology is transferred to state and local governments or to the private sector for solution of key problems and commercialization. The transfer of technology encourages the full use of DOE's investment in research programs and staff development at PNL. This year, technologies in all five research areas were transferred to other agencies or organizations. PNL staff members consult with these clients on an ongoing basis.

#### Arid Land Sciences

A device developed by PNL for measuring plant stem diameter (U.S. Patent No. 4,549,355) is

being used by EPA to detect and measure the short-term physiological responses of various economically important plant species to ozone. The agricultural and forest industries have also expressed interest in using the device for measuring water, nutrient, and contaminant stress.

The "briobarrier" technology developed by PNL is licensed to E. I. du Pont de Nemours & Co. (Inc.) and is being used by a sewer gasket manufacturer to develop root-inhibiting gaskets for sewer pipes. The sewer gasket slowly releases a controlled amount of herbicide, which prevents plant roots from penetrating into sewer lines. This technology is estimated to save the industry an estimated \$20 billion in future damage.

Rockwell Hanford Operations, UNC Nuclear Industries, Inc., and the NRC are using the BIOPORT computer code developed by PNL to estimate the quantities of radioactive materials that are vertically transported and redistributed by plants and burrowing animals in the vicinity of shallow-land waste burial sites.

A phytoassay technique developed on DOE programs was adopted by EPA as part of its suite of routine test protocols for assessing toxicity of hazardous chemicals in soils.

#### Marine Sciences

Information from the marine chemistry program on the distribution, sources, and fate of contaminants in Puget Sound is being used by EPA and local regulatory agencies to plan cleanup strategies for the Puget Sound region.

Surface hydrologic and chemical modeling capabilities are being used by the petroleum industry to predict the fate of surface contaminants on the Pacific Coast. Japanese utilities are using the methodology to predict the fate of radionuclides from the nuclear industry.

Information on the interactions between environmental stress and disease is being used by the Washington Department of Fisheries to help predict the effects of disease on natural populations of animals and by the National Marine Fisheries Service to prevent diseases in valuable salmon resources.

## Biogeochemical Phenomena in Indirect Exposure Pathways

Models incorporating basic soil microbial and plant metabolic processes elaborated in laboratory studies are being used to formulate changes in dose-to-man estimates for plutonium, neptunium, and technetium.

OHER studies on organic matter degradation have provided basic information necessary for the Electric Power Research Institute to test the concept of using microbiological degradation as a viable first-stage process for coal liquefaction.

Research programs have provided the data necessary to develop and test innovative in situ biological decontamination methods. The Defense Waste and Byproducts Management Office of DOE has expressed interest in using these methods as viable alternatives to conventional clean-up practices.

Coupled geochemical-hydrologic models based on fundamental principles and developed on iterative laboratory-field studies were shown to be a more desirable alternative to simple assays proposed under RCRA for assessing the composition of energy-derived waste leachates over the long-term. This information is being used by the petroleum (oil shale) and utility (coal) industries in design and siting of waste-disposal facilities.

Soil microbiological studies have provided a basis for development of gene probes to detect genetically modified microorganisms in the environment.

Geochemical models that incorporate aqueous speciation have recently been incorporated by EPA in proposed revisions of RCRA. They are also being used by EPA and the Corps of Engineers to identify chemical constituents that are toxic to biota. Results will be

used to develop better environmental regulations and treatment systems for protecting our nation's recreational and commercial resources.

The Department of Defense is using fundamental data generated by OHER projects to evaluate the environmental fate and effects of obscurants and other materials of importance to national security.

## Subsurface Transport

Hydrologic and geochemical models developed on OHER projects are being used by NRC and industry to determine the performance of low-level waste and hazardous waste disposal sites.

## Theoretical (Quantitative) Ecology

Research on sampling for contaminant patterns resulted in technology which allowed EPA to use honeybees to quantitatively define the source and dispersion of airborne heavy metals over the Puget Sound region (7500 km<sup>2</sup>).

Results from DOE-sponsored research on survey designs is being used by EPA to assess the biological hazard of chemical waste sites.

Studies of double sampling have provided NRC with a method whereby analytic costs can be dramatically reduced when sampling low-level radioactive waste sites.

Basic research on the "vital statistics" of large-mammal populations has resulted in models that allow game managers to make use of limited data for important management decisions.

Theoretical statistical simulations have provided a method usable on a hand calculator whereby the possibility of success for an impact study can be completed a priori.





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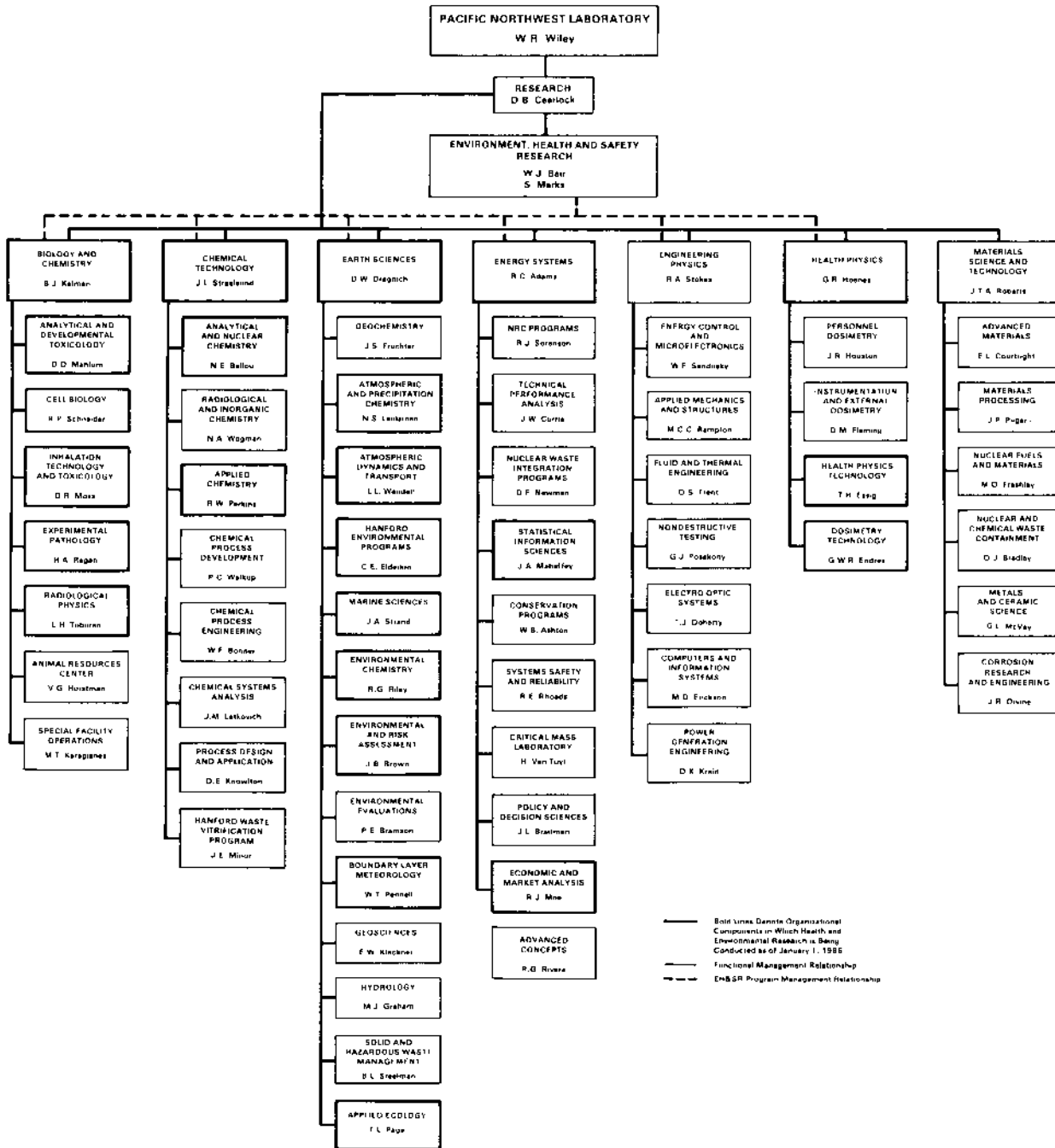


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