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Developing Biological and Chemical Methods
for Environmental Monitoring of DOE Waste
Disposal and Storage Facilities

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Progress Report

April 1, 1985 - October 30, 1985

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Introduction

During the first year of this contract great effort were made to develop methods for (1) characterizing bacteria from soil and sediment, (2) evaluating the ability of single and mixed soil bacterial isolates to, (a) bioconcentrate, (b) biodegrade and/or (c) precipitate inorganic and organic pollutants and (3) expanding current concepts for treating waste in aqueous (i.e. biological waste treatment system) and solid media (i.e. in situ (soil) treatment system). The development of the above methods are in the final stages of completion and we have as a result of these efforts isolated from soil (1) a mixed culture which precipitate toxic metals (i.e. mercury cadmium, lead etc.) and (2) single isolates which bioconcentrate a variety of toxic metals. Methods for screening soil bacterial isolates for their ability to concentrate, degrade and/or precipitate environmental pollutants have been developed. The development of those methods will allow the staff at ORRI to quickly screen hundred of samples in our attempt to isolate bacteria capable of degrading, concentrating and/or precipitating inorganics and organics in aqueous and solid waste. The results of these studies are summarized below.

We have established during the first year contractual agreements with the Oak Ridge Research Institute and Meharry Medical School, Tuskegee Institute, Kentucky State University, and Knoxville College. These agreements include research studies on bacterial identification, biochemical pathways and genetic characterization. These bacterial studies will be performed on bacteria isolated by the Oak Ridge Research Institute from Y-12 sites. A complete discription of these studies is enclosed.

II. a) In Situ Treatment

The flood plain of East Fork Poplar Creek contains a large amount of mercury, as a result of past releases from the Department of Energy's Y-12 plant, which is located at the headwaters of the creek. Oak Ridge Research Institute has been engaged in a survey of microbial activity in the soil of the flood plain. One of our goals is to determine whether heavy metals in the soil have a significant impact on microbial populations and their metabolic capabilities. We believe our results point to a possible approach to remediation of heavy metal contamination of the soil. This discussion will focus on mercury, although we realize that other metals are also present at significant levels.

The risk associated with this environmental contamination depends on several factors, including concentration of metal in the soil, chemical nature and mobility of various mercury species in the soil, and toxicity of the present mercury species and their potential metabolites. Mercury may be present in the soil in the elemental form, as inorganic salts, or in organic compounds. Studies with mice show that the alimentary absorption of elemental and inorganic mercury is fairly low, whereas absorption of organic mercurials is high. Inorganic mercury binds avidly to soil, whereas organic mercurials are more mobile. Therefore factors affecting the chemical nature of mercury in the soil will determine the biological availability of mercury in the soil and water, and these factors should be of primary concern in assessing risk.

Potential options for remediation include removal of the contaminated soil or removing the contaminant in situ. The former option would entail the transfer of large quantities of soil to another site, whereas the latter is probably technically impossible because of the avid binding of mercury to the soil - even concentrated mineral acid does not remove more

than about 40% of mercury added to soil samples in laboratory experiments. Restricting the use of the affected land or capping it would leave open the possibility of vertical or lateral movement of the metals by leaching into groundwater.

Another potential option is to immobilize the contaminants in situ so that they are biologically unavailable. This would require converting them to a form that is not absorbed after ingestion or inhalation. We suggest that it would be prudent to determine what conditions are required to enhance or maintain the soil mercury in a metabolically and toxicologically unavailable form, in particular by encouraging its precipitation as mercuric sulfide and by inhibiting methylation. Such conditions might readily be imposed on the soil relatively economically and without damaging its fertility.

Most of the biological activity in soil is carried out by the microorganisms prevalent there. These microorganisms are often specialized in their metabolic pathways, and often several kinds occur together in communities, each benefitting from the metabolic capabilities of the others. These communities create microenvironments, whose chemical properties are appropriate to the organisms found there. Among the important activities of soil microorganisms are the cyclical oxidation and reduction of various elements, especially carbon, nitrogen, and sulfur. We believe the sulfur cycle can be exploited in reducing the concentration of available inorganic mercury in the soil.

Certain bacteria, the dissimilatory sulfate reducers, carry out a respiratory process in which sulfate is reduced to sulfide, which appears extracellularly. We have observed that this process can remove mercury from solution by precipitation of mercuric sulfide, whose low solubility removes it from further metabolic activity. We have also shown that

absorption of mercuric sulfide in the intestinal tract of the mouse is extremely low, indicating its metabolic inactivity.

Sulfate-reducing bacteria are present in soil, and their activity in soil and sediments has shown to be enhanced by increased concentrations of oxidized sulfur compounds. We have found sulfate-reducing bacteria in every soil sample we have taken from the flood plain of East Fork Poplar Creek. However, in each case, the addition of sulfate was required for sulfate-reducing activity to be observed. We suggest that amending the soil with sulfate would increase sulfate-reducing activity in the contaminated soil and reduce the concentration of available mercury in the soil, thereby reducing the risk that mercury will be metabolized to organic forms, which are soluble, mobile, and toxic.

In addition to the sulfate-reducers, we have found bacteria in soil which synthesize metal-binding proteins and thereby concentrate mercury in a combined form. There are also soil bacteria that can demethylate mercury and also reduce mercury salts to elemental mercury, which is biologically inert. Amendment of soil to enhance these activities should also help decrease the risk associated with the contamination.

Laboratory studies need to be continued and extended to define the conditions necessary to increase the desirable biological activities described here. In addition, field studies should be conducted to determine the feasibility and cost of soil amendment, and guidelines must be established for determining whether the procedures are successful in preventing release of mercury to the food chain.

Laboratory studies have to be conducted to determine what concentrations of sulfate must be added to soil to enhance reduction. We have also determine whether the addition of organic acids as a nutrient source is necessary. The impact of these additions on soil pH will be

measured, and we will determine whether sulfate reduction has an effect on other metals, especially arsenic, beryllium, silver, and thallium. It will be crucial to be certain that precipitation of mercury is not accompanied by mobilization of other toxic metals.

Soil samples will be taken to determine cross-sections of metal and sulfate concentrations in the flood plain. In this way we will be able to correlate sulfate levels with heavy metal concentrations and identify remediation areas. We will also have to determine the levels of organic mercurials at these sites, since it has been reported that at low concentrations of sulfate, sulfate-reducing bacteria can methylate mercury.

We are to carry out leaching studies with columns (30 cm) of soil. We are assessing rates of leaching of mercury and other metals in both normal and waterlogged soil, with and without sulfate amendment. These studies will help us determine whether amending the soil has an effect on the mobility of mercury and other metals, and on the formation of organic metal complexes. We will also monitor sulfate reduction, total bacterial populations, and changes in population distributions as a result of the amendment.

Field studies will have to be based on the results of the pilot-scale leaching studies. We expect that amendment would take the form of surface application of gypsum (i.e. CaSO_4) or some other slowly released sulfate. It will be necessary to monitor sulfate concentrations in the amended soil to determine whether periodic reapplication will be needed. The concentrations of soluble metals will also have to be monitored, as well as formation of organo-metal complexes.

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Results from preliminary studies show that soil from East Fork Poplar Creek has relatively low levels of sulphate and sulphide (Appendix A). Studies have also shown that amending soil samples from East Fork Poplar Creek with sulphate increases the activity of sulphate reducing bacteria. Results from these latter studies are supported by data from soil columns (soil samples from East Fork Poplar Creek) which show that soil amendment with sulphate reduces the level mercury is leachate following the addition of deionized water to the top of the column. (these studies are described in appendix A.)

Effect of sulfate on leaching of metals from soil.

Sulfate-reducing bacteria are a ubiquitous class of soil organisms. They are an important factor in the sulfur cycle in soil. These bacteria carry out a type of respiration in which the reduction of sulfate to sulfide is coupled with oxidation of organic acids. Other bacteria, in turn, can oxidize the sulfide back to elemental sulfur or to sulfate, thus maintaining the sulfur cycle.

We have shown that the growth of sulfate-reducing bacteria in media containing heavy metals results in the precipitation of metal sulfides. In this way, for example, the concentration of soluble mercuric ion in the medium can be reduced to less than 0.1 ppb.

The production of sulfide in soil laden with heavy-metal ion could similarly lead to precipitation of the metal ions. Any toxic effects of heavy metals in the soil might be reduced by this process. However, the level of sulfur available to the sulfur cycle would also be reduced. It has been shown that in the absence of sufficient available sulfate, sulfate-reducing bacteria produce a metabolite that is able to methylate mercuric ion, producing the highly toxic compound methyl mercury. Thus by a combination of these two processes, the metabolism of a limited amount of sulfate by sulfate-reducing bacteria could first decrease, then markedly increase, the potential toxicity of heavy metals in the soil.

Our experiments have shown that all of the soil samples tested to date contain sulfate-reducing bacteria. In each case substantial sulfate respiration by bacteria in slurries of the soil samples could be observed only if sulfate was added to the slurry. This result, together with the observed low levels of soluble sulfate in the soil, indicate that sulfate

may be a limiting constituent for the growth of sulfate-reducing bacteria in the flood plain of EFPC. Thus amendment of the soil with sulfate might both enhance the immobilization of heavy metals by precipitation and prevent the formation of methyl mercury.

As an initial test of the effects of sulfate amendment of soil, we have constructed columns containing soil from a site with a high concentration of mercury. The columns were supplemented with CaSO_4 , the chemical constituent of gypsum, and water is being percolated through the columns at approximately the rate of local rainfall. Ongoing studies will include measurements of leached sulfate and heavy metals. Initial results are shown in Table . These results show that CaSO_4 applied to the surface of the column began to appear in the effluent in approximately 20-30 days, indicating that powdered gypsum applied to the soil should penetrate the soil readily to supply sulfate to the sulfate-reducing bacteria. There are indications from these preliminary results that the treatments might have had a slightly inhibitory effect on leaching of metals from the soil. More extensive analysis will be required to substantiate this result. These studies will be continued and expanded to include analyses for other heavy metals.

II. b) East Fork Poplar Creek Assessment for Microbial Populations and Chemical Properties of Soil from the Flood Plains.

The goal of our soil studies is to assess the biological activities of soil microbes found in several locations along East Fork Poplar Creek, in the area impacted by the historic metal discharges from the Y-12 plant. These studies are intended to determine whether gross changes in soil chemistry, microbial flora, or microbial biochemical activity appear to have occurred as a result of accumulation of pollutants in the soil. Recommendations for remediation may be based on these findings. We further expect these studies to lead to the development of biological treatment systems for the removal of heavy metals from metal-laden waste water, and to the development of systems in which soil microbes can be used to degrade or concentrate hydrocarbons, chlorinated hydrocarbons, or other organic pollutants. This report will summarize our sampling and analytical methods, and give a preliminary assessment of the results.

Twelve sites in the flood plain of East Fork Poplar Creek were chosen from sites previously sampled by this laboratory for a study of heavy metal concentrations. These sites are indicated in Appendix B Figure 1. At each of six locations samples were taken in duplicate on each side of the stream, within 10 m of the stream. Each sample comprised a core 5 cm in diameter, taken to a depth of 45 cm by use of a core sampler equipped with a plastic liner tube. The sample depths used in calculations have been corrected for the compaction observed during sampling.

The core tubes were returned to the laboratory, and samples were taken at the top, middle, and bottom of each core. These samples were used to assess physical, chemical, and biological properties of the soil.

Samples were slurried in water (1:1 w:v) for measurements of pH and conductivity, which were measured immediately and after 17 hrs, with a pH meter and a conductivity meter. These suspensions were then used for measurements (by a barium chloranilate colorimetric method) of soluble sulfate. Moisture was measured by drying a weighed sample of soil for 16 hrs at 110°C and then weighing again to determine the mass of water lost. Moisture is expressed as percent of wet weight. Acid-soluble sulfide was measured by use of a colorimetric assay on acid extracts of soil. Volatile organics were measured by gas chromatography using a purge-and-trap method with water suspensions of soil. Heavy metals were measured by atomic absorption spectroscopy of concentrated nitric acid digests of soil, uranium and nitrate were measured by use of colorimetric assays, and CO₂ evolution was measured by trapping the CO₂ in filter paper with a known amount of NaOH, followed by titration with HCl. To measure metal-binding proteins, cell suspensions were centrifuged and washed, and the pellets were resuspended in buffer with 0.1% toluene to permeabilize the cells. The suspensions were centrifuged and the supernates treated with 2% trichloroacetic acid, which precipitates many proteins but does not precipitate metallothionein, a mammalian metal-binding protein. The resulting suspension was exposed to ²⁰³HgCl₂ to label the metal-binding proteins, and bound ²⁰³Hg was measured by gamma spectrometry.

To enumerate different classes of organisms, soil suspensions were diluted and plated on agar plates containing media selective for aerobic heterotrophs, anaerobic heterotrophs, nitrogen-fixing bacteria, or fungi. CO₂ production was measured by putting 10 g soil samples in wide-mouth jars, each with a trap for atmospheric CO₂. Samples were incubated at room temperature, with measurements made at 4, 8, and 12 days. The

cumulative total CO_2 production was used in calculation of correlations. The ability of the microbes in soil samples to reduce nitrate to nitrite and oxidize urea to nitrite were measured by suspending 10 g of soil in 50 ml water containing the appropriate substrate at 100 ppm N. Incubation was continued in sealed containers at room temperature, with samples taken for chemical assay at weekly intervals for four weeks. Because nitrite can in turn be reduced to N_2 or oxidized to nitrate, the maximum value observed was used in calculation of correlations.

The metal-binding capacities of organisms in the soil samples were measured by growing the microbes in a non-selective growth medium after inoculation with soil. Uranium binding was measured under two conditions: cells were grown aerobically in medium containing 10 ppm uranium as uranyl acetate, or stationary-phase cultures were centrifuged and the washed cells were suspended in buffer with 10 ppm uranium. The concentration of uranium left in the cell-free supernate was then measured. Metallothionein-like proteins were measured as described above, using cultures grown either aerobically or anaerobically, each in the presence of either 10 ppm arsenic, mercury and lead and 1 ppm cadmium or 5 ppm mercury and 1 ppm cadmium, conditions which ought to induce metal-binding proteins. Results were expressed as ng mercury bound per mg protein.

Correlation coefficients were calculated as an aid to discerning effects of metal levels or other factors on microbial activity. Detailed analyses (i.e. partial correlations) have not been completed, so all conclusions at this time are very tentative. However, certain observations can be made. First, the concentrations of the four metals were positively correlated, and numbers of microbes were correlated among all four types enumerated. Numbers of aerobic organisms were inversely correlated with

depth, as would be expected since oxygen concentration should decrease with depth. A single value for metal concentration was used for all depths at each site, so metal concentration and depth should not be correlated. In fact, the correlation coefficients of metals with depth were all close to zero. These observations serve as checks on the validity of the results.

Second, certain other groups of variables seem to be inter-related. For example, arsenic, cadmium, lead, mercury, pH and conductivity were all positively correlated. There are several possible explanations for this inter-relationship, i.e., local soil chemistry could have had an effect on the deposition and retention of metals, deposited metals may have changed other parameters of soil chemistry, or local geologic or other factors leading to metal deposition may also have affected pH and conductivity. The number of anaerobes recovered was positively correlated with pH, arsenic, lead, and mercury. Again, it is not clear which, if any, of these parameters may have had a direct effect on anaerobes. These and other correlations must be analyzed in further detail to provide some clues as to the physiological mechanisms involved.

In general, we conclude that there are no indications that any biological activities we measured were depressed as a result of metal accumulation in the soil. The distribution of some activities may be affected to some extent by soil metal levels, but for the most part, any correlations seem to be toward higher levels of biological activity.

II. c) Biological properties of Pseudomonas syringe a bacterium capable of bioconcentrating toxic metals.

Introduction - It has been known for some time that microorganisms live in ecosystems polluted with heavy metals such as mercury (Hg), lead (Pb), zinc (zn), and cadmium (cd). These microorganisms have developed resistance mechanisms that allow them to grow and flourish in these environments. Heavy metal resistance has not been confined to one specific type of microorganism, but a good representation of the microbial world possess these traits. Gram positive and gram negative bacteria, as well as fungi and actinomycetes have been isolated from polluted environments.

How can we best exploit these resistant microorganisms, and utilize their ability to survive in contaminated environments for useful purposes? Towards this end we are developing microbial strains that can concentrate and/or metabolize heavy metal pollutants. These strains can then be used in biological waste treatment systems to eliminate toxic substances from soil. We have isolated a member of the genus, Pseudomonas, that has superior properties of multiple heavy metal and antibiotic resistance. This isolate was obtained from soil samples taken from the flood plains of East Fork Poplar Creek. For the past several months we have observed this culture growing under laboratory conditions of stress in the presence of heavy metals and/or antibiotics and under non-stress conditions. The following report contains the results of these observations.

Growth Conditions -

Pseudomonas Q₂ (ORRI strain designation) is a gram negative, rod shaped (2.0 X 1.0 μ m), motile, strictly aerobic bacterium which contains dark intracellular granules. General properties of the bacterium include its ability to utilize glucose, maltose, dulcitol, and xylose as carbon sources. It can hydrolyze gelatin and casein but not starch or urea. It is oxidase negative, catalase positive and does not reduce nitrate. Hydrogen sulfide is not produced from ferrous ammonium sulfate or sodium thiosulfate nor is Q₂ able to utilize citrate. It has a sodium chloride tolerance of 5.0% and fluoresces on Bacto Pseudomonas Agar F but not on standard agar. Based on these characteristics the culture was identified as Pseudomonas syringe.

Pseudomonas Q₂ grows well in NR1 synthetic medium (Table 1), at 32°C, under rotary shaker aeration (100 rpm). Within 17 hrs of inoculation (20% v/v) this culture can obtain an absorbance at 600 nm of between 1-2 O.D. units. Under heavy growth conditions it produces either a yellow or greenish pigment. The culture can be induced (stress conditions) in synthetic medium to grow in the presence of various heavy metals (Hg, Zn, Pb, Cd) of environmental concern (see Table 4). It also grows in the presence of high concentrations of antibiotics (ampicillin, 250 μ g/ml; tetracycline 4 μ g/ml; streptomycin, 75 μ g/ml). Although Q₂ grows well in the presence of these antibiotics, only ampicillin stimulates its growth in the presence of Hg and other heavy metals (Zn, Pb, and Cd). Oxygen also stimulates growth of Q₂ in the presence of the heavy metals.

Amplification -

We are developing various methods for amplifying growth of Pseudomonas O₂ in the presence of heavy metals alone or in combination with each other. Two approaches to amplification are under investigation, one based on cell growth and physiology and the other on genetic manipulation (gene amplification).

Gene Amplification -

Antibiotic stimulation - Historically, multiple heavy metal resistance of environmental microbes (i.e. bacteria) has been attributed to the existence of plasmid genomes in the cell. Many heavy metal resistant isolates have also been antibiotic resistant, a trait invariably coded for by plasmids. Plasmids are autonomously replicating DNA elements coding for genetic traits separate from the chromosome. Genetic information located on plasmids can be amplified by stimulating replication of plasmid copies per cell. Stimulation can be accomplished by growing cells harboring plasmids in a substance that requires plasmid gene activity for degradation or cell utilization. It is believed, but not yet verified, that Pseudomonas O₂ contains plasmids coding for genes that allows it to grow in the presence of heavy metals and antibiotics. Ampicillin (amp) stimulates plasmid replication in O₂. It is also believed that the amp resistant and Hg resistant genes are located on the same plasmid since only the ampicillin antibiotic stimulates growth in the presence of Hg and the other heavy metals. We have found that Pseudomonas O₂ growing in NR1 plus ampicillin is resistant to Hg up to 2.4 mM. The MIC of an uninduced O₂ culture or other test isolates to Hg is around 0.09 mM. Pseudomonas O₂ growing in the presence of ampicillin plus cd, zn, and Pb in NR1 is

resistant up to 5 mM Hg. The additional growth stimulation of Pseudomonas O₂ in NR1 plus mixed metals and ampicillin is believed to be due to zinc or other metals, possibly assisting in gene expression.

Chemical Mutagenesis -

Many environmental pollutants are also chemical mutagens. Thus, within the environment, natural mutations occur on a frequent basis due to contaminant exposure. Many of these mutations are stable and are transferred through cell generations. In the laboratory, mutagenesis can be forced upon a culture by treating it with standard commercial mutagens such as nitrosoguanidine, methyl methane sulfonate, and ethyl methane sulfonate. Isolates following treatment are screened for increased heavy metal resistance over the parent culture (alteration of the cells genetic make-up i.e. genetic manipulation). We are in the beginning stages of developing techniques for chemically mutating single isolates and mixed cell populations for pollutant resistance.

Amplification Based on Metabolic Stimulation.

Resistance of bacterial cultures to heavy metals can be amplified by optimizing physiological conditions that highly influence cell growth and survival in the presence of pollutants. Physiological parameters such as pH, Oxygen uptake, temperature, and growth medium are a few of the external factors influencing growth rate (i.e. cell number) of a microbial population. We have found that growth of Pseudomonas O₂ in the presence of Hg is enhanced or amplified by increasing the oxygen supply to the cells (see Table 6). The data shows that forcing air through the NR1 growth medium supplies enough dissolved oxygen for Pseudomonas O₂ to grow in as

high as 0.3 mM Hg. A combination of forced air and ampicillin increased Q₂ Hg tolerance up to 1.0 mM.

Media Cost Analysis Study

Growth medium of Pseudomonas Q₂ will be an important factor in its utilization in a biological waste treatment system. Medium constituents and whether they will support growth and bioconcentration of pollutants will be a major concern. Of even more importance will be medium cost, "how cost-effective is the medium on a large scale volume?" Media cost analysis studies such as the one generated for table 7 are being done to determine the most efficient and cost-effective medium in which strains such as Pseudomonas Q₂ can be grown on a large scale. The data in table 7 shows that a cost savings of \$.27 per liter of medium can be netted by using medium NR2 over NR1. This amount seems small when dealing with small quantities of medium (1 liter). But if (for example) the required amount of medium needed to run a waste treatment facility was 100,000 liters, then a cost savings of \$27,000 would be realized by using NR2 over NR1; a significant savings for the waste treatment operation.

Uptake of heavy metals from growth medium by *Pseudomonas* O_2 .

We wanted to know the mechanism of resistance of *Pseudomonas* O_2 to Hg and Cd in growth medium. Does O_2 exist in the presence of these heavy metals based in impermeability of the cell membrane to metal uptake or are the metals taken up by the cells and converted to non-toxic forms? Tables 9 and 10 provide data to indicate the latter, metal uptake occurs. *Pseudomonas* O_2 cells were exposed to radioactive Hg and Cd under various growth conditions in NR1 synthetic medium. The amount of radioactivity taken up by the cells and the amount incorporated into TCA precipitable material (i.e. protein) was determined. Almost all radioactivity (95%) ended up in TCA precipitable material indicating metal binding to protein. Table 11 shows stimulation of protein synthesis following exposure of O_2 to ampicillin or Hg. This further provides evidence that an inducible system based on protein synthesis is turned on to support heavy metal or antibiotic resistance.

III. Proposed Studies Nov. 1, 1985 - April 30, 1986

During the past 12 months considerable knowledge has been gained on (1) methods for isolating bacteria capable of concentrating, degrading and/or precipitating inorganics, radionuclides and organics, (2) systems for treating aqueous waste and (3) in situ treatment for immobilizing inorganic (and radionuclides) and degrading organics. One of the important steps in developing 2 and 3 is the method used for isolating bacteria capable of the above functions. During the past year efforts were made to isolate specific strains of bacteria capable of these functions. This approach although technical sound is time consuming, and limits the number of soil samples that can be processed. Our approach in the future will include the characterization of mixed populations for the above functions. An example of how we purpose to isolate and characterize soil bacteria (both aerobes and anaerobes) for the above functions is shown below:

Example

Method - for inorganic and radionuclides

Broth +
Soil Extract +
Antibiotic +
Soil

Incubate at
30°C for 36 hrs

Plate and cell
counts

Add mixed metals
to 1/2 of culture

Add acid to
culture

Centrifuge and
measure the
concentration of
the mixed metals
in the pellet

Calculate mixed
metals per colony
and per cell

Broth +
Soil Extract +
Mixed metals
(i.e. Hg, Cd, Pb, etc.)
+ soil

Incubate at
30°C for 36 hrs

Plate and cell
counts

Add acid to 1/2
culture

Centrifuge and
measure the
concentration
of mixed metals
in the pellet

Calculate mixed
metals per colony
and per cell

Method for organics

Broth +
Soil Extract +
Organic (i.e.
(perchloroethylene)
soil

Incubate at
30°C for 36 hrs

Plate and cell
counts

Centrifuged 1/2
to culture

Measure (GC) the
organic and
possible
metabolites in
the supernatant

Calculate the
level of the
parent organic
and metabolite
per colony and
per cell

If the rate for concentrating, degrading or precipitating these toxic pollutants is greater than 30% attempts will be made to amplify this rate in the mixed culture. The amplification methods include the addition to the culture of antibiotics and/or mutagens. We describe in this report results which show that ampicillin a antibiotic amplifies the bioconcentrating rate of pseudomonas syringe. Other investigators with different isolates have made similar observations.

Major focus during the next 12 months will be to identified mixed microbial populations that biodegrade organics such as halogenated hydrocarbons, alkanes, alkenes, aromatic and polycyclic aromatic hydrocarbons. Attempts will be made to isolate mixed population capable of degrading organics from the Y-12 burial ground. We have made arrangements to collect soil and samples from the Y-12 burial grounds. We also propose during the period to determine if soil amendment (i.e East Fork Poplar Creek) with calcium sulfate increases the activity of sulfate reducing bacteria resulting in a increase in the formation of metal sulfide. As discussed above the formation of heavy metal sulfide specifically, mercury sulfide would be method for reducing the environmental toxicity of this element (i.e mercury, sulfide is very insoluble in aqueous solution). During this period attempt will be made to measure in soil from East Fork Poplar Creek to rate of methylmercury formation and the possible microbial population responsible for this activity.

APPENDIX A

SOLUBILITY OF MERCURY IN SOIL FROM EAST FORK POPLAR CREEK

Site	Total	Water	Organic	HNO ₃
(Soil flood	Mercury		Solvent	
<u>Plains)</u>	<u>(ppm)</u>	<u> </u>	<u> </u>	<u> </u>
		<u>% of total</u>		
NOAA	974	< 1	< 1	46
K-Mart	61	< 1	< 1	40
Bruners	1799	< 1	< 1	48
Sewage	43	< 1	< 1	40
Treatment				
Plant				
Bear Creek	11	< 1	< 1	40

SOLUBILITY OF MERCURY IN VARIOUS SOLVENTS

Water and/ or HCL	Organic Solvents	HNO ₃	H ₂ SO ₄ , HCL, NaS
Most Salts (except HgO HgO)	Organic Mercurials eg. CH ₃ Hg	All Salts, HgO and HgO (except HgS)	All compounds of Mercury including HgS

REMEDIAL ACTION OPTIONS FOR MERCURY IN SOIL

FROM EAST FORK POPLAR CREEK

- (1) Remove and store soil with concentration greater than 12 ppm (approximately 1 million cubic yards)
- (2) Cover with topsoil. (approximate area = 400 acres)
- (3) Buy property and restrict use (approximate area = 400 acres)
- (4) Leave as is
- (5) In situ treatment (Sulfur Reduction - Oxidation Amplification)

MERCURY METABOLISM IN SOIL

Possible Pathways

(1)

(2)

BIOLOGICAL SYSTEM FOR IN SITU TREATMENT
OF HEAVY METALS
(AMPLIFY IN SOIL AND/OR SEDIMENT ALONG EAST FORK
POPLAR CREEK BACTERIA CAPABLE OF IMMOBILIZING
HEAVY METALS)

Cost estimates for amplification of sulfate reducing bacteria in soil from East Fork Poplar Creek to immobilize toxic metals.

- o Area affected with high levels of toxic metals

400 acres (Approx.)

- o Sulphate required to increase the soil level to 500 ppm at a depth of 40 cm.

500 lbs / acre

- o Cost (Approx.) per lb to apply sulphate.

\$1.00 per lb (includes costs for materials and labor)

- o Cost (Approx.) for treating 400 acres with sulphate

\$200,000 per treatment

Sulphate and sulphide concentrations in soil samples from
East Fork Poplar Creek

Site	Sulphate	ppm	Sulphide
1	15		1
3	10		1
4	11		1
6	15		1
7	36		2
9	15		0
10	10		0
12	12		1
13	13		0
15	15		0
16	14		0
18	12		0

Sulphate and sulphide were measured in triplicate in soil samples from East Fork Poplar Creek. The zero indicate that analysis were not performed on these samples.

Sulphate reducing activity in soil samples amended with and without sulphate.

	Sulphate added to soil (ppm)		
	0	100	200
1			
3	—	+	+
4	—	+	+
6	—	+	+
7	—	+	+
9	—	+	+
10	—	—	+
12	—	+	+
13	—	+	+
15	—	—	+
16	—	+	+
18	—	—	+

Soil samples were amended with sulphate at the concentration shown above by the addition of ferric ammonium sulphate and magnesium sulphate (1:1 w/w). The presence + or absence - of sulphate reducing activity was determined by a black ppt in the soil sample.

Soil column study for determining the diffusion of mercury and sulfate following the amendment of soil with sulfate.

Deionized waste was added to each column at 7, 18 and 31 days and collected immediately through drain and mercury and sulphate determined in the leachate.

<u>Columns</u>		A	B	C
Sulphate				
ug/ml	7	34	30	564
	18	10	48	527
	31	13	73	484
Mercury				
ng/ml	7	3.9	1.1	1.3
	18	ND	0.3	0.3
	31	ND	0.3	ND

Table . Soil Column Studies

Column (1)	Time (days)	CaSO ₄ location (2)	Sulfate effluent (ppm)	Mercury in effluent (ppb)
1	7	—	34	3.9
2	7	T	30	1.1
3	7	D	564	1.3
1	18	—	10	—
2	18	T	48	0.3
3	18	D	527	0.3
1	31	—	13	—
2	31	T	73	0.3
3	31	D	484	—
4	31	—	6	3.7
5	31	T	35	1.8

(1) Columns 1-3 were leached with 75 ml deionized water applied on Monday, Wednesday, and Friday of each week. Columns 4 and 5 were leached with deionized water applied according to records of local rainfall for 1985.

(2) — = no CaSO₄; T = 0.5 g CaSO₄/kg soil, applied to soil surface; D = 0.5 g CaSO₄/kg soil, mixed through soil.

APPENDIX B

SAMPLE SITE LOCATIONS, EPPC

CHARACTERIZATION OF SOIL SAMPLES

Date: 7/16/85

Location: Site 1 (Guard post, left)

		1	2
Depth of sampler (cm)		45	45
Depth of core (cm)		29	31
Compaction ratio (sampler/core)		1.55	1.45
Levels sampled (cm)	Top	25	29
(from top)		[6.2]	[2.9]
	Middle	16	14
		[20.15]	[24.65]
	Bottom	4	5
		[38.75]	[37.7]

Physical description of cores:

Site very moist, mucky.

Mid level gravelly.

2 Mid clay-yellow

1 Mid, 1 & 2 Btm gray-brown

Btm sulfurous in odor

Temp in core hole 21.5; conductivity 1000 uMho.

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	4.60	4.86
	17 hr	5.20	4.90
Middle	Initial	4.70	4.80
	17 hr	5.11	5.00
Bottom	Initial	4.76	5.07
	17 hr	5.40	5.40
B. Conductivity			
Top		36	25
Middle		44	30
Bottom		28	26
C. Moisture			
Top			
Initial		10.0	10.0
Final		6.4	6.2
Difference		3.6	3.8
% Moisture		36.0	38.0
Middle			
Initial		10.0	10.0
Final		6.8	7.3
Difference		3.2	2.7
% Moisture		32.0	27.0
Bottom			
Initial		10.0	10.0
Final		8.1	8.6
Difference		1.9	1.4
% Moisture		19.0	14.0

Site 1 Date 7-16-85

D. Soluble sulfate (ppm)

Top	<10	<10
Middle	<10	15
Bottom	13	29

E. Acid-soluble sulfide

Top	<1	--
Middle	<1	<1
Bottom	--	2.5

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml)		
Top	1.3(.21)e7	1.1(.007)e6
Middle	3.4(.57)e6	1.4(.07)e6
Bottom	8.9(.85)e5	1.21(.14)e5
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	1 e 5	5 e 4
Middle	1 e 4	1 e 4
Bottom	2 e 4	0 e 4
C. NIF agar (Avg colonies/ml)		
Top	9.2(.42)e5	2.2(.35)e6
Middle	7.2(.02)e5	8.5(2.1)e4
Bottom	4.1(1.1)e5	0 e 4
D. Rose Bengal agar (Avg colonies/ml)		
Top	4.2(.07)e4	1.3(.07)e5
Middle	3.1(1.2)e4	3.5(3.5)e3
Bottom	8.0(2.8)e3	0.5(.71)e3

3. Metabolic Activity of Samples

A. Carbon mineralization (umole CO₂/g soil)

		1	2
Top			
Day 4	Increment	10.0	20.0
	(Cumulative)	10.0	20.0
Day 8	Increment	0.0	0.0
	(Cumulative)	10.0	20.0
Day 12	Increment	1.0	9.0
	(Cumulative)	11.0	29.0
Middle			
Day 4	Increment	0.0	4.0
	(Cumulative)	0.0	4.0
Day 8	Increment	0.0	0.0
	(Cumulative)	0.0	4.0
Day 12	Increment	5.0	7.5
	(Cumulative)	5.0	11.5
Bottom			
Day 4	Increment	6.0	11.0
	(Cumulative)	6.0	11.0
Day 8	Increment	0.0	0.0
	(Cumulative)	6.0	11.0
Day 12	Increment	7.0	4.0
	(Cumulative)	13.0	15.0

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	4.60	4.86
	17 hr	5.20	4.90
Middle	Initial	4.70	4.80
	17 hr	5.11	5.00
Bottom	Initial	4.76	5.07
	17 hr	5.40	5.40
B. Conductivity			
Top		36	25
Middle		44	30
Bottom		28	26
C. Moisture			
Top			
Initial		10.0	10.0
Final		6.4	6.2
Difference		3.6	3.8
% Moisture		36.0	38.0
Middle			
Initial		10.0	10.0
Final		6.8	7.3
Difference		3.2	2.7
% Moisture		32.0	27.0
Bottom			
Initial		10.0	10.0
Final		8.1	8.6
Difference		1.9	1.4
% Moisture		19.0	14.0

Site 1 Date 7-16-85

D. Soluble sulfate (ppm)

Top	<10	<10
Middle	<10	15
Bottom	13	29

E. Acid-soluble sulfide

Top	<1	--
Middle	<1	<1
Bottom	--	2.5

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml)		
Top	1.3(.21)e7	1.1(.007)e6
Middle	3.4(.57)e6	1.4(.07)e6
Bottom	8.9(.85)e5	1.21(.14)e5
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	1 e 5	5 e 4
Middle	1 e 4	1 e 4
Bottom	2 e 4	0 e 4
C. NIF agar (Avg colonies/ml)		
Top	9.2(.42)e5	2.2(.35)e6
Middle	7.2(.02)e5	8.5(2.1)e4
Bottom	4.1(1.1)e5	0 e4
D. Rose Bengal agar (Avg colonies/ml)		
Top	4.2(.07)e4	1.3(.07)e5
Middle	3.1(1.2)e4	3.5(3.5)e3
Bottom	8.0(2.8)e3	0.5(.71)e3

3. Metabolic Activity of Samples

A. Carbon mineralization (umole CO₂/g soil)

		1	2
Top			
Day 4	Increment	10.0	20.0
	(Cumulative)	10.0	20.0
Day 8	Increment	0.0	0.0
	(Cumulative)	10.0	20.0
Day 12	Increment	1.0	9.0
	(Cumulative)	11.0	29.0
Middle			
Day 4	Increment	0.0	4.0
	(Cumulative)	0.0	4.0
Day 8	Increment	0.0	0.0
	(Cumulative)	0.0	4.0
Day 12	Increment	5.0	7.5
	(Cumulative)	5.0	11.5
Bottom			
Day 4	Increment	6.0	11.0
	(Cumulative)	6.0	11.0
Day 8	Increment	0.0	0.0
	(Cumulative)	6.0	11.0
Day 12	Increment	7.0	4.0
	(Cumulative)	13.0	15.0

B. Nitrogen mineralization

1. Oxidation of urea (ppb NO₂)

	1	2
Top		
Day 0	48.4	15.1
Day 7	19.0	4.5
Day 14	8.4	2.8
Day 21	5.9	8.8
Middle		
Day 0	15.9	15.1
Day 7	7.0	9.0
Day 14	19.6	17.7
Day 21	6.6	5.9
Bottom		
Day 0	28.0	20.4
Day 7	30.0	13.0
Day 14	5.6	8.4
Day 21	5.9	8.9

2. Reduction of nitrate (ppb NO₂)

Top		
Day 0	12.8	12.1
Day 7	67.0	12.0
Day 14	12.1	5.6
Day 21	2.2	11.0
Middle		
Day 0	21.2	12.1
Day 7	5.0	2.0
Day 14	2.8	7.4
Day 21	5.9	4.4
Bottom		
Day 0	0.76	15.1
Day 7	51.0	19.0
Day 14	31.7	8.7
Day 21	5.1	4.4

4. Metabolic activities of mixed cultures

A. Binding of uranium; ppm U in supernate

Pellets

Top	1.75	1.00
Middle	1.61	1.08
Bottom	1.40	1.05

Growing cells

Top	0.12	0.08
Middle	0.11	0.08
Bottom	0.10	0.13

B. Metal binding proteins

Aerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	34,593	35,062
203Hg in void volume/ml	3,115	2,609
ng protein/column	6.0	3.2
ng Hg/mg protein	1.95	2.77

Middle

203Hg in TCA sup/ml	33,661	34,626
203Hg in void volume/ml	3,281	2,567
ng protein/column	5.0	5.0
ng Hg/mg protein	2.53	1.73

Bottom

203Hg in TCA sup/ml	35,636	35,517
203Hg in void volume/ml	3,198	3,074
ng protein/column	3.7	1.7
ng Hg/mg protein	3.29	6.75

Aerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	33,355	33,979
203Hg in void volume/ml	3,095	4,302
ng protein/column	3.1	1.4
ng Hg/mg protein	3.74	13.1

Middle

203Hg in TCA sup/ml	33,478	33,954
203Hg in void volume/ml	2,846	3,581
ng protein/column	2.7	3.0
ng Hg/mg protein	3.78	4.77

Bottom

203Hg in TCA sup/ml	35,507	36,494
203Hg in void volume/ml	2,564	3,321
ng protein/column	1.9	2.5
ng Hg/mg protein	4.54	5.15

Metal binding proteins

Anaerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	36,127	35,236
203Hg in void volume/ml	2,718	2,037
mg protein/ml	5.1	3.1
ng Hg/mg protein	1.86	1.83

Middle

203Hg in TCA sup/ml	35,151	35,931
203Hg in void volume/ml	2,157	2,343
mg protein/ml	3.0	1.8
ng Hg/mg protein	2.11	4.10

Bottom

203Hg in TCA sup/ml	36,057	34,136
203Hg in void volume/ml	2,203	2,561
mg protein/ml	2.3	1.7
ng Hg/mg protein	2.87	5.06

Anaerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	36,325	36,434
203Hg in void volume/ml	1,890	1,902
mg protein/ml	3.3	1.4
ng Hg/mg protein	1.47	3.51

Middle

203Hg in TCA sup/ml	35,848	36,758
203Hg in void volume/ml	1,818	3,435
mg protein/ml	1.7	1.1
ng Hg/mg protein	2.62	12.3

Bottom

203Hg in TCA sup/ml	35,822	36,600
203Hg in void volume/ml	2,017	2,746
mg protein/ml	1.3	0.9
ng Hg/mg protein	4.28	10.7

CHARACTERIZATION OF SOIL SAMPLES

Date: 7/11/1985

Location: Site 3 (Guard post, right)

	1	2
Depth of sampler (cm)	25	25
Depth of core (cm)	16	20
Compaction ratio (sampler/core)	1.56	1.25
Levels sampled (cm)	15	18
(from top)	[1.6]	[2.5]
Middle	4.5	5.5
	[17.9]	[18.1]

Physical description of cores:

Soil temperature at bottom of core 25.8. Conductance of creek 400 umho.

Site marshy, sampled following heavy rain.

Sampler hit rock layer at 25 cm; therefore samples are designated Top and Middle only.

Top highly organic, muddy. Middle sticky red clay with gravel embedded, plus mineral deposits white to yellow-white. Odor similar to sewer pipes.

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	6.13	6.26
	17 hr	6.63	6.71
Middle	Initial	6.8	7.2
	17 hr	6.91	7.13
B. Conductivity			
Top		360	160
Middle		240	240
C. Moisture			
Top			
Initial		10.0	10.0
Final		6.1	6.1
Difference		3.9	3.9
% Moisture		39.0	39.0
Middle			
Initial		10.0	10.0
Final		8.0	8.5
Difference		2.0	1.5
% Moisture		20.0	15.0

Site 3 Date 7-11-85

E. Soluble sulfate (ppm)

Top	21	10
Middle	<10	<10

F. Acid-soluble sulfide

Top	<1	<1
Middle	<1	<1

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml)		
Top	1.1(.33)e6	1.5(.30)e6
Middle	7.9(1.1)e5	7.0(.14)e5
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	2 e 4	1 e 4
Middle	5 e 4	2 e 4
C. NIF agar (Avg colonies/ml)		
Top	1.4(.09)e6	1.6(.17)e6
Middle	4.6(.35)e5	7.1(.14)e5
D. Rose Bengal agar (Avg colonies/ml)		
Top	8.0(2.8)e4	6.3(.92)e4
Middle	8.5(2.1)e3	9.5(2.1)e3

3. Metabolic Activity of Samples

A. Carbon mineralization (umole CO₂/g soil)

			1	2
Top				
Day	4	Increment	4.0	6.0
		(Cumulative)	4.0	6.0
Day	8	Increment	15.0	19.5
		(Cumulative)	19.0	25.5
Day	12	Increment	7.5	17.5
		(Cumulative)	26.5	43.0
Middle				
Day	4	Increment	0.0	0.5
		(Cumulative)	0.0	0.5
Day	8	Increment	28.5	8.0
		(Cumulative)	28.5	8.5
Day	12	Increment	0.5	10.0
		(Cumulative)	29.0	18.5

B. Nitrogen mineralization

1. Oxidation of urea (ppb NO₂)

	1	2
Top		
Day 0	8.4	3.8
Day 7	37.8	684.0
Day 14	684.0	541.0
Day 21	0.0	0.0
Middle		
Day 0	7.6	3.8
Day 7	6.0	0.0
Day 14	11.0	3430
Day 21	0.0	0.0

2. Reduction of nitrate (ppb NO₂)

Top		
Day 0	22.8	26.4
Day 7	6.8	1.5
Day 14	21.0	xs color
Day 21	3.7	13.0
Middle		
Day 0	30.0	8.4
Day 7	5.2	4.4
Day 14	5.0	20.0
Day 21	44.8	0.0

4. Metabolic activities of mixed cultures

A. Binding of uranium: ppm U in supernate

Pellets

Top	1.89,----	1.74,0.42
Middle	0.63,0.6	0.62,0.74

Growing cells

Top	0.16,0.20	0.10,0.16
Middle	0.36,0.29	0.16,0.18

B. Metal binding proteins

Aerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	32,738	33,926
203Hg in void volume/ml	4,455	5,964
mg protein/ml	8.00	4.00
ng Hg/mg protein	2.40	6.92

Middle

203Hg in TCA sup/ml	33,250	33,508
203Hg in void volume/ml	4,379	2,985
mg protein/ml	7.45	6.00
ng Hg/mg protein	2.51	1.83

Aerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	33,263	36,081
203Hg in void volume/ml	3,457	3,767
mg protein/ml	11.00	4.00
ng Hg/mg protein	1.24	3.84

Middle

203Hg in TCA sup/ml	33,110	33,706
203Hg in void volume/ml	4,094	3,931
mg protein/ml	9.50	7.50
ng Hg/mg protein	1.81	2.17

Metal binding proteins

Anaerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	34,303	38,485
203Hg in void volume/ml	3,329	3,685
mg protein/ml	2.00	0.32
ng Hg/mg protein	6.45	47.0

Middle

203Hg in TCA sup/ml	37,661	38,127
203Hg in void volume/ml	3,514	3,412
mg protein/ml	0.24	0.42
ng Hg/mg protein	58.1	32.0

Anaerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	37,395	38,548
203Hg in void volume/ml	3,435	5,706
mg protein/ml	1.02	0.22
ng Hg/mg protein	13.3	119.2

Middle

203Hg in TCA sup/ml	38,362	38,250
203Hg in void volume/ml	3,850	2,802
mg protein/ml	0.54	0.34
ng Hg/mg protein	29.0	29.3

CHARACTERIZATION OF SOIL SAMPLES

Date: 7/9/85

Location: Site 4 (NOAA Left)

	1	2
Depth of sampler (cm)	45	45
Depth of core (cm)	26	32.5
Compaction ratio (sampler/core)	1.73	1.40
Levels sampled (cm)	25	28
(from top)	[1.7]	[6.3]
Middle	15.5	14
	[18.2]	[25.9]
Bottom	4	5
	[40.0]	[37.2]

Physical description of cores:

Top layer gray-brown
Highly organic

Bottom layer charcoal
brown, slightly grainy

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	6.45	7.15
	17 hr	7.10	7.29
Middle	Initial	6.62	7.03
	17 hr	7.10	7.36
Bottom	Initial	6.75	6.85
	17 hr	7.20	7.49
B. Conductivity (uMho)			
Top		120	140
Middle		120	220
Bottom		220	260
C. Moisture			
Top	Initial	10.0	10.0
	Final	6.6	6.9
	Difference	3.4	3.1
	% Moisture	34.0	31.0
Middle	Initial	10.0	10.0
	Final	6.7	6.3
	Difference	3.3	3.7
	% Moisture	33.0	37.0
Bottom	Initial	10.0	10.0
	Final	6.5	6.9
	Difference	3.5	3.1
	% Moisture	5.0	31.0

E. Soluble sulfate (ppm)

Top	<10	12
Middle	10	14
Bottom	11	<10

F. Acid-soluble sulfide

Top	1	1
Middle	1	1
Bottom	1	1

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml)		
Top	3.1(1.5)e7	1.26(.11)e7
Middle	5.7(.64)e6	1.3(.13)e6
Bottom	4.8(.56)e5	6.3(1.1)e5
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	6.0e6	1.9e6
Middle	8.3e5	1.8e5
Bottom	2.0e5	1.2e6
C. NIF agar (Avg colonies/ml)		
Top	5.2(2.1)e6	9.0(3.4)e6
Middle	5.6(.42)e6	1.9(1.3)e6
Bottom	3.5(1.6)e5	9.0(3.3)e6
D. Rose Bengal agar (Avg colonies/ml)		
Top	6.5(.71)e4	1.8(.35)e5
Middle	6.9(1.1)e4	8.5(3.5)e3
Bottom	2.0(0)e3	1.5(.71)e3

3. Metabolic Activity of Samples

A. Carbon mineralization (umole CO₂/g soil)

		1	2
Top			
Day 4	Increment	27.5	30.0
	(Cumulative)	27.5	30.0
Day 8	Increment	6.5	9.0
	(Cumulative)	34.0	39.0
Day 12	Increment	7.5	8.0
	(Cumulative)	41.5	47.0
Middle			
Day 4	Increment	20.0	30.0
	(Cumulative)	20.0	30.0
Day 8	Increment	7.5	2.5
	(Cumulative)	27.5	32.5
Day 12	Increment	1.0	8.0
	(Cumulative)	28.5	40.5
Bottom			
Day 4	Increment	16.5	26.5
	(Cumulative)	16.5	26.5
Day 8	Increment	1.0	6.5
	(Cumulative)	17.5	33.0
Day 12	Increment	0.0	0.0
	(Cumulative)	17.5	33.0

B. Nitrogen mineralization

1. Oxidation of urea (ppb NO₂)

	1	2
Top		
Day 0	78.8	97.2
Day 7	13.6	31.8
Day 14	542.0	1215.0
Day 21	2.8	4.7
Middle		
Day 0	87.0	97.2
Day 7	6.0	34.0
Day 14	377.0	3054.0
Day 21	broken	3322.0
Bottom		
Day 0	45.2	63.0
Day 7	18.8	7.6
Day 14	2964.0	20.0
Day 21	3159.5	2004.0

2. Reduction of nitrate (ppb NO₂)

Top		
Day 0	62.4	67.8
Day 7	6.8	126.0
Day 14	10.0	0.0
Day 21	0.0	0.9
Middle		
Day 0	27.4	25.4
Day 7	6.0	181.0
Day 14	21.0	24.0
Day 21	18.6	7.4
Bottom		
Day 0	28.8	23.2
Day 7	26.5	171.0
Day 14	22.0	0.0
Day 21	0.9	25.5

4. Metabolic activities of mixed cultures

A. Binding of uranium: ppm U in supernate

Pellets

Top	1.71,0.50	1.02,0.63
Middle	0.87,0.73	1.39,0.70
Bottom	0.72,0.60	0.69,1.26

Growing cells

Top	0.23,0.41	0.75,0.32
Middle	0.20,0.18	0.17,0.22
Bottom	0.09,0.67	0.18,0.16

B. Metal binding proteins

No data available.

CHARACTERIZATION OF SOIL SAMPLES

Date: 7/3/85

Location: Site 6 (NOAA Right)

	1	2
Depth of sampler (cm)	45	45
Depth of core (cm)	30.5	29
Compaction ratio (sampler/core)	1.48	1.55
Levels sampled (cm)		
[from top]		
Top	25	28
	[6.7]	[11.6]
Middle	16	14
	[21.5]	[23.2]
Bottom	3.5	5
	[40.0]	[37.2]

Physical description of cores:

Top gravelly, brown loose soil. Bottom darker brown (slightly blackish), very moist.

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	7.43	7.54
	17 hr	7.27	7.35
Middle	Initial	7.66	7.62
	17 hr	7.33	7.53
Bottom	Initial	7.68	7.55
	17 hr	7.98	7.00
B. Conductivity (umho)			
Top		160	120
Middle		120	160
Bottom		100	120
C. Moisture			
Top			
Initial		10.2	10.2
Final		8.8	8.6
Difference		1.4	1.6
% Moisture		13.7	15.7
Middle			
Initial		10.1	10.2
Final		7.6	7.4
Difference		2.5	2.8
% Moisture		24.8	27.4
Bottom			
Initial		10.1	10.2
Final		7.7	6.5
Difference		2.4	3.7
% Moisture		23.8	36.3

Site 6 Date 7-3-85

E. Soluble sulfate (ppm)

Top	16	11
Middle	<10	17
Bottom	14	17

F. Acid-soluble sulfide

Top	---	---
Middle	---	<1
Bottom	<1	<1

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml(S.D.))		
Top	5.4(.35)e6	4.8(.28)e6
Middle	4.8(.35)e6	1.1(.49)e7
Bottom	8.6(1.4)e5	3.5(1.3)e6
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	2 e 5	7.4e5
Middle	1.7e6	3.8e5
Bottom	2.7e6	3.5e5
C. NIF agar (Avg colonies/ml)		
Top	7.7(.85)e5	1.7(.65)e6
Middle	1.6(.48)e6	2.1(.35)e6
Bottom	1.2(.25)e6	8.9(2.0)e5
D. Rose Bengal agar (Avg colonies/ml)		
Top	3.5(.71)e4	1.4(.14)e5
Middle	7.2(3.4)e4	4(1.4)e4
Bottom	1.7(.35)e4	3.5(.28)e4

3. Metabolic Activity of Samples

A. Carbon mineralization (umole CO₂/g soil)

			1	2
Top				
Day	5	Increment	10.0	21.0
		(Cumulative)	10.0	21.0
Day	8	Increment	17.0	5.0
		(Cumulative)	27.0	26.0
Day	12	Increment	0.0	3.0
		(Cumulative)	27.0	29.0
Middle				
Day	5	Increment	18.5	0.0
		(Cumulative)	18.5	0.0
Day	8	Increment	14.5	3.5
		(Cumulative)	23.0	3.5
Day	12	Increment	2.0	12.0
		(Cumulative)	25.0	15.5
Bottom				
Day	5	Increment	29.5	27.0
		(Cumulative)	29.5	27.0
Day	8	Increment	15.0	11.0
		(Cumulative)	44.5	38.0
Day	12	Increment	2.0	13.5
		(Cumulative)	46.5	51.5

B. Nitrogen mineralization

1. Oxidation of urea (ppb NO₂)

	1	2
Top		
Day 0	70.6	99.2
Day 7	1470.0	2908.0
Day 14	3000.0	3010.0
Day 21	115.0	36.0
Middle		
Day 0	103.4	124.2
Day 7	87.6	91.8
Day 14	3054.0	2914.0
Day 21	3510.0	12.0
Bottom		
Day 0	106.0	130.2
Day 7	131.6	220.0
Day 14	2248.0	125.0
Day 21	349.0	20.0

2. Reduction of nitrate (ppb NO₂)

Top		
Day 0	53.0	57.0
Day 7	78.8	33.6
Day 14	76.0	60.0
Day 21	100.0	80.0
Middle		
Day 0	53.0	68.0
Day 7	60.2	61.6
Day 14	60.0	38.0
Day 21	380.0	260.0
Bottom		
Day 0	60.0	72.0
Day 7	76.0	124.6
Day 14	53.0	98.0
Day 21	100.0	140.0

4. Metabolic activities of mixed cultures

A. Binding of uranium: ppm U in supernate

Pellets

Top	3.57,1.42	1.85,2.38
Middle	2.45,2.70	2.04,1.30
Bottom	1.74,2.16	1.25,1.59

Growing cells

Top	0.67,0.28	0.18,----
Middle	0.51,0.15	0.15,0.20
Bottom	0.27,0.23	0.16,0.34

B. Metal binding proteins

Aerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	31,872	34,899
203Hg in void volume/ml	1,494	2,552
mg protein/ml	3.7	7.5
ng Hg/mg protein	4.55	1.14

Middle

203Hg in TCA sup/ml	32,253	34,524
203Hg in void volume/ml	1,180	2,091
mg protein/ml	3.00	5.90
ng Hg/mg protein	1.47	1.01

Bottom

203Hg in TCA sup/ml	34,845	33,845
203Hg in void volume/ml	1,989	2,007
mg protein/ml	4.20	6.00
ng Hg/mg protein	1.29	0.92

Aerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	---	31,229
203Hg in void volume/ml	---	2,656
mg protein/ml	---	5.40
ng Hg/mg protein	---	1.69

Middle

203Hg in TCA sup/ml	30,874	30,508
203Hg in void volume/ml	1,482	1,624
mg protein/ml	5.60	7.50
ng Hg/mg protein	0.46	0.48

Bottom

203Hg in TCA sup/ml	31,271	---
203Hg in void volume/ml	1,755	---
mg protein/ml	8.20	---
ng Hg/mg protein	0.50	---

Metal binding proteins

Anaerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	31,960	31,747
203Hg in void volume/ml	3,126	4,029
mg protein/ml	3.60	3.70
ng Hg/mg protein	3.27	4.55

Middle

203Hg in TCA sup/ml	32,094	32,311
203Hg in void volume/ml	2,978	3,140
mg protein/ml	3.90	3.30
ng Hg/mg protein	2.81	3.59

Bottom

203Hg in TCA sup/ml	32,169	31,842
203Hg in void volume/ml	2,896	2,963
mg protein/ml	3.10	3.40
ng Hg/mg protein	3.38	3.19

Anaerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	31,807	32,629
203Hg in void volume/ml	2,849	4,615
mg protein/ml	2.90	2.20
ng Hg/mg protein	3.52	9.14

Middle

203Hg in TCA sup/ml	31,400	32,158
203Hg in void volume/ml	3,009	4,393
mg protein/ml	4.10	3.00
ng Hg/mg protein	2.71	6.29

Bottom

203Hg in TCA sup/ml	32,048	32,614
203Hg in void volume/ml	2,488	2,222
mg protein/ml	3.80	2.40
ng Hg/mg protein	2.16	2.80

CHARACTERIZATION OF SOIL SAMPLES

Date: 7/1/85

Location: Site 7 (K-Mart left)

	1	2
Depth of sampler (cm)	45	45
Depth of core (cm)	33	29
Compaction ratio (sampler/core)	1.36	1.55
Levels sampled (cm)		
[from top]		
Top	25	26
	(10.9)	(4.6)
Middle	16	14
	(23.1)	(23.2)
Bottom	6	6
	(36.7)	(35.6)

Physical description of cores:

Uniform color; tannish brown clay.

Upper layer damp, middle more friable, lower sticky.

Samples had been taken subsequent to heavy rain.

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	6.82	7.00
	17 hr	6.88	7.05
Middle	Initial	7.00	7.60
	17 hr	7.01	7.40
Bottom	Initial	7.20	7.00
	17 hr	7.21	7.33
B. Conductivity			
Top		65	72
Middle		57	100
Bottom		56	82
C. Moisture			
Top			
Initial		10.2	10.0
Final		8.2	8.0
Difference		2.0	2.0
% Moisture		19.6	20.0
Middle			
Initial		10.2	10.2
Final		8.6	8.3
Difference		1.6	1.9
% Moisture		15.6	18.7
Bottom			
Initial		10.1	10.1
Final		8.5	8.5
Difference		1.6	1.6
% Moisture		15.8	15.8

Site 7 Date 7-1-85

E. Soluble sulfate (ppm)

Top	18	41
Middle	32	24
Bottom	85	20

F. Acid-soluble sulfide

Top	<1	<1
Middle	<1	<1
Bottom	2	2

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml)		
Top	7.35(.07)e6	9.7(2.9)e6
Middle	1.4(.63)e7	1.2(.53)e5
Bottom	2.6(2.1)e6	2.3(0.8)e6
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	3.1e6	1.1e6
Middle	3.3e6	3.8e5
Bottom	9 e 5	2.9e5
C. NIF agar (Avg colonies/ml)		
Top	3.5 (0) e6	2.3(.78)e6
Middle	3.4(.42)e6	1.0(.15)e7
Bottom	2.8(.42)e5	5.0(2.8)e5
D. Rose Bengal agar (Avg colonies/ml)		
Top	8.0(4.2)e4	1.35(.2)e5
Middle	2.2(.57)e4	1.2(.42)e4
Bottom	2.5(.71)e3	1.2(.14)e4

3. Metabolic Activity of Samples

A. Carbon mineralization (umole CO₂/g soil)

		1	2
Top			
Day 4	Increment	25.5	12.5
	(Cumulative)	25.5	12.5
Day 8	Increment	12.5	6.5
	(Cumulative)	38.0	19.0
Day 11	Increment	0.0	12.0
	(Cumulative)	38.0	31.0
Middle			
Day 4	Increment	6.5	12.5
	(Cumulative)	6.5	12.5
Day 8	Increment	11.0	0.0
	(Cumulative)	17.5	12.5
Day 12	Increment	3.5	15.0
	(Cumulative)	21.0	27.5
Bottom			
Day 4	Increment	3.0	2.5
	(Cumulative)	3.0	2.5
Day 8	Increment	4.0	0.0
	(Cumulative)	7.0	2.5
Day 12	Increment	15.5	21.0
	(Cumulative)	22.5	23.5

B. Nitrogen mineralization

1. Oxidation of urea (ppb NO₂)

	1	2
Top		
Day 0	76.8	131.0
Day 7	39.0	31.6
Day 14	822.0	51.0
Day 21	0.0	9.0
Middle		
Day 0	117.2	78.4
Day 7	324.0	54.8
Day 14	2706.0	2956.0
Day 21	102.0	2760.0
Bottom		
Day 0	69.0	63.0
Day 7	28.8	67.0
Day 14	2918.0	2930.0
Day 21	18.0	11.0

2. Reduction of nitrate (ppb NO₂)

Top		
Day 0	63.0	50.0
Day 7	353.4	304.0
Day 14	5.2	3.8
Day 21	6.6	0.0
Middle		
Day 0	43.2	41.4
Day 7	28.0	16.4
Day 14	5.2	7.6
Day 21	36.0	30.0
Bottom		
Day 0	30.2	26.8
Day 7	24.0	52.0
Day 14	6.8	18.0
Day 21	7.0	0.0

4. Metabolic activities of mixed cultures

A. Binding of uranium: U in supernate (ppm)

Pellets

Top	0.57,0.89	1.15,0.62
Middle	1.35,0.80	1.22,3.87
Bottom	1.77,1.26	1.11,1.06

Growing cells

Top	0.16,0.24	0.15,0.16
Middle	0.11,0.15	0.13,0.11
Bottom	0.20,0.24	0.20,0.13

B. Metal binding proteins

Aerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	32,759	32,863
203Hg in void volume/ml	1,568	1,497
mg protein/ml	4.30	5.20
ng Hg/mg protein	0.71	0.51

Middle

203Hg in TCA sup/ml	34,729	33,210
203Hg in void volume/ml	1,337	1,301
mg protein/ml	3.20	3.70
ng Hg/mg protein	0.55	0.42

Bottom

203Hg in TCA sup/ml	32,730	33,239
203Hg in void volume/ml	1,650	1,497
mg protein/ml	3.10	1.70
ng Hg/mg protein	1.13	1.56

Aerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	32,920	31,464
203Hg in void volume/ml	1,792	1,824
mg protein/ml	3.70	5.60
ng Hg/mg protein	1.16	0.80

Middle

203Hg in TCA sup/ml	31,819	30,627
203Hg in void volume/ml	4,253	1,143
mg protein/ml	5.60	7.00
ng Hg/mg protein	3.23	0.095

Bottom

203Hg in TCA sup/ml	32,327	30,512
203Hg in void volume/ml	1,701	1,104
mg protein/ml	6.10	6.30
ng Hg/mg protein	0.62	0.07

Metal binding proteins

Anaerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	31,328	31,147
203Hg in void volume/ml	1,479	722
mg protein/ml	3.10	3.30
ng Hg/mg protein	0.82	0

Middle

203Hg in TCA sup/ml	31,171	30,481
203Hg in void volume/ml	799	707
mg protein/ml	3.40	6.40
ng Hg/mg protein	0	0

Bottom

203Hg in TCA sup/ml	31,492	31,877
203Hg in void volume/ml	1,321	859
mg protein/ml	3.50	2.60
ng Hg/mg protein	0.48	0

Anaerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	31,180	34,513
203Hg in void volume/ml	664	1,119
mg protein/ml	2.20	2.90
ng Hg/mg protein	0	0.18

Middle

203Hg in TCA sup/ml	31,582	32,830
203Hg in void volume/ml	886	1,066
mg protein/ml	3.10	2.60
ng Hg/mg protein	0	0.09

Bottom

203Hg in TCA sup/ml	31,543	32,827
203Hg in void volume/ml	913	1,140
mg protein/ml	2.50	1.90
ng Hg/mg protein	0	0.34

CHARACTERIZATION OF SOIL SAMPLES

Date: JULY 22, 1985

Location: Site 9 (K-Mart, right)

	1	2
Depth of sampler (cm)	45	45
Depth of core (cm)	29	36
Compaction ratio (sampler/core)	1.55	1.25
Levels sampled (cm)		
(from top)		
Top	25	35
	(6.2)	(1.2)
Middle	15	21
	(21.7)	(18.7)
Bottom	4	5
	(38.7)	(38.7)

Physical description of cores:

Top highly organic, shading to sandy in middle and bottom.
Fewer roots in middle and bottom than at other sites.

Temp in sample hole 25 C.

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	6.70	7.20
	17 hr	6.85	7.18
Middle	Initial	6.90	7.00
	17 hr	7.26	7.20
Bottom	Initial	7.20	7.30
	17 hr	7.27	7.40
B. Conductivity			
Top		100	120
Middle		120	160
Bottom		75	46
C. Moisture			
Top			
Initial		10.0	10.0
Final		7.9	7.8
Difference		2.1	2.2
% Moisture		21.0	22.0
Middle			
Initial		10.0	10.0
Final		8.0	8.1
Difference		2.0	1.9
% Moisture		20.0	19.0
Bottom			
Initial		10.0	10.0
Final		8.6	8.7
Difference		1.4	1.3
% Moisture		14.0	13.0

E. Soluble sulfate (ppm)

Top	14	23
Middle	13	12
Bottom	15	13

F. Acid-soluble sulfide

Top	<1	---
Middle	<1	---
Bottom	---	---

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml)		
Top	7.7(2.3)e6	3.2(.21)e6
Middle	3.2(.35)e6	8.2(2.4)e6
Bottom	3.6(1.5)e6	4.5(.99)e6
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	7 e 5	2 e 4
Middle	5 e 4	3 e 4
Bottom	3 e 5	2 e 4
C. NIF agar (Avg colonies/ml)		
Top	2.5(.57)e6	2.45(1.2)e6
Middle	5.2(1.4)e5	4.8(.85)e5
Bottom	3.6(.14)e5	5.5(.49)e5
D. Rose Bengal agar (Avg colonies/ml)		
Top	4.0(.28)e4	5.2(1.4)e4
Middle	2.7(1.4)e4	2.4(.57)e4
Bottom	1.9(.07)e4	2.6(.70)e4

3. Metabolic Activity of Samples

A. Carbon mineralization (umole CO₂/g soil)

			1	2
Top				
Day 4	Increment		1.5	30.0
	(Cumulative)		1.5	30.0
Day 8	Increment		5.0	8.5
	(Cumulative)		6.5	38.5
Day 12	Increment		0.0	0.0
	(Cumulative)		6.5	38.5
Middle				
Day 4	Increment		17.5	22.0
	(Cumulative)		17.5	22.0
Day 8	Increment		9.0	13.5
	(Cumulative)		26.5	35.5
Day 12	Increment		5.0	0.0
	(Cumulative)		31.5	35.5
Bottom				
Day 4	Increment		12.5	6.0
	(Cumulative)		12.5	6.0
Day 8	Increment		11.5	11.0
	(Cumulative)		24.0	17.0
Day 12	Increment		1.0	5.0
	(Cumulative)		25.0	22.0

B. Nitrogen mineralization

1. Oxidation of urea (ppb NO₂)

	1	2
Top		
Day 0	2.0	7.0
Day 7	0.0	7.4
Day 14	8.1	7.3
Day 21	47.4	42.8
Middle		
Day 0	7.0	8.0
Day 7	1.8	3.7
Day 14	13.2	20.6
Day 21	45.9	3051
Bottom		
Day 0	27.0	Broken
Day 7	20.5	3.7
Day 14	65.3	1520
Day 21	2838	2137

2. Reduction of nitrate (ppb NO₂)

Top		
Day 0	1.0	20.0
Day 7	0.0	0.0
Day 14	6.6	3.7
Day 21	54.3	8.4
Middle		
Day 0	11.0	0.0
Day 7	29.8	6.9
Day 14	56.5	25.7
Day 21	110.1	838.8
Bottom		
Day 0	16.0	23.0
Day 7	3.7	2.8
Day 14	1.5	1.5
Day 21	7.65	56.6

4. Metabolic activities of mixed cultures

A. Binding of uranium: U in supernate (ppm)

Pellets

Top	2.21, 1.14	2.44, 2.72
Middle	2.58, 2.54	2.35, 2.63
Bottom	2.13, 2.70	2.35, 2.39

Growing cells

Top	0.21, 0.05	0.09, 0.43
Middle	0.03, 0.12	0.03, 0.10
Bottom	0.07, 0.03	0.10, 0.12

B. Metal binding proteins

Aerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	32,549	32,654
203Hg in void volume/ml	5,368	2,010
mg protein/ml	5.60	4.40
ng Hg/mg protein	4.34	1.25

Middle

203Hg in TCA sup/ml	32,969	32,975
203Hg in void volume/ml	2,414	2,214
mg protein/ml	4.60	4.00
ng Hg/mg protein	1.69	1.67

Bottom

203Hg in TCA sup/ml	33,843	32,883
203Hg in void volume/ml	2,686	2,004
mg protein/ml	4.80	6.20
ng Hg/mg protein	1.94	0.88

Aerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	30,815	32,350
203Hg in void volume/ml	2,623	2,844
mg protein/ml	5.60	5.70
ng Hg/mg protein	1.60	1.79

Middle

203Hg in TCA sup/ml	31,933	32,284
203Hg in void volume/ml	2,470	2,270
mg protein/ml	5.80	5.80
ng Hg/mg protein	8.10	1.20

Bottom

203Hg in TCA sup/ml	33,148	32,660
203Hg in void volume/ml	2,029	2,068
mg protein/ml	4.90	6.60
ng Hg/mg protein	1.15	0.89

Metal binding proteins

Anaerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	33,062	33,395
203Hg in void volume/ml	1,985	1,775
mg protein/ml	3.52	3.06
ng Hg/mg protein	1.53	1.37

Middle

203Hg in TCA sup/ml	33,809	33,907
203Hg in void volume/ml	2,274	1,413
mg protein/ml	2.50	3.80
ng Hg/mg protein	2.80	0.57

Bottom

203Hg in TCA sup/ml	34,015	33,333
203Hg in void volume/ml	1,723	2,237
mg protein/ml	2.80	4.20
ng Hg/mg protein	1.40	1.62

Anaerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	33,393	34,164
203Hg in void volume/ml	1,707	1,266
mg protein/ml	4.60	3.40
ng Hg/mg protein	0.83	0.40

Middle

203Hg in TCA sup/ml	33,134	34,694
203Hg in void volume/ml	1,576	1,359
mg protein/ml	2.80	5.60
ng Hg/mg protein	1.10	0.34

Bottom

203Hg in TCA sup/ml	33,620	34,782
203Hg in void volume/ml	1,451	1,541
mg protein/ml	1.60	2.80
ng Hg/mg protein	1.49	1.03

CHARACTERIZATION OF SOIL SAMPLES

Date: 6/24/85

Location: Site 10 (Bruner Left)

	1	2
Depth of sampler (cm)	45	45
Depth of core (cm)	30	30
Compaction ratio (sampler/core)	1.5	1.5
Levels sampled (cm)		
(from top)		
Top	25	28.5
Middle	15	14
Bottom	3.5	5
	[7.5]	[2.2]
	[22.5]	[24.0]
	[40.0]	[37.5]

Physical description of cores:

Surface loamy, greyish.

At depth of about 15 cm, a layer (about 5 cm) of densely black soil.

At depth of about 40 cm, a stratum of clay.

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	6.30	6.80
	17 hr	7.42	7.28
Middle	Initial	6.50	6.90
	17 hr	7.16	7.29
Bottom	Initial	6.70	7.00
	17 hr	7.45	7.45
B. Conductivity			
Top		140	84
Middle		73	110
Bottom		120	120
C. Moisture			
Top			
Initial		10.01	10.02
Final		7.6	7.4
Difference		2.41	2.62
% Moisture		24.1	26.1
Middle			
Initial		10.00	10.0
Final		6.9	6.8
Difference		3.1	3.2
% Moisture		31.0	32.0
Bottom			
Initial		10.03	10.0
Final		7.1	6.9
Difference		2.93	3.1
% Moisture		29.2	31.0

Site 10 Date 6-24-85

	1	2
E. Soluble sulfate (ppm)		
Top	14	35
Middle	<10	25
Bottom	<10	21
F. Acid-soluble sulfide		
Top	-----	-----
Middle	-----	-----
Bottom	-----	-----

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml)		
Top	1.41(.17)e7	9.3(1.2)e6
Middle	2.5(2.0)e7	1.4(0.6)e7
Bottom	2.2(1.1)e6	1.2(.16)e6
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	1.0e7	9.3e6
Middle	3.2e6	6.4e6
Bottom	2.2e6	2.6e6
C. NIF agar (Avg colonies/ml)		
Top	1.4(.01)e7	2.9(.2)e6
Middle	1.2(.13)e7	3.13(.26)e6
Bottom	1.2(.06)e6	5.1(.9)e5
D. Rose Bengal agar (Avg colonies/ml)		
Top	1.9(.21)e5	1.4(.14)e5
Middle	1.9(.64)e5	5.5(3.5)e5
Bottom	1.7(.28)e4	1.6(.35)e4

3. Metabolic Activity of Samples

A. Carbon mineralization (umole CO₂/g soil)

		1	2
Top			
Day 4	Increment	16.5	3.0
	(Cumulative)	16.5	3.0
Day 7	Increment	9.5	8.0
	(Cumulative)	26.0	11.0
Day 12	Increment	26.5	10.0
	(Cumulative)	52.5	21.0
Middle			
Day 4	Increment	0.0	3.0
	(Cumulative)	0.0	3.0
Day 7	Increment	4.0	9.5
	(Cumulative)	4.0	12.5
Day 12	Increment	4.0	15.0
	(Cumulative)	8.0	27.5
Bottom			
Day 4	Increment	4.0	0.0
	(Cumulative)	4.0	0.0
Day 7	Increment	6.0	9.5
	(Cumulative)	10.0	9.5
Day 12	Increment	2.5	6.5
	(Cumulative)	12.5	16.0

B. Nitrogen mineralization

1. Oxidation of urea (ppb NO₂)

	1	2
Top		
Day 0	80.2	142.2
Day 7	59.4	176.8
Day 14	175.4	3062.0
Day 21	3.8	7.6
Middle		
Day 0	84.4	156.8
Day 7	103.4	2660.0
Day 14	2648.0	568.0
Day 21	152.0	7.6
Bottom		
Day 0	136.2	182.8
Day 7	265.4	3292.0
Day 14	3038.0	1246.0
Day 21	2922.0	237.0

2. Reduction of nitrate (ppb NO₂)

Top		
Day 0	37.0	97.4
Day 7	88.0	84.4
Day 14	42.4	80.2
Day 21	9.2	135.2
Middle		
Day 0	76.8	73.2
Day 7	58.6	81.8
Day 14	7.6	2.8
Day 21	97.6	43.2
Bottom		
Day 0	76.8	113.0
Day 7	93.0	81.0
Day 14	35.0	56.8
Day 21	40.0	59.6

4. Metabolic activities of mixed cultures

A. Binding of uranium: ppm U in supernate

Pellets

Top	2.25, 1.58	0.78, 1.30
Middle	2.16, 1.45	1.30, 1.74
Bottom	1.90, 1.72	1.92, 2.46

Growing cells

Top	0.13, 0.16	0.09, 0.07
Middle	0.13, 0.14	0.14, 0.20
Bottom	0.18, 0.14	0.20, 0.20

B. Metal binding proteins

Aerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	-----	19,622
203Hg in void volume/ml	-----	2,092
mg protein/ml	-----	5.10
ng Hg/mg protein	-----	1.17

Middle

203Hg in TCA sup/ml	-----	13,284
203Hg in void volume/ml	-----	990
mg protein/ml	-----	5.00
ng Hg/mg protein	-----	0

Bottom

203Hg in TCA sup/ml	-----	9,155
203Hg in void volume/ml	-----	780
mg protein/ml	-----	3.80
ng Hg/mg protein	-----	0

Aerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	13,928	12,393
203Hg in void volume/ml	1,096	3,124
mg protein/ml	3.20	3.50
ng Hg/mg protein	0.13	3.36

Middle

203Hg in TCA sup/ml	13,783	36,044
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Site 10 Date 6-24-85

203Hg in void volume/ml	1,248	739
mg protein/ml	3.10	5.20
ng Hg/mg protein	0.40	0

Bottom

203Hg in TCA sup/ml	11,718	33,971
203Hg in void volume/ml	2,701	1,168
mg protein/ml	2.70	2.80
ng Hg/mg protein	3.48	0.29

Metal binding proteins

Anaerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	9,928	15,990
203Hg in void volume/ml	810	1,216
mg protein/ml	2.90	3.30
ng Hg/mg protein	0	0.33

Middle

203Hg in TCA sup/ml	9,510	13,747
203Hg in void volume/ml	1,264	1,078
mg protein/ml	2.60	2.80
ng Hg/mg protein	0.52	0.03

Bottom

203Hg in TCA sup/ml	15,258	13,007
203Hg in void volume/ml	1,968	1,124
mg protein/ml	4.70	3.00
ng Hg/mg protein	1.12	0.19

Anaerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	13,633	21,302
203Hg in void volume/ml	3,010	1,452
mg protein/ml	2.90	2.50
ng Hg/mg protein	3.84	0.96

Middle

203Hg in TCA sup/ml	14,043	39,475
203Hg in void volume/ml	2,813	1,320
mg protein/ml	2.20	2.90
ng Hg/mg protein	4.55	0.57

Bottom

203Hg in TCA sup/ml	13,431	26,991
203Hg in void volume/ml	1,024	1,592
mg protein/ml	3.10	3.10
ng Hg/mg protein	0	1.03

CHARACTERIZATION OF SOIL SAMPLES

Date: 6/17/85

Location: Site 12 (Bruner right)

	1	2
Depth of sampler (cm)	45	45
Depth of core (cm)	30	25
Compaction ratio (sampler/core)	1.5	1.8
Levels sampled (cm)		
(from top)		
Top	26	22.5
Middle	18	17
Bottom	10	5
	(6.0)	(4.5)
	(18.0)	(14.4)
	(30.0)	(36.0)

Physical description of cores:

Soil friable, sandy loam.

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	7.80	7.57
	17 hr	7.50	7.57
Middle	Initial	8.44	7.35
	17 hr	7.34	7.35
Bottom	Initial	8.14	7.59
	17 hr	7.50	7.59
B. Conductivity - meter not available			
C. Moisture			
Top			
	Initial	10.15	10.00
	Final	6.98	6.39
	Difference	3.17	3.61
	% Moisture	31.2	36.1
Middle			
	Initial	10.66	10.10
	Final	7.18	6.98
	Difference	2.48	3.12
	% Moisture	23.3	30.9
Bottom			
	Initial	10.57	10.23
	Final	7.83	8.40
	Difference	2.74	1.83
	% Moisture	25.9	17.9

Site 12 Date 6-17-85

	1	2
E. Soluble sulfate (ppm)		
Top	16	16
Middle	<10	Lost
Bottom	<10	<10
F. Acid-soluble sulfide		
Top	<1	<1
Middle	<1	<1
Bottom	<1	<1

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml)		
Top	1.1(.22)e7	1.93(.04)e6
Middle	1.8(.25)e6	1.68(.26)e6
Bottom	4.9(2.0)e6	1.23(.28)e6
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	1.0e7	2.4e6
Middle	5.3e6	8.3e5
Bottom	2.1e6	7.2e5
C. NIF agar (Avg colonies/ml)		
Top	4.0(.85)e6	7.0(2.8)e5
Middle	2.1(.21)e6	2.35(.07)e6
Bottom	1.3(.64)e6	1.4(.28)e6
D. Rose Bengal agar (Avg colonies/ml)		
Top	5.5(4.9)e4	2.0(1.4)e4
Middle	4.0(1.4)e4	2.5(2.1)e4
Bottom	1.0(2.8)e4	4.0(2.8)e4

3. Metabolic Activity of Samples

A. Carbon mineralization (umole CO₂/g soil)

			1	2
Top				
Day 4	Increment		22.9	20.65
	(Cumulative)		22.9	20.65
Day 8	Increment		22.25	21.0
	(Cumulative)		45.15	41.65
Day 12	Increment		0.0	8.25
	(Cumulative)		45.15	49.90
Middle				
Day 4	Increment		9.0	16.0
	(Cumulative)		9.0	16.0
Day 8	Increment		10.5	19.65
	(Cumulative)		19.5	35.65
Day 12	Increment		0.5	16.0
	(Cumulative)		20.0	51.65
Bottom				
Day 4	Increment		6.75	18.25
	(Cumulative)		6.75	18.25
Day 8	Increment		4.75	11.25
	(Cumulative)		11.50	29.50
Day 12	Increment		4.25	6.25
	(Cumulative)		15.75	35.75

B. Nitrogen mineralization

1. Oxidation of urea (ppb NO₂)

	1	2
Top		
Day 0	290.0	220.0
Day 7	262.0	122.0
Day 14	115.6	136.2
Day 21	104.2	99.4
Middle		
Day 0	235.0	545.0
Day 7	140.0	175.0
Day 14	4370.0	76.8
Day 21	12500.0	69.8
Bottom		
Day 0	645.0	0.0
Day 7	575.0	20.0
Day 14	3466.0	394.0
Day 21	89.0	528.0

2. Reduction of nitrate (ppb NO₂)

Top		
Day 0	185.0	315.0
Day 7	85.0	160.0
Day 14	94.0	185.4
Day 21	113.0	184.4
Middle		
Day 0	295.0	245.0
Day 7	78.0	18.0
Day 14	51.8	44.0
Day 21	99.4	130.2
Bottom		
Day 0	~4000	555.0
Day 7	445.0	125.0
Day 14	44.8	38.0
Day 21	78.8	18.4

4. Metabolic activities of mixed cultures

A. Binding of uranium: ppm U in supernate

Pellets

Top	0.39, 0.39	1.01, 1.14
Middle	0.17, 0.39	0.37, 0.69
Bottom	0.54, 0.42	0.25, 0.25

Growing cells

Top	0.30, 0.44	0.47, 0.42
Middle	0.15, 0.64	0.57, 0.60
Bottom	0.13, 0.35	0.54, 1.14

B. Metal binding proteins

Specific activity of ^{203}Hg 0.833 cpm/ng

Aerobically grown cells

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

^{203}Hg in TCA sup/ml	114,181	32,284
^{203}Hg in void volume/ml	13,570	3,086
ng protein/ml	4.00	6.75
ng Hg/ng protein	17.60	1.71

Middle

^{203}Hg in TCA sup/ml	34,708	36,992
^{203}Hg in void volume/ml	3,304	4,732
ng protein/ml	3.75	20.0
ng Hg/ng protein	3.40	1.04

Bottom

^{203}Hg in TCA sup/ml	33,801	34,853
^{203}Hg in void volume/ml	3,396	3,150
ng protein/ml	5.50	2.00
ng Hg/ng protein	2.42	5.95

Aerobically grown cells

Induced with 5 ppm Hg, 1 ppm Cd

Top

^{203}Hg in TCA sup/ml	36,897	36,137
^{203}Hg in void volume/ml	3,090	3,134
ng protein/ml	1.00	1.25
ng Hg/ng protein	11.50	9.45

Middle

^{203}Hg in TCA sup/ml	35,977	35,055
^{203}Hg in void volume/ml	3,294	3,114

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mg protein/ml	1.00	1.00
ng Hg/mg protein	12.43	11.7

Bottom

203Hg in TCA sup/ml	35,354	34,919
203Hg in void volume/ml	3,402	3,180
mg protein/ml	1.25	1.25
ng Hg/mg protein	11.00	9.66

Metal binding proteins

Anaerobically grown cells

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	34,066	34,605
203Hg in void volume/ml	2,752	3,298
mg protein/ml	4.50	3.50
ng Hg/mg protein	2.15	3.64

Middle

203Hg in TCA sup/ml	41,947	35,056
203Hg in void volume/ml	4,742	3,336
mg protein/ml	1.00	3.00
ng Hg/mg protein	21.00	4.31

Bottom

203Hg in TCA sup/ml	38,373	34,022
203Hg in void volume/ml	3,202	2,676
mg protein/ml	3.50	4.00
ng Hg/mg protein	3.48	2.31

Anaerobically grown cells

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	35,992	35,312
203Hg in void volume/ml	3,122	3,318
mg protein/ml	1.50	1.0
ng Hg/mg protein	7.80	13.0

Middle

203Hg in TCA sup/ml	35,592	33,357
203Hg in void volume/ml	3,332	2,542
mg protein/ml	1.25	1.25
ng Hg/mg protein	10.3	6.80

Bottom

203Hg in TCA sup/ml	34,609	35,066
203Hg in void volume/ml	3,150	2,858
mg protein/ml	1.25	1.50
ng Hg/mg protein	9.52	6.85

CHARACTERIZATION OF SOIL SAMPLES

Date: JULY 25, 1985

Location: Site 13 (sewage plant, left)

	1	2
Depth of sampler (cm)	45	45
Depth of core (cm)	30	31
Compaction ratio (sampler/core)	1.5	1.45
Levels sampled (cm)		
Top	25.5	29
Middle	15.5	15
Bottom	4	5

Physical description of cores:

Sampled on day following 2 inches of rain. Site was high, soft but not waterlogged. Soil was sandy organic at top, grading to clayish sandy at bottom.

Temp in sample hole 23 C. Stream conductivity 375 uMho.

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	6.94	6.80
	17 hr	6.87	6.80
Middle	Initial	6.88	7.00
	17 hr	7.04	6.89
Bottom	Initial	6.92	6.85
	17 hr	6.80	6.78
B. Conductivity			
Top		120	100
Middle		40	45
Bottom		50	50
C. Moisture			
Top			
Initial		10.0	10.0
Final		8.8	8.3
Difference		1.2	1.7
% Moisture		12.0	17.0
Middle			
Initial		10.0	10.0
Final		8.5	8.8
Difference		1.5	1.2
% Moisture		15.0	12.0
Bottom			
Initial		10.0	10.0
Final		9.1	9.1
Difference		0.9	0.9
% Moisture		9.0	9.0

E. Soluble sulfate (ppm)

Top	13	<10
Middle	21	13
Bottom	10	13

F. Acid-soluble sulfide

Top	-----	-----
Middle	-----	-----
Bottom	-----	-----

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml)		
Top	8.7(2.0)e6	1.7(.08)e7
Middle	5.3(.07)e6	1.5(.18)e6
Bottom	1.1(.06)e6	1.3(.09)e6
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	-----	-----
Middle	-----	-----
Bottom	-----	-----
C. NIF agar (Avg colonies/ml)		
Top	5.8(.99)e6	1.1(.06)e7
Middle	3.3(.64)e6	5.1(.28)e6
Bottom	2.0(.21)e6	1.1(.98)e6
D. Rose Bengal agar (Avg colonies/ml)		
Top	4.3(.64)e5	2.1(.07)e5
Middle	5.8(1.1)e4	3.3(.92)e5
Bottom	2.2(.42)e4	1.8(.28)e4

3. Metabolic Activity of Samples

A. Carbon mineralization (umole CO₂/g soil)

			1	2
Top				
Day 4	Increment		10.5	10.5
	(Cumulative)		10.5	10.5
Day 8	Increment		7.5	17.5
	(Cumulative)		18.0	28.0
Day 12	Increment		5.0	13.0
	(Cumulative)		23.0	41.0
Middle				
Day 4	Increment		9.5	0.0
	(Cumulative)		9.5	0.0
Day 8	Increment		LOST	22.0
	(Cumulative)		----	22.0
Day 12	Increment		22.5	11.5
	(Cumulative)		----	33.5
Bottom				
Day 4	Increment		1.5	0.0
	(Cumulative)		1.5	0.0
Day 8	Increment		16.5	LOST
	(Cumulative)		18.0	----
Day 12	Increment		1.5	8.0
	(Cumulative)		19.5	----

B. Nitrogen mineralization

1. Oxidation of urea (ppb NO₂)

	1	2
Top		
Day 0	66.0	15.0
Day 7	988	7.4
Day 14	2062	2297
Day 21	0.0	7.4
Middle		
Day 0	12.0	11.0
Day 7	0.9	4.7
Day 14	1025	1901
Day 21	11.5	16.1
Bottom		
Day 0	27.0	8.0
Day 7	0.9	0.0
Day 14	164	63.1
Day 21	211	52.8

2. Reduction of nitrate (ppb NO₂)

Top		
Day 0	14.0	16.0
Day 7	0.0	0.0
Day 14	2.2	3.7
Day 21	9.9	19.9
Middle		
Day 0	7.0	13.0
Day 7	1.8	0.9
Day 14	2.2	2.2
Day 21	0.0	9.2
Bottom		
Day 0	6.0	13.0
Day 7	0.0	0.0
Day 14	0.7	0.7
Day 21	3.8	2.3

4. Metabolic activities of mixed cultures

A. Binding of uranium: ppm U in supernates

Pellets

Top	1.28,0.80	1.24,1.87
Middle	0.94,1.40	1.25,2.41
Bottom	1.20,1.16	2.10,2.96

Growing cells

Top	0.16,0.29	0.23,0.17
Middle	0.06,0.20	0.17,0.14
Bottom	0.13,0.07	0.15,0.38

B. Metal binding proteins

Aerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	32,477	32,842
203Hg in void volume/ml	2,349	1,666
mg protein/ml	5.00	7.00
ng Hg/mg protein	1.48	0.51

Middle

203Hg in TCA sup/ml	36,117	34,992
203Hg in void volume/ml	2,104	1,789
mg protein/ml	5.00	5.00
ng Hg/mg protein	1.21	0.86

Bottom

203Hg in TCA sup/ml	32,717	34,160
203Hg in void volume/ml	2,278	1,729
mg protein/ml	4.40	4.20
ng Hg/mg protein	1.60	0.94

Aerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	34,754	33,873
203Hg in void volume/ml	1,979	1,557
mg protein/ml	6.40	6.20
ng Hg/mg protein	0.84	0.48

Middle

203Hg in TCA sup/ml	36,520	33,561
203Hg in void volume/ml	1,763	1,606
mg protein/ml	5.20	6.00
ng Hg/mg protein	0.80	0.54

Bottom

203Hg in TCA sup/ml	36,345	32,275
203Hg in void volume/ml	1,981	2,569
mg protein/ml	4.40	5.82
ng Hg/mg protein	1.22	1.49

Metal binding proteins

Anaerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	37,529	37,102
203Hg in void volume/ml	2,538	2,302
mg protein/ml	4.00	3.42
ng Hg/mg protein	2.12	2.09

Middle

203Hg in TCA sup/ml	34,539	33,810
203Hg in void volume/ml	1,640	837
mg protein/ml	3.40	3.02
ng Hg/mg protein	1.01	0

Bottom

203Hg in TCA sup/ml	37,919	35,172
203Hg in void volume/ml	2,368	2,009
mg protein/ml	3.20	3.20
ng Hg/mg protein	2.35	1.72

Anaerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	33,926	36,181
203Hg in void volume/ml	1,741	2,801
mg protein/ml	6.80	3.42
ng Hg/mg protein	0.59	2.91

Middle

203Hg in TCA sup/ml	37,342	37,637
203Hg in void volume/ml	2,791	1,052
mg protein/ml	4.20	3.80
ng Hg/mg protein	2.36	0.04

Bottom

203Hg in TCA sup/ml	34,737	34,553
203Hg in void volume/ml	2,901	2,531
mg protein/ml	3.80	3.86
ng Hg/mg protein	2.77	2.19

CHARACTERIZATION OF SOIL SAMPLES

Date: 8/5/85

Location: Site 15 (sewage plant, right)

	1	2
Depth of sampler (cm)	45	45
Depth of core (cm)	23.5	25
Compaction ratio (sampler/core)	1.91	1.8
Levels sampled (cm)		
Top	22	24
Middle	15	13.5
Bottom	3.5	4

Physical description of cores:

Moist, organic grading to sandy clay.
Few roots.

Core 2 contained 2 worms, the only
sample (of 24) with worms.

Temperature in sample hole 21.5 C.
Temperature of creek 21.5 C;
conductivity 420 uMho.

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	7.01	7.05
	17 hr	7.24	7.18
Middle	Initial	7.09	7.11
	17 hr	7.29	7.20
Bottom	Initial	7.15	7.07
	17 hr	7.28	7.18
B. Conductivity			
Top		140	160
Middle		71	100
Bottom		68	100
C. Moisture			
Top			
Initial		10.0	10.0
Final		8.4	7.9
Difference		1.6	2.1
% Moisture		16.0	21.0
Middle			
Initial		10.0	10.0
Final		8.3	8.3
Difference		1.7	1.7
% Moisture		17.0	17.0
Bottom			
Initial		10.0	10.0
Final		7.7	7.6
Difference		2.3	2.4
% Moisture		23.0	24.0

E. Soluble sulfate (ppm)

Top	<10	<10
Middle	<10	10
Bottom	<10	11

F. Acid-soluble sulfide

Top	-----	-----
Middle	-----	-----
Bottom	-----	-----

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml)		
Top	2.1(.49)e7	1.7(.45)e7
Middle	1.2(.04)e7	1.9(1.5)e7
Bottom	4.5(.57)e6	5.7(.92)e6
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	6 e 4	3.4e5
Middle	2 e 4	2 e 4
Bottom	1 e 5	5 e 4
C. NIF agar (Avg colonies/ml)		
Top	4.9(4.6)e6	5.6(.85)e6
Middle	6.0(.78)e6	2.7(.14)e6
Bottom	3.3(.57)e6	2.3(.14)e6
D. Rose Bengal agar (Avg colonies/ml)		
Top	1.8(.64)e5	9.2(.64)e4
Middle	1.5(.71)e5	8.1(.21)e4
Bottom	1.1(.06)e5	4.5(.07)e4

3. Metabolic Activity of Samples

A. Carbon mineralization (umole CO₂/g soil)

			1	2
Top				
Day 4	Increment		8.0	11.0
	(Cumulative)		8.0	11.0
Day 8	Increment		11.0	8.0
	(Cumulative)		19.0	19.0
Day 12	Increment		4.0	29.5
	(Cumulative)		23.0	48.5
Middle				
Day 4	Increment		7.0	8.5
	(Cumulative)		7.0	8.5
Day 8	Increment		4.0	3.5
	(Cumulative)		11.0	12.0
Day 12	Increment		1.0	20.0
	(Cumulative)		12.0	32.0
Bottom				
Day 4	Increment		5.0	4.0
	(Cumulative)		5.0	4.0
Day 8	Increment		2.0	0.5
	(Cumulative)		7.0	4.5
Day 12	Increment		0.5	3.5
	(Cumulative)		7.5	8.0

B. Nitrogen mineralization

1. Oxidation of urea (ppb NO₂)

	1	2
Top		
Day 0	3.7	5.1
Day 7	6.1	42.1
Day 14	20.2	99.6
Day 21	6.7	22.4
Middle		
Day 0	9.5	3.7
Day 7	8.4	42.1
Day 14	102.3	94.1
Day 21	6.7	3.7
Bottom		
Day 0	5.1	2.2
Day 7	9.2	3.8
Day 14	91.1	88.1
Day 21	17.9	0.0

2. Reduction of nitrate (ppb NO₂)

Top		
Day 0	4.4	2.2
Day 7	2.3	LOST
Day 14	10.5	46.3
Day 21	7.5	17.9
Middle		
Day 0	5.9	0.7
Day 7	3.1	6.9
Day 14	0.0	5.2
Day 21	29.9	31.4
Bottom		
Day 0	0.0	0.0
Day 7	22.2	20.6
Day 14	1.5	0.0
Day 21	9.0	6.0

4. Metabolic activities of mixed cultures

A. Binding of uranium: ppm U in supernate

Pellets

Top	1.66, 1.30	2.14, 1.55
Middle	2.06, 1.90	1.76, 1.32
Bottom	0.34, 2.58	0.41, 0.55

Growing cells

Top	0.28, 0.31	0.25, 0.53
Middle	0.12, 0.18	0.31, 0.19
Bottom	0.30, ----	0.15, 0.16

B. Metal binding proteins

Aerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	32,818	32,939
203Hg in void volume/ml	3,688	3,760
mg protein/ml	4.60	5.70
ng Hg/mg protein	3.24	2.69

Middle

203Hg in TCA sup/ml	32,904	32,400
203Hg in void volume/ml	3,920	3,144
mg protein/ml	5.70	3.80
ng Hg/mg protein	2.85	3.12

Bottom

203Hg in TCA sup/ml	33,533	31,691
203Hg in void volume/ml	3,631	3,599
mg protein/ml	4.80	5.80
ng Hg/mg protein	3.04	2.49

Aerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	31,653	31,742
203Hg in void volume/ml	3,654	3,866
mg protein/ml	6.10	7.60
ng Hg/mg protein	2.41	2.09

Middle

203Hg in TCA sup/ml	31,524	31,186
203Hg in void volume/ml	4,057	3,956
mg protein/ml	7.20	7.00
ng Hg/mg protein	2.36	2.35

Bottom

203Hg in TCA sup/ml	32,631	31,419
203Hg in void volume/ml	3,598	3,621
mg protein/ml	5.40	6.00
ng Hg/mg protein	2.67	2.42

Metal binding proteins

Anaerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	33,314	33,517
203Hg in void volume/ml	3,485	3,445
mg protein/ml	2.20	3.10
ng Hg/mg protein	6.26	4.37

Middle

203Hg in TCA sup/ml	32,680	32,696
203Hg in void volume/ml	3,672	3,239
mg protein/ml	2.80	2.80
ng Hg/mg protein	5.80	4.93

Bottom

203Hg in TCA sup/ml	32,959	32,884
203Hg in void volume/ml	3,517	3,910
mg protein/ml	3.80	2.60
ng Hg/mg protein	3.67	6.22

Anaerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	32,438	32,022
203Hg in void volume/ml	3,146	3,317
mg protein/ml	3.40	3.60
ng Hg/mg protein	3.50	3.57

Middle

203Hg in TCA sup/ml	32,374	32,530
203Hg in void volume/ml	3,603	3,008
mg protein/ml	3.20	4.20
ng Hg/mg protein	4.51	2.65

Bottom

203Hg in TCA sup/ml	32,730	32,912
203Hg in void volume/ml	3,763	3,007
mg protein/ml	3.40	3.30
ng Hg/mg protein	4.51	3.37

CHARACTERIZATION OF SOIL SAMPLES

Date: 8/1/85

Location: Site 16 (Turnpike bridge, left)

	1	2
Depth of sampler (cm)	45	45
Depth of core (cm)	25.5	21
Compaction ratio (sampler/core)	1.76	2.14
Levels sampled (cm)		
Top	24	19
Middle	16	12
Bottom	4.5	2.5

Physical description of cores:

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	5.80	6.09
	17 hr	5.93	6.21
Middle	Initial	5.95	6.14
	17 hr	6.03	6.21
Bottom	Initial	6.10	6.19
	17 hr	6.39	6.27
B. Conductivity			
Top		76	54
Middle		86	54
Bottom		34	42
C. Moisture			
Top			
Initial		10.0	10.0
Final		7.5	7.7
Difference		2.5	2.3
% Moisture		25.0	23.0
Middle			
Initial		10.0	10.0
Final		7.6	7.7
Difference		2.4	2.3
% Moisture		24.0	23.0
Bottom			
Initial		10.0	10.0
Final		8.4	8.3
Difference		1.6	1.7
% Moisture		16.0	17.0

E. Soluble sulfate (ppm)

Top	18	20
Middle	<10	<10
Bottom	Lost	13

F. Acid-soluble sulfide

Top	-----	-----
Middle	-----	-----
Bottom	-----	-----

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml)		
Top	1.1(.19)e7	1.9(.14)e7
Middle	1.9(.71)e7	7.7(.28)e6
Bottom	3.1(1.1)e6	4.3(.49)e6
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	2.7e5	6.9e5
Middle	1.8e5	2.0e5
Bottom	9 e 4	7 e 4
C. NIF agar (Avg colonies/ml)		
Top	7.2(.14)e6	1.0(.04)e6
Middle	9.7(1.3)e6	6.6(.92)e6
Bottom	1.8(.09)e6	2.9(.97)e6
D. Rose Bengal agar (Avg colonies/ml)		
Top	2.9(.64)e5	1.4(1.1)e5
Middle	2.2(.28)e5	1.6(.42)e5
Bottom	1.6(1.1)e4	7.0(1.4)e4

3. Metabolic Activity of Samples

A. Carbon mineralization (umole CO₂/g soil)

			1	2
Top				
Day 4	Increment		11.5	10.5
	(Cumulative)		11.5	10.5
Day 8	Increment		11.0	8.0
	(Cumulative)		22.5	18.5
Day 12	Increment		3.5	0.0
	(Cumulative)		26.0	18.5
Middle				
Day 4	Increment		8.0	5.5
	(Cumulative)		8.0	5.5
Day 8	Increment		4.0	3.5
	(Cumulative)		12.0	9.0
Day 12	Increment		1.0	4.5
	(Cumulative)		13.0	13.5
Bottom				
Day 4	Increment		9.5	9.0
	(Cumulative)		9.5	9.0
Day 8	Increment		2.0	0.5
	(Cumulative)		11.5	9.5
Day 12	Increment		3.0	7.0
	(Cumulative)		14.5	16.5

B. Nitrogen mineralization

1. Oxidation of urea (ppb NO₂)

	1	2
Top		
Day 0	1.8	1.8
Day 7	19.8	20.6
Day 14	22.9	28.3
Day 21	11.9	11.9
Middle		
Day 0	0.0	0.0
Day 7	8.1	63.9
Day 14	335.7	11.5
Day 21	9.7	48.5
Bottom		
Day 0	0.0	0.0
Day 7	5.1	36.7
Day 14	28.3	16.8
Day 21	0.0	3.7

2. Reduction of nitrate (ppb NO₂)

Top		
Day 0	4.7	6.5
Day 7	19.1	16.9
Day 14	11.5	8.4
Day 21	14.9	18.7
Middle		
Day 0	0.0	LOST
Day 7	25.7	8.1
Day 14	9.9	16.8
Day 21	9.0	11.2
Bottom		
Day 0	0.0	5.6
Day 7	7.3	0.7
Day 14	3.8	9.9
Day 21	10.5	24.6

4. Metabolic activities of mixed cultures

A. Binding of uranium

Pellets

Top	0.85,0.57	0.85,0.92
Middle	1.01,0.85	1.86,1.76
Bottom	1.06,1.09	1.00,1.14

Growing cells

Top	0.05,0.08	0.04,0.10
Middle	0.04,0.04	0.08,0.12
Bottom	0.10,0.09	0.03,0.02

B. Metal binding proteins

Aerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	33,422	32,074
203Hg in void volume/ml	4,352	4,046
mg protein/ml	4.64	5.42
ng Hg/mg protein	4.02	3.12

Middle

203Hg in TCA sup/ml	32,240	31,355
203Hg in void volume/ml	3,241	3,310
mg protein/ml	4.00	5.20
ng Hg/mg protein	3.10	2.46

Bottom

203Hg in TCA sup/ml	32,639	31,520
203Hg in void volume/ml	3,930	3,451
mg protein/ml	5.32	5.84
ng Hg/mg protein	3.06	2.33

Aerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	34,057	30,990
203Hg in void volume/ml	4,116	3,593
mg protein/ml	6.50	7.20
ng Hg/mg protein	2.66	2.00

Middle

203Hg in TCA sup/ml	30,373	32,242
203Hg in void volume/ml	4,300	4,144
mg protein/ml	5.90	6.60
ng Hg/mg protein	3.11	2.65

Bottom

203Hg in TCA sup/ml	31,418	31,160
203Hg in void volume/ml	3,969	4,296
mg protein/ml	6.60	7.40
ng Hg/mg protein	2.50	2.48

Metal binding proteins

Anaerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	36,053	33,500
203Hg in void volume/ml	1,452	1,521
mg protein/ml	2.40	2.60
ng Hg/mg protein	1.00	1.07

Middle

203Hg in TCA sup/ml	33,438	36,988
203Hg in void volume/ml	1,500	1,719
mg protein/ml	2.20	2.40
ng Hg/mg protein	1.21	1.62

Bottom

203Hg in TCA sup/ml	35,575	39,109
203Hg in void volume/ml	1,603	1,704
mg protein/ml	3.00	2.86
ng Hg/mg protein	1.08	1.33

Anaerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	35,814	36,536
203Hg in void volume/ml	1,666	1,529
mg protein/ml	3.90	3.90
ng Hg/mg protein	0.92	0.72

Middle

203Hg in TCA sup/ml	34,715	34,158
203Hg in void volume/ml	3,187	3,554
mg protein/ml	5.80	3.60
ng Hg/mg protein	2.09	4.58

Bottom

203Hg in TCA sup/ml	33,824	33,160
203Hg in void volume/ml	3,911	3,725
mg protein/ml	1.60	3.30
ng Hg/mg protein	10.1	4.58

CHARACTERIZATION OF SOIL SAMPLES

Date: JULY 29, 1985

Location: Site 18 (Turnpike bridge, right)

	1	2
Depth of sampler (cm)	45	45
Depth of core (cm)	27	28
Compaction ratio (sampler/core)	1.67	1.61
Levels sampled (cm)		
Top	25	23
Middle	16	15
Bottom	4	2

Physical description of cores:

Top sandy organic, grading to slightly less organic toward bottom. Gravel at bottom of core 2.

Temp. 22 C

1. Chemical Characterization

		1	2
A. pH			
Top	Initial	6.69	6.73
	17 hr	6.43	6.48
Middle	Initial	6.95	6.90
	17 hr	6.64	6.62
Bottom	Initial	6.85	6.70
	17 hr	6.56	6.37
B. Conductivity (uMho)			
Top		140	50
Middle		44	40
Bottom		46	50
C. Moisture			
Top			
	Initial	10.0	10.0
	Final	8.0	7.5
	Difference	2.0	2.5
	% Moisture	20.0	25.0
Middle			
	Initial	10.0	10.0
	Final	8.0	7.9
	Difference	2.0	2.1
	% Moisture	20.0	21.0
Bottom			
	Initial	10.0	10.0
	Final	8.4	8.6
	Difference	1.6	1.4
	% Moisture	16.0	14.0

E. Soluble sulfate (ppm)

Top	Lost	17
Middle	<10	58
Bottom	Lost	<10

F. Acid-soluble sulfide

Top	-----	-----
Middle	-----	-----
Bottom	-----	-----

2. Enumeration of Bacteria and Fungi

	1	2
A. Aerobic plate-count medium (Avg colonies/ml)		
Top	1.5(.16)e7	3.0(.35)e6
Middle	8.1(1.4)e6	2.1(.31)e6
Bottom	2.0(.18)e6	1.7(.11)e6
B. Anaerobic plate-count medium (Avg colonies/ml)		
Top	1.4e5	7 e 4
Middle	6.2e5	2.4e5
Bottom	6.0e5	1.0e6
C. NIF agar (Avg colonies/ml)		
Top	1.0(.06)e7	6.8(.92)e6
Middle	6.2(.28)e6	5.3(.23)e6
Bottom	1.6(.04)e6	1.3(.03)e6
D. Rose Bengal agar (Avg colonies/ml)		
Top	2.0(.82)e5	1.3(.14)e5
Middle	1.6(.71)e5	4.0(.07)e4
Bottom	2.0(.07)e4	1.8(.59)e4

3. Metabolic Activity of Samples

A. Carbon mineralization ($\mu\text{mole CO}_2/\text{g soil}$)

			1	2
Top				
Day 4	Increment		10.5	26.5
	(Cumulative)		10.5	26.5
Day 8	Increment		0.5	7.5
	(Cumulative)		11.0	34.0
Day 12	Increment		10.5	8.5
	(Cumulative)		21.5	42.5
Middle				
Day 4	Increment		1.5	5.5
	(Cumulative)		1.5	5.5
Day 8	Increment		14.5	1.0
	(Cumulative)		16.0	6.5
Day 12	Increment		12.0	13.0
	(Cumulative)		28.0	19.5
Bottom				
Day 4	Increment		4.0	19.0
	(Cumulative)		4.0	19.0
Day 8	Increment		3.0	6.5
	(Cumulative)		7.0	25.5
Day 12	Increment		15.5	13.0
	(Cumulative)		22.5	38.5

B. Nitrogen mineralization

1. Oxidation of urea (ppb NO₂)

	1	2
Top		
Day 0	2.8	0.0
Day 7	41.1	54.3
Day 14	8.1	31.3
Day 21	56.0	74.6
Middle		
Day 0	7.4	1.8
Day 7	7.3	5.1
Day 14	5.4	7.6
Day 21	80.6	74.6
Bottom		
Day 0	0.9	3.7
Day 7	2.9	3.7
Day 14	65.8	3.1
Day 21	93.3	

2. Reduction of nitrate (ppb NO₂)

Top		
Day 0	Broken	3.7
Day 7	4.4	0.0
Day 14	3.1	0.0
Day 21	0.1	28.4
Middle		
Day 0	0.0	15.8
Day 7	2.9	2.9
Day 14	0.0	26.0
Day 21	0.1	2.2
Bottom		
Day 0	0.0	3.7
Day 7	0.0	0.0
Day 14	0.0	3.1
Day 21	17.9	15.7

4. Metabolic activities of mixed cultures

A. Binding of uranium

Pellets

Top	1.70,0.44	2.15,2.03
Middle	0.93,1.09	1.93,1.78
Bottom	2.29,2.01	1.14,1.64

Growing cells

Top	0.15,0.21	0.19,0.19
Middle	0.15,0.13	0.10,0.20
Bottom	0.10,0.18	0.18,0.08

B. Metal binding proteins

Aerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	28,988	25,286
203Hg in void volume/ml	1,723	1,536
mg protein/ml	5.90	5.70
ng Hg/mg protein	0.66	0.50

Middle

203Hg in TCA sup/ml	35,448	32,490
203Hg in void volume/ml	2,150	1,472
mg protein/ml	6.30	6.80
ng Hg/mg protein	1.00	0.37

Bottom

203Hg in TCA sup/ml	32,531	32,757
203Hg in void volume/ml	1,780	1,738
mg protein/ml	5.90	4.30
ng Hg/mg protein	0.72	0.93

Aerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	32,121	32,013
203Hg in void volume/ml	4,485	2,566
mg protein/ml	2.00	5.50
ng Hg/mg protein	9.69	1.57

Middle

203Hg in TCA sup/ml	31,955	31,868
203Hg in void volume/ml	1,918	1,620
mg protein/ml	5.60	6.20
ng Hg/mg protein	0.89	0.54

Bottom

203Hg in TCA sup/ml	34,578	34,216
203Hg in void volume/ml	1,966	1,675
mg protein/ml	6.20	6.40
ng Hg/mg protein	0.85	0.57

Metal binding proteins

Anaerobically grown

Induced with 10 ppm Pb, Hg, As, 1 ppm Cd

Top

203Hg in TCA sup/ml	33,985	34,892
203Hg in void volume/ml	1,749	2,263
mg protein/ml	1.55	2.10
ng Hg/mg protein	2.62	3.30

Middle

203Hg in TCA sup/ml	39,219	35,857
203Hg in void volume/ml	2,212	2,433
mg protein/ml	2.07	1.75
ng Hg/mg protein	3.21	4.51

Bottom

203Hg in TCA sup/ml	33,916	35,222
203Hg in void volume/ml	2,400	1,716
mg protein/ml	1.38	3.10
ng Hg/mg protein	5.58	1.25

Anaerobically grown

Induced with 5 ppm Hg, 1 ppm Cd

Top

203Hg in TCA sup/ml	34,697	33,787
203Hg in void volume/ml	2,355	1,529
mg protein/ml	2.00	1.80
ng Hg/mg protein	3.73	1.57

Middle

203Hg in TCA sup/ml	35,491	40,005
203Hg in void volume/ml	1,558	1,719
mg protein/ml	2.07	2.40
ng Hg/mg protein	1.44	1.62

Bottom

203Hg in TCA sup/ml	28,475	36,486
203Hg in void volume/ml	1,211	1,593
mg protein/ml	3.00	2.70
ng Hg/mg protein	0.35	1.18

TABLE 1. NR1 Medium

<u>Ingredients</u>	<u>Concentrations (grams/liter)</u>
(NH ₄) ₂ SO ₄	0.1
MgSO ₄ 7H ₂ O	0.2
NaCl	1.0
KCl	1.0
NH ₄ Cl	1.0
Glucose	5.0
Na-B-glycerophosphate	0.67
Alanine	0.17
Arginine	0.10
Methionine	0.10
Phenylalanine	0.90
Serine	0.22
Valine	0.12
Tris	6.06

pH 7.2

TABLE 2. Heavy metal or ampicillin-induced cultures of *Pseudomonas O₂* in presence of two heavy metals combined or a heavy metal plus ampicillin.

Induced Culture (induction conc.)	Heavy Metal (common per flask)	Heavy Metal or Antibiotic Supplement	Growth (Abs. 600 nm)		
			0	Hrs 24	48
Zn (1.0 mM)	Zn (1.0 mM)	0	0.104	0.849	1.920
Cd (0.44 mM)	Zn (1.0 mM)	Cd (0.44 mM)	0.132	0.539	1.140
Pb (0.41 mM)	Zn (1.0 mM)	Pb (0.2 mM)	0.553	1.135	1.282
Amp (250 ug/ml)	Zn (1.0 mM)	Amp (250 ug/ml)	0.090	1.194	1.691
non-induced	Zn (1.0 mM)	0	0.193	0.195	0.180
Zn (1.0 mM)	0	0	0.112	1.277	1.823
Zn (1.0 mM)	Cd (0.44 mM)	Zn (1.0 mM)	0.085	0.863	1.774
Cd (0.44 mM)	Cd (0.44 mM)	0	0.066	0.076	1.124
Pb (0.41 mM)	Cd (0.44 mM)	Pb (0.2 mM)	0.334	0.666	0.865
Amp (250 ug/ml)	Cd (0.44 mM)	Amp (250 ug/ml)	0.094	0.096	1.805
non-induced	Cd (0.44 mM)	0	0.172	0.163	0.170
Cd (0.44 mM)	0	0	0.058	2.007	2.165
Zn (1.0 mM)	Pb (0.2 mM)	Zn (1.0 mM)	0.184	1.522	1.875
Cd (0.44 mM)	Pb (0.2 mM)	Cd (0.44 mM)	0.074	0.084	2.397
Pb (0.41 mM)	Pb (0.2 mM)	0	0.193	0.264	1.758*
Amp (250 ug/ml)	Pb (0.2 mM)	Amp (250 ug/ml)	0.203	0.941	2.190
non-induced	Pb (0.2 mM)	0	0.272	0.298	0.298*
Pb (0.41 mM)	0	0	0.195	0.122	1.320*

- a *Pseudomonas O₂* was induced to grow in high concentrations of heavy metals, zn (zinc), cd (cadmium), Pb (lead), or the antibiotic, Amp (ampicillin).
- b Once induced, the cultures were inoculated (20% v/v) in NR-1 synthetic medium, plus either Zn, Cd, or Pb and one additional heavy metal or Amp.
- c Chemical forms of heavy metals added to growth medium, CdCl₂, ZnCl₂, Pb(NO₃)₂. * 66 Hr. Readings
- d 1.0 ml samples were taken from growth medium at 0,24,48 hrs, and the Abs. at 600 nm were read for each.

TABLE 3. Growth of Zn-induced *Pseudomonas* O₂ culture in the presence of mixed heavy metals and ampicillin.

Flask No.	Hg (mM)	Supplements ^b			Zn (mM)	Amp (Ug/ml)	Absorbance (600nm) ^c	
		Cd (mM)	Pb (mM)				0 time	72 hrs
I	0.4	0.44	0.2		1.0	250	0.275	2.068
II	0.5	0.44	0.2		1.0	250	0.243	1.071
III	0.6	0.44	0.2		1.0	250	0.236	0.697
IV	0.7	0.44	0.2		1.0	250	0.260	0.820
V	0.8	0.44	0.2		1.0	250	0.243	0.665
VI	0.9	0.44	0.2		1.0	250	0.352	0.907
VII	1.0	0.44	0.2		1.0	250	0.405	1.540
VIII	2.0	0.44	0.2		1.0	250	0.440	0.706
IX	3.0	0.44	0.2		1.0	250	0.792	1.328
X	4.0	0.44	0.2		1.0	250	0.776	1.084
XI	5.0	0.44	0.2		1.0	250	0.757	1.298
XII	0	0	0		0	0	0.127	3.643

^a *Pseudomonas* O₂ was induced to grow in high concentrations of Zn (1.0mM). After induction cells were inoculated into 20 ml of NR1 synthetic medium (20% v/v) plus mixed heavy metals and ampicillin concentrations mentioned above. Incubation was at 32°C, shaking for 72 hrs.

^b Chemical forms of heavy metals added to the medium were HgCl₂, CdCl₂, Pb(NO₃)₂, ZnCl₂.

^c 1.0 ml samples were taken from each flask at 0 time and 72 hrs and absorbance read at 600 nm.

TABLE 4. Facts About Pseudomonas O₂

- 1) Pseudomonas O₂ is resistant to several heavy metals, Zn (1.5 mM), Cd (1.0 mM), Pb (1.6 mM) and Hg (0.4 mM) and to the antibiotic, ampicillin (250 ug/ml). conc.
- 2) Growth of Pseudomonas O₂ in synthetic medium, NRL, containing zn, cd, or Pb (induction) enables the culture to be resistant to any of these heavy metals in growth medium.
- 3) Pseudomonas O₂ can grow in the presence of mixed heavy metals (Zn + Pb + Hg + Cd) at high concentrations when ampicillin is in the medium. In the presence of ampicillin the culture can grow as high as 4.0 mM Hg.
- 4) Without ampicillin in NRL synthetic medium, Pseudomonas O₂ has difficulty growing under shaker aeration (100 rpm) at 32°C in presence of Hg.
- 5) Pseudomonas O₂ can be induced to grow on Ag (40 ug/ml), Au (82.04 ug/ml), and Ur (50.25 ug/ml).
- 6) Growth of Pseudomonas O₂ has been obtained in synthetic medium containing a combination of Ag (10 ug/ml), Au (6 ug/ml), and Ur (10.05 ug/ml) plus ampicillin (250 ug/ml).
- 7) Pseudomonas O₂ is resistant to other drugs besides ampicillin (Streptomycin, Kanamycin, and tetracycline) but sensitive to erythromycin.
- 8) Although the culture is resistant to other drugs, ampicillin is the only antibiotic tested that allows Pseudomonas O₂ to grow in high Hg concentrations in NRL synthetic medium.

TABLE 5. Technical Assistance

Tanya Osborne

Samuel Ebong

Suzanne Benson (culture isolation)

TABLE 6

Growth of *Pseudomonas* O₂ in the Presence and Absence of Forced Aeration (Bubbling) in NRL Synthetic Medium

Sampling Time (Hrs)	Growth (Abs. 600 nm) ^a							
	<u>Hg Conc. (mM)</u>							
	0	0.1	0.2	0.3	0.4			
<u>Bubbling^b</u>								
0	0.005	0.006	0.013	0.006	0.004			
18	1.367	0.016	0.090	0.004	0.008			
25	1.450	0.106	0.010	0.003	0.003			
41	1.801	1.469	1.720	0.265	0.012			
48	1.826	1.588	1.748	1.200	0			
<u>No Bubbling</u>								
0	0	0	0	0	0			
18	0.972	0	0	0	0			
25	1.005	0	0	0	0			
41	1.039	0	0	0	0			
48	1.066	0	0	0	0			
<u>Bubbling + Ampicillin^c</u>								
	<u>Hg Conc. (mM)</u>							
	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
0	0.054	0.049	0.035	0.042	0.041	0.050	0.054	0.045
15	0.457	0.049	0.038	0.015	0.299	0.065	0.046	0.036
22	0.675	0.075	0.011	1.169	0.308	0.035	0.030	0.014
39	2.089	1.073	0.391	1.930	2.185	1.490	1.604	0.410
46	2.240	1.553	0.644	1.946	2.050	1.850	2.229	1.280
69	2.100	2.115	1.830	1.893	1.900	1.933	2.189	2.15

^a *Pseudomonas* O₂ was inoculated (20% v/v) in NRL synthetic medium plus HgCl₂ at various concentrations. 1.0 ml samples were taken at times indicated above and absorbances read at 600 nm.

^b Air was bubbled for 2 minute time periods into NRL medium containing culture and HgCl₂ at sampling times shown above. Between bubbling periods, cultures were placed on a rotary shaker (100 rpm) at 32°C.

^c Ampicillin was added at 250 ug/ml to each culture.

TABLE 7

COST ANALYSIS OF REGULAR NR1 AND CASA-NR1 (OR NR2)

MEDIA

Casa NR1 Ingredients	Quantity (gm)	Unit Cost \$	Quantity (gm) liter	Cost of Ingredient liter
(NH ₄) ₂ SO ₄	500 gm	6.90	0.1	0.0014
MgSO ₄ ·7H ₂ O	500 gm	17.25	0.2	0.0069
NaCl	500 gm	5.80	1.0	0.0116
NH ₄ Cl	100 gm	3.75	1.0	0.0375
Glucose	100 gm	3.65	5.0	0.1825
Na-B-glycerophosphate	100 gm	18.75	0.67	0.1256
Casamino Acid	100 gm	3.95	1.65	0.0652
Methionine	100 gm	17.80	0.02	0.0036
*Tris	500 gm	19.50	6.06	0.2363
KCl	250 gm	4.80	1.0	0.0192
TOTAL				0.6898
Regular NR1 Ingredients				
(NH ₄) ₂ SO ₄	500	6.90	0.1	0.0014
MgSO ₄ ·7H ₂ O	500	17.25	0.2	0.0069
NaCl	500	5.80	1.0	0.0116
NH ₄ Cl	100	3.75	1.0	0.0375
Glucose	100	3.65	5.0	0.1825
Na-B-glycerophosphate	100	18.75	0.67	0.1256
Alanine	100	19.00	0.17	0.0323
Arginine	100	9.00	0.10	0.0090
Methionine	100	17.80	0.10	0.0178
Phenylalanine	100	21.00	0.90	0.1890
Serine	100	27.75	0.22	0.0611
Valine	100	19.50	0.12	0.0234
KCl	250	4.80	1.0	0.0192
*Tris	500	19.50	6.06	0.2363
TOTAL				0.9536

COST DIFFERENCE: NR1 - NR2 \$0.9536 - \$0.6898 = \$0.26

*Unit Cost from Bethesda Research Lab. Others from Sigma.

TABLE 8

The effect of mercury chloride (HgCl_2), cadmium chloride (CdCl_2), zinc chloride (ZnCl_2) and ampicillin on growth of Pseudomonas syringae at various time intervals.

Culture Conditions	(Absorbance 600 nm)		
	24	48	72
Bacteria	1.6	2.0	1.9
Bacteria + Ampicillin (167 ug/ml)	2.0	2.1	1.8
Bacteria + HgCl_2 (27 ppm)	0.8	1.2	1.3
Bacteria + CdCl_2 (18 ppm)	0.4	0.8	1.0
Bacteria + ZnCl_2 (136 ppm)	1.7	1.8	1.6
Bacteria + ZnCl_2 (136 ppm) + HgCl_2 (54 ppm)	0.6	0.8	0.7
Bacteria + ZnCl_2 (136 ppm) + CdCl_2 (36 ppm)	0.4	0.5	0.5
Bacteria + Ampicillin + HgCl_2 (163 ppm)	0.8	1.0	1.1
Bacteria + Ampicillin + CdCl_2 (180 ppm)	1.0	1.3	1.4
Bacteria + ZnCl_2 (136 ppm) + Ampicillin + HgCl_2 (652 ppm)	0.8	1.2	1.0
Bacteria + ZnCl_2 (136 ppm) + Ampicillin + CdCl_2 (73 ppm)	0.6	0.8	0.7
Bacteria + ZnCl_2 (136 ppm) + Ampicillin + HgCl_2 (109 ppm) + PbCl_2 (111 ppm)	0.5	0.8	0.8

Culture were grown in a minimal broth containing the toxic metals and antibiotics and at various time intervals an aliquot of culture was taken and the optical density determined. The appropriate control cultures (i.e. cultures minus Pseudomonas syringae) were subtracted from the test culture at the various time intervals.

TABLE 9

The removal of toxic metals from growth medium by Pseudomonas syringe

Culture Conditions	Filtration	TCA Precipitation and filtration % of total radioactivity (added to culture) on the filter	Boling and filtration
Bacteria + $^{203}\text{HgCl}_2$	38	39	26
Bacteria + $^{109}\text{CdCl}_2$	26	30	30
Bacteria + Ampicillin + ZnCl_2 (136 ppm) $^{203}\text{HgCl}_2$ + HgCl_2 (652 ppm)	48	95	95
Bacteria + Ampicillin + $^{109}\text{CdCl}_2$ + CdCl_2 (180 ppm)	51	95	99

Pseudomonas syringe was grown for 48 hrs in the presence of these metals at 32°C. Aliquots of the culture were filtered (0.45 μm) and the filters were counted in a gamma counter.

TABLE 10

Pseudomonas syringae growth and mercury binding (^{203}Hg) in cultures containing aqueous extracts of mercury contaminated soil.

Culture conditions	% Soil Extract added to cultures					
	10		30		50	
	A	B	A	B	A	B
Bacteria only	0.86 ± 0.10	29 ± 2.11	0.53 ± 0.07	29 ± 1.15	0.53 ± 0.12	35 ± 1.31
Bacteria + Ampicillin	0.82 ± 0.11	28 ± 1.34	0.78 ± 0.15	38 ± 1.27	0.50 ± 0.36	45 ± 3.4
Bacteria + Ampicillin + 1 mM ZnCl_2	0.60 ± 0.08	50 ± 5.36	0.55 ± 0.13	55 ± 3.46	0.66 ± 0.40	65 ± 5.3
Bacteria + Ampicillin + 0.5 mM mixed metals (HgCl_2 , CdCl_2 , PbCl_2 and ZnCl_2)	0.45 ± 0.07	23 ± 1.18	0.18 ± 0.01	29 ± 2.3	0.43 ± 0.31	29 ± 3.0

Mean $\pm$ SD for 3 experiments. A = optical density and B = % ^{203}Hg bound to the cells. Cells were grown in a medium containing ^{203}Hg and extracts of soil for 24 hours. At the end of the experiment the optical density, and the binding of ^{203}Hg to bacteria were determined. Mercury binding was determined by taking an aliquot of the culture and filtering through a 0.45 μm filter and then measuring ^{203}Hg on the filter.

TABLE 11

The incorporation of ^{35}S into proteins of Pseudomonas syringe following the exposure to ampicillin and mercury (Hg)

<u>Culture</u> <u>Conditions</u>	<u>Radionuclide</u>	
	<u>$\text{Na}^{35}\text{SO}_4$</u>	<u>^{35}S-Methionine</u>
	cpm / mg protein	
Bacteria only	105 ± 30	890 ± 300
Bacteria + Ampicillin	280 ± 169	5500 ± 600
Bacteria + Hg (80 ppm)	332 ± 113	3800 ± 263
Bacteria + Hg (80 ppm) + Ampicillin (167 ug/ml)	300 ± 215	12890 ± 460

Cultures of Pseudomonas syringe were grown for 48 hrs in the presence or absence of ampicillin and mercury with radiolabelled NaSO_4 and methionine added. At 48 hours culture were centrifuged and the pellet was added to chloroform methanol (2:1 v/v) to extract all lipids. After leaving overnight at 40°C the precipitate was removed and dissolved in 9m urea. Protein and ^{35}S were determined in the dissolved precipitate. Mean $\pm$ SD

FIG. 1. Heavy metal or ampicillin-induced cultures of *Pseudomonas* G₂ in presence of Zn (10 mM) compared with another heavy metal or ampicillin.

Incubation = 32°C, shaking

FIG. 2. Heavy metal or ampicillin-induced cultures of *Pseudomonas* in presence of Cd(0.44mM) combined with one other heavy metal or ampicillin. Incubation - 32°C, shaking

FIG. 5. Heavy metal or ampicillin-induced cultures of *Pseudomonas* O₂ in presence of Pb (102 μ M) combined with one other heavy metal or ampicillin.
Incubation - 32°C, shaking

FIG 4.

Pseudomonas O_2

Will Cells Grow In Presence of
 Hg , Au , Cd , Zn , Pb ?

APPENDIX D

SUBCONTRACT NO. ORRI-85-2

between

OAK RIDGE RESEARCH INSTITUTE

and

TUSKEGEE INSTITUTE

This agreement entered into by and between the Oak Ridge Research Institute, with its principal offices in Oak Ridge, Tennessee (hereinafter referred to as "Contractor") and Tuskegee Institute, having offices in Tuskegee, Alabama (hereinafter referred to as "Subcontractor");

WITNESSETH THAT:

WHEREAS, the Contractor has heretofore entered into Contract No. DE-AC05-84OR21492 (hereinafter called the "Principal Contract") with the UNITED STATES OF AMERICA (hereinafter called the "Government") represented by the UNITED STATES DEPARTMENT OF ENERGY (hereinafter called "DOE"); and

WHEREAS, in furtherance of the Principal Contract, the parties hereto desire to provide for the Subcontractor's performing the research as hereinafter described; and

WHEREAS, the Subcontractor represents that it is ready, willing and able to undertake a portion of said efforts as hereinafter set forth;

NOW THEREFORE, in consideration of the premises, as well as the obligations made and undertaken, the parties hereto mutually agree as follows:

Article I. Statement of Work

Subcontractor shall perform the effort set forth in the Statement of Work attached hereto as Exhibit A. Said Exhibit A is incorporated herein by reference.

Article II. Period of Performance

The Agreement shall commence effective July 1, 1985. All work hereunder shall be completed on or before September 30, 1988.

Article III. Subcontract Type and Price

A. This is a firm fixed price type subcontract, issued pursuant to applicable Federal Acquisition Regulations. The total price for the services set forth in Article I hereof is One Hundred Forty Thousand and No/100 (\$140,000.00) Dollars U.S. Payment will be made in accordance with the Schedule attached hereto as Exhibit B, which is incorporated herein by reference. Cost billed for the period from July 1, 1985 through October 31, 1985 shall not exceed \$19,110. Costs billed thereafter in any contract year (November 1 to October 1) shall not exceed the amounts indicated.

B. Payment under this subcontract shall be made upon certification of invoices submitted, not more often than monthly, for percentage of work completed during the preceding month, to the Contractor's Principal Office, 113 Union Valley Road, Oak Ridge, Tennessee, ATTN: Dr. Nathaniel Revis.

Article IV. Inspection

The contractor, through its authorized representative, has the right at all reasonable times to inspect or otherwise evaluate the work performed or being performed by the Subcontractor.

Article V. Interest of Subcontractor

The Subcontractor covenants that he presently has no interest direct or indirect which would conflict in any manner or degree with the performances of his services hereunder. The Subcontractor further covenants that in the performance of this Agreement no such person having such interest shall be employed.

Article VI. Publicity

Subcontractor agrees that no advertising or publicity matter having or containing any reference to the Oak Ridge Research Institute or in which the name is mentioned, shall be made use of by the Subcontractor or anyone in the Subcontractor's behalf unless and until the same shall have first been submitted to and received the approval of the Oak Ridge Research Institute. The same shall apply to the Oak Ridge Research Institute or any of its divisions or units.

Article VII. Termination

The Contractor may, by written notice to the Subcontractor, terminate this Contract in whole or in part at any time, either for the Contractor's convenience or because of the failure of the Subcontractor to fulfill its contract obligations.

Article VIII. Changes

The Contractor may, from time to time, require changes in the scope of the services of the Subcontractor to be performed hereunder. Such changes shall be incorporated by written amendment into this agreement.

Article IX. Independent Contractor and Labor Requirements

A. The Subcontractor is furnishing his services hereunder as an independent contractor, and the Subcontractor shall not be considered an employee of the Contractor.

B. The Subcontractor shall be responsible for compliance with all requirements and obligations relating to such services under local, state or federal law including but not limited to minimum wage, social security, unemployment insurance, income tax and worker's compensation. The Subcontractor shall also be responsible for all licensing requirements of any local, state, or federal jurisdiction to which the performance of his services may be subject.

Article X. Entire Agreement

This subcontract constitutes the sole and entire Agreement between the parties with respect to the subject matter contained herein. This Subcontract supersedes any prior Agreement, offer, or proposal between the parties with respect thereto, and may be amended only with the consent of each party in advance in writing.

Article XI. Applicable Law

This subcontract shall be construed and performance hereinafter shall be determined according to the Laws of the State of Tennessee.

Article XII. Assignment

This subcontract shall inure to the benefit of and shall be binding upon the respective successors and assigns of the parties hereto, but may not be voluntarily assigned in whole or in part by either party without the prior written consent of the other.

Article XIII. Institute Held Harmless

Subcontractor shall hold Contractor harmless from and shall indemnify Contractor for any and all claims, demands, and actions upon or arising out of any services performed by Subcontractor under this Agreement and shall defend any and all actions brought against Contractor based upon any such claims or demands.

Article XIV. Applicability of Terms and Conditions

It is the intent of the parties that this Agreement shall not contain anything inconsistent with or contrary to the provisions that may be set forth in prime contract No. DE-AC05-84OR21492. Consequently, the parties hereto agree that the Subcontractor shall, upon request, negotiate with the Contractor in good faith upon such amendments to this Agreement as may be necessary to make this Agreement consistent with the requirements of the Prime Contract noted herein.

The provisions of the following attached articles and documents are hereby made a part of this contract:

- (a) Terms and Conditions FERD (8-82).
- (b) Terms and Conditions Change Sheet (3-84).
- (c) Exhibit 1C - Patent Rights (6-83).
- (d) Exhibit 20 - Rights in Technical Data (Short Form) (8-83).

XV. Close-out Procedures

Subcontractor will submit to Contractor, within 30 days of actual notification, all close-out documents required hereunder.

WHEREFORE, the parties have caused this agreement to be executed as set forth below.

CONTRACTOR

OAK RIDGE RESEARCH INSTITUTE

By: James A. R...

Title: Director

Date: June 20, 1985

SUBCONTRACTOR

TUSKEGEE INSTITUTE

By: J. A. Williams

Vice President for Business Affairs

Date: June 25, 1985

SSN No. 63-0288878

Statement of Work

The Oak Ridge Research Institute will provide bacterial strains to the sub-contractor. The sub-contractor will identify and characterize the strains and provide information on the base composition of their DNA.

The sub-contractor will provide information on the effects of certain environmental pollutants, especially mercury, cadmium, and halogenated hydrocarbons, on the differential rates of synthesis of DNA, RNA, and protein by bacterial strains provided by the Oak Ridge Research Institute. The sub-contractor will determine whether exposure to mercury or cadmium induces the synthesis of metal-binding proteins by these bacteria.

The sub-contractor will provide the Oak Ridge Research Institute semi-annual progress reports on these experiments. These reports will describe methods used and results obtained.

This Statement of Work will be subject to modification by mutual agreement between the sub-contractor and the Oak Ridge Research Institute.

SUBCONTRACT NO. ORRI-85-4

between

OAK RIDGE RESEARCH INSTITUTE

and

MEHARRY MEDICAL COLLEGE

This agreement entered into this 1st day of October, 1985 by and between the Oak Ridge Research Institute, with its principal offices in Oak Ridge, Tennessee (hereinafter referred to as "Contractor") and Meharry Medical College, having offices in Nashville, Tennessee (hereinafter referred to as "Subcontractor");

WITNESSETH THAT:

WHEREAS, the Contractor has heretofore entered into Contract No. DE-AC05-84OR21492 (hereinafter called the "Principal Contract") with the UNITED STATES OF AMERICA (hereinafter called the "Government") represented by the UNITED STATES DEPARTMENT OF ENERGY (hereinafter called "DOE"); and

WHEREAS, in furtherance of the Principal Contract, the parties hereto desire to provide for the Subcontractor's performing the research as hereinafter described; and

WHEREAS, the Subcontractor represents that it is ready, willing and able to undertake a portion of said efforts as hereinafter set forth;

NOW THEREFORE, in consideration of the premises, as well as the obligations made and undertaken, the parties hereto mutually agree as follows:

Article I. Statement of Work

Subcontractor shall perform the effort set forth in the Statement of Work attached hereto as Exhibit A. Said Exhibit A is incorporated herein by reference.

Article II. Period of Performance

The Agreement shall commence effective October 1, 1985. All work hereunder shall be completed on or before September 30, 1988.

Article III. Subcontract Type and Price

A. This is a firm fixed price type subcontract, issued pursuant to applicable Federal Acquisition Regulations. The total price for the services set forth in Article I hereof is One Hundred Fourteen Thousand (\$114,000.00) Dollars. Payment will be made in accordance with the Schedule attached hereto as Exhibit B, which is incorporated herein by reference. Costs billed in any contract year (November 1 to October 1) shall not exceed the amounts indicated.

B. Payment under this subcontract shall be made upon certification of invoices submitted, not more often than monthly, for percentage of work completed during the preceding month, to the Contractor's principal office, 113 Union Valley Road, Oak Ridge, Tennessee, ATTN: Dr. Nathaniel Revis.

Article IV. Inspection

The contractor, through its authorized representative, has the right at all reasonable times to inspect or otherwise evaluate the work performed or being performed by the Subcontractor.

Article V. Interest of Subcontractor

The Subcontractor covenants that he presently has no interest direct or indirect which would conflict in any manner or degree with the performances of his services hereunder. The Subcontractor further covenants that in the performance of this Agreement no such person having such interest shall be employed.

Article VI. Publicity

Subcontractor agrees that no advertising or publicity matter having or containing any reference to the Oak Ridge Research Institute or in which the name is mentioned, shall be made use of by the Subcontractor or anyone in the Subcontractor's behalf unless and until the same shall have first been submitted to and received the approval of the Oak Ridge Research Institute. The same shall apply to the Oak Ridge Research Institute or any of its divisions or units.

Article VII. Termination

The Contractor may, by written notice to the Subcontractor, terminate this Contract in whole or in part at any time, either for the Contractor's convenience or because of the failure of the Subcontractor to fulfill its contract obligations.

Article VIII. Changes

The Contractor may, from time to time, require changes in the scope of the services of the Subcontractor to be performed hereunder. Such changes shall be incorporated by written amendment into this agreement.

Article IX. Independent Contractor and Labor Requirements

A. The Subcontractor is furnishing its services hereunder as an independent contractor, and the Subcontractor shall not be considered an employee of the Contractor.

B. The Subcontractor shall be responsible for compliance with all requirements and obligations relating to such services under local, state or federal law including but not limited to minimum wage, social security, unemployment insurance, income tax and worker's compensation. The Subcontractor shall also be responsible for all licensing requirements of any local, state, or federal jurisdiction to which the performance of his services may be subject.

Article X. Entire Agreement

This subcontract constitutes the sole and entire Agreement between the parties with respect to the subject matter contained herein. This Subcontract supersedes any prior Agreement, offer, or proposal between the parties with respect thereto, and may be amended only with the consent of each party in advance in writing.

Article XI. Applicable Law

This subcontract shall be construed and performance hereinafter shall be determined according to the Laws of the State of Tennessee.

Article XII. Assignment

This subcontract shall inure to the benefit of and shall be binding upon the respective successors and assigns of the parties hereto, but may not be voluntarily assigned in whole or in part by either party without the prior written consent of the other.

Article XIII. Institute Held Harmless

Subcontractor shall hold Contractor harmless from and shall indemnify Contractor for any and all claims, demands, and actions upon or arising out of any services performed by Subcontractor under this Agreement and shall defend any and all actions brought against Contractor based upon any such claims or demands.

Article XIV. Applicability of Terms and Conditions

It is the intent of the parties that this Agreement shall not contain anything inconsistent with or contrary to the provisions that may be set forth in prime contract No. DE-AC05-84OR21492. Consequently, the parties hereto agree that the Subcontractor shall, upon request, negotiate with the Contractor in good faith upon such amendments to this Agreement as may be necessary to make this Agreement consistent with the requirements of the Prime Contract noted herein.

The provisions of the following attached articles and documents are hereby made a part of this contract:

- (a) Terms and Conditions FERD (8-82).
- (b) Terms and Conditions Change Sheet (3-84).
- (c) Exhibit 1C - Patent Rights (6-83).
- (d) Exhibit 20 - Rights in Technical Data (Short Form) (8-83).

XV. Close-out Procedures

Subcontractor will submit to Contractor, within 30 days of actual notification, all close-out documents required hereunder.

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XVI. Equipment

All equipment acquired by the Subcontractor under this subcontract shall vest in the Contractor. Upon completion of the Subcontract, the Contractor shall direct the Subcontractor on the disposition of the equipment.

WHEREFORE, the parties have caused this agreement to be executed as set forth below.

CONTRACTOR

SUBCONTRACTOR

OAK RIDGE RESEARCH INSTITUTE

MEHARRY MEDICAL COLLEGE

By: [Signature]

7P ^{AKC} By: [Signature]
David Satcher, M.D., Ph.D.

Title: DIRECTOR

Date: 10/30/85

Date: OCT. 1, 1985

SSN No. _____

APPENDIX A

STATEMENT OF WORK

OBJECTIVES

Resistance to mercury and organomercurials in microorganisms occurs by detoxification mechanisms. Detoxification is achieved by volatilization of mercury following activity of organomercurial lyase and/or mercuric reductase enzymes. In most bacteria thus far studied, these enzymes are inducible and encoded by plasmid genes. The goals of this study are: 1) to determine the mechanism(s) by which a community of bacteria isolated from Hg-contaminated soil may achieve resistance to Hg; 2) to determine whether a community of naturally selected resistant organisms may be manipulated to convert Hg to insoluble complexes and maintain these complexes as a potential decontamination mechanism; and 3) to determine whether standard, non-recombinant DNA genetic techniques may be used to increase the production of detoxifying enzymes to an extent useful in environmental decontamination. The initial objectives to achieve the first two stated goals are:

1. Assay sample isolates from the community of mercury-resistant (Hg^{r}) bacteria found at a toxic waste site at Oak Ridge, Tn., to measure organomercurial lyase and mercuric reductase enzyme activities. Bacterial isolates will be screened to confirm whether Hg^{r} is broad or narrow spectrum. Lyase and reductase enzyme levels will be measured by standard methods to correlate enzyme levels with the spectra of resistance.
2. Determine the level of Hg^{r} among sulfate-reducing bacteria not exposed to high levels of mercury, and compare to Hg^{r} of similar organisms isolated from the Oak Ridge site. The principal question is whether high production of H_2S causes precipitation of HgS or similar insoluble complexes sufficiently to protect the bacteria from mercury toxicity even in the absence of organomercurial lyase and mercuric reductase.
3. Measure the extent to which and the rates at which Hg^{r} soil organisms, e.g. *Thiobacillus* sp., can oxidize HgS complexes to form soluble mercuric salts and organomercurials. Significant activity of this type might counter the potential utility for production of insoluble mercury complexes as a decontamination mechanism. Oxidation of HgS will form the basis to test the influence of other metals, e.g. Fe^{2+} as part of a HgS complex, upon the rate of reoxidation of the insoluble salts of mercury.
4. Search for sequences homologous to the mer probe in the community of bacterial isolates from a mercury contamination site at Oak Ridge, Tn. The gram-negative bacterial community of isolates will be probed for the presence of Hg^{r} genes by the colony hybridization technique, or by probe hybridization to sheared DNA preparations from crude extracts. Mercury-resistant and mercury-sensitive strains will be screened.
5. Determine whether mercury-resistance is of plasmid or chromosomal origin in the Oak Ridge-site strains. Plasmids from strains with DNA which hybridizes with the mer probe will be isolated to detect whether the mer probe hybridizes with plasmid DNA, chromosomal DNA, or both. If the mer probe hybridizes to DNA of mercury-sensitive strains, it may reveal sequences used to evolve mercury-resistance.

SUBCONTRACT NO. ORRI-85-1

between

OAK RIDGE RESEARCH INSTITUTE

and

KENTUCKY STATE UNIVERSITY

This agreement into by and between the Oak Ridge Research Institute, with its principal offices in Oak Ridge, Tennessee (hereinafter referred to as "Contractor") and Kentucky State University, having offices in Frankfort, Kentucky (hereinafter referred to as "Subcontractor");

WITNESSETH THAT:

WHEREAS, the Contractor has therefore entered into Contract No. DE-AC05-84OR21492 (hereinafter called the "Principal Contract") with the UNITED STATES OF AMERICA (hereinafter called the "Government") represented by the UNITED STATES DEPARTMENT OF ENERGY (hereinafter called "DOE"); and

WHEREAS, in furtherance of the Principal Contract, the parties hereto desire to provide for the Subcontractor's performing the research as hereinafter described; and

WHEREAS, the Subcontractor represents that it is ready, willing and able to undertake a portion of said efforts as hereinafter set forth;

NOW THEREFORE, in consideration of the premises, as well as the obligations made and undertaken, the parties hereto mutually agree as follows:

Article I. Statement of Work

Subcontractor shall perform the effort set forth in the Statement of Work attached hereto as Exhibit A. Said Exhibit A is incorporated herein by reference.

Article II. Period of Performance

The Agreement shall commence effective June 1, 1985. All work hereunder shall be completed on or before September 30, 1988.

Article III. Subcontract Type and Price

A. This is a firm fixed price type subcontract, issued pursuant to applicable Federal Acquisition Regulations. The total price for the services set forth in Article I hereof is One Hundred Forty thousand and No/100 (\$140,000.00) Dollars U.S. Cost billed in any contract year is not to exceed \$35,000.

B. Payment under this subcontract shall be made upon certification of invoices submitted, not more often than monthly, for percentage of work completed during the preceding month, to the Contractor's Principal Office, 113 Union Valley Road, Oak Ridge, Tennessee, ATTN: Dr. Nathaniel Revis.

Article IV. Inspection

The contractor, through its authorized representative, has the right at all reasonable times to inspect or otherwise evaluate the work performed or being performed by the Subcontractor.

Article V. Interest of Subcontractor

The Subcontractor covenants that he presently has no interest direct or indirect which would conflict in any manner or degree with the performances of his services hereunder. The Subcontractor further covenants that in the performance of this Agreement no such person having such interest shall be employed.

Article VI. Publicity

Subcontractor agrees that no advertising or publicity matter having or containing any reference to the Oak Ridge Research Institute or in which the name is mentioned, shall be made use of by the Subcontractor or anyone in the Subcontractor's behalf unless and until the same shall have first been submitted to and received the approval of the Oak Ridge Research Institute. The same shall apply to the Oak Ridge Research Institute or any of its divisions or units.

Article VII. Termination

The Contractor may, by written notice to the Subcontractor, terminate this Contract in whole or in part at any time, either for the Contractor's convenience or because of the failure of the Subcontractor to fulfill its contract obligations. Upon receipt of such notice, the Subcontractor shall: (1) immediately discontinue all services affected (unless the notice directs summaries, computer software, and such other information and material as may have been prepared for and/or accumulated by the Subcontractor in performing this Agreement, whether completed or in process.

Article VIII. Changes

The Contractor may, from time to time, require changes in the scope of the services of the Subcontractor to be performed hereunder. Such changes shall be incorporated by written amendment to this agreement.

Article IX. Independent Contractor and Labor Requirements

A. The Subcontractor is furnishing his services hereunder as an independent contractor, and the Subcontractor shall not be considered an employee of Contractor.

B. The Subcontractor shall be responsible for compliance with all requirements and obligations relating to such services under local, state or federal law including but not limited to minimum wage, social security, unemployment insurance, income tax and worker's compensation. The Subcontractor shall also be responsible for all licensing requirements of any local, state, or federal jurisdiction to which the performance of his services may be subject.

Article X. Entire Agreement

This subcontract constitutes the sole and entire Agreement between the parties with respect to the subject matter contained herein. This Subcontract supersedes any prior Agreement, offer, or proposal between the parties with respect thereto, and may be amended only with the consent of each party in advance in writing.

Article XI. Applicable Law

This subcontract shall be construed and performance hereunder shall be determined according to the Laws of the State of Georgia.

Article XII. General Provisions

The attached clauses (Exhibit B) are hereby incorporated by reference and made binding upon Subcontractor with the same force and effect as if set forth in full text.

Article XIII. Assignment

This subcontract shall inure to the benefit of and shall be binding upon the respective successors and assigns of the parties hereto, but may not be voluntarily assigned in whole or in part by either party without the prior written consent of the other.

Article XIV. Institute Held Harmless

Subcontractor shall hold Contractor harmless from and shall indemnify Contractor for any and all claims, demands, and actions upon or arising out of any services performed by Subcontractor under this Agreement and shall defend any and all actions brought against Contractor based upon any such claims or demands.

Article XV. Applicability of Terms and Conditions

It is the intent of the parties that this Agreement shall not contain anything inconsistent with or contrary to the provisions that may be set forth in prime contract No. DE-AC05-84OR21492. Consequently, the parties hereto agree that the Subcontractor shall, upon request, negotiate with the Contractor in good faith upon such amendments to this Agreement as may be necessary to make this Agreement consistent with the requirements of the Prime Contract noted herein.

Article XVI. Close-out Procedures

Subcontractor will submit to Contractor, within 30 days of actual notification, all close-out documents required hereunder.

WHEREFORE, the parties have caused this agreement to be executed as set forth below.

CONTRACTOR

OAK RIDGE RESEARCH INSTITUTE

By: Michael R. Rame

Title: Director

Date: May 2, 1985

SUBCONTRACTOR

KENTUCKY STATE UNIVERSITY

By: R. A. M. Dune

Date: 5-7-85

SSN No. 402-72-3329

The provisions of the following attached articles and documents are hereby made a part of this contract:

- a) Terms and Conditions FERD (8-82).
- b) Terms and Conditions Change Sheet (3-84).
- c) Exhibit 1C - Patent Rights (6-83).
- d) Exhibit 20 - Rights in Technical Data (Short Form) (8-83).

EXHIBIT A

Statement of Work

The physiological diversity of bacteria results in their wide distribution throughout the various types of soil, with many different genera being present. Since soil contains aerobic and anaerobic microhabitats, both aerobes and anaerobes are present. In acid or alkaline soils with an unusually high mineral content, specifically adapted organisms will be present as the indigenous population. Therefore the microbial flora is strongly influenced by soil condition. Some of the more frequently isolated bacteria from the soil are *Arthrobacter*, *Azotobacter*, *Bacillus*, *Clostridium*, *Micrococcus*, *Nitrosomonas*, *Pseudomonas* and *Rhizobium*. The purpose of this proposal is to identify and characterize the microorganisms sent to us by the Oak Ridge Research Institute. The identification will be done using morphological, biochemical, physiological and microscopic studies.

Identification of pure culture isolates of these bacteria will be established by the determination of staining characteristics e.g. Gram, acid fast, etc., cellular and colonial characteristics on different media, oxidase reaction, fermentation or oxidation of carbohydrates, and optimal growth pH and temperature. After determining the above characteristics, the organisms will be tested further using selected biochemical methods and will be identified. There might be some organisms that will require additional tests for definitive speciation, such as biochemicals, flagellar staining, spore staining, and etc..

Subject: Subcontract No. ORRI-85-1

This modification(1) is issued to subject subcontract as follows:

Article III Subcontract Type and Price

Cost billed from the period June 1, 1985 thru September 30, 1985 shall not exceed \$35,000. Cost billed thereafter in any contract year (October 1 - September 30) shall not exceed \$35,000.

Summary

<u>Period</u>	<u>Total Billing Allowed</u>
6/1/85 - 9/30/85	\$ 35,000
10/1/85 - 9/30/86	35,000
10/1/86 - 9/30/87	35,000
10/1/87 - 9/30/88	35,000
Total Contract	<u>\$140,000</u>

CONTRACTOR

OAK RIDGE RESEARCH INSTITUTE

By: Ms. Ronald Hicks

Title: Controller

Date: May 9, 1985

SUBCONTRACTOR

KENTUCKY STATE UNIVERSITY

By: Roy M. Doe

Date: 5-17-85

SSN No. 402-71-3329

SUBCONTRACT NO. ORR1-85-3

between

OAK RIDGE RESEARCH INSTITUTE

and

KNOXVILLE COLLEGE

This agreement entered into this 1st day of October, 1985 by and between the Oak Ridge Research Institute, with its principal offices in Oak Ridge, Tennessee (hereinafter referred to as "Contractor") and Knoxville College, having offices in Knoxville, Tennessee (hereinafter referred to as "Sub-contractor");

WITNESSETH THAT:

WHEREAS, the Contractor has heretofore entered into Contract No. DE-AC05-84OR21492 (hereinafter called the "Principal Contract") with the UNITED STATES OF AMERICA (hereinafter called the "Government") represented by the UNITED STATES DEPARTMENT OF ENERGY (hereinafter called "DOE"); and

WHEREAS, in furtherance of the Principal Contract, the parties hereto desire to provide for the Subcontractor's performing the research as hereinafter described; and

WHEREAS, the Subcontractor represents that it is ready, willing and able to undertake a portion of said efforts as hereinafter set forth;

NOW THEREFORE, in consideration of the premises, as well as the obligations made and undertaken, the parties hereto mutually agree as follows:

SCHEDULE

ARTICLE I - STATEMENT OF WORK

The Subcontractor shall, to the best of its ability, furnish personnel, facilities, equipment, materials, supplies, and services necessary for the performance of the research provided for in APPENDIX "A", attached hereto and made a part hereof, and shall perform the research and shall report thereon pursuant to the provisions of this contract. Except as otherwise provided, the Subcontractor shall, when directed by the Contractor, and may, but only when authorized by the Contractor enter into sub-subcontracts for the performance of any part of the work under this article.

ARTICLE II - PERIOD OF PERFORMANCE

The period of performance under this subcontract shall commence on October 1, 1985 and shall expire on September 30, 1988, unless sooner terminated in accordance with the provisions of this subcontract pertaining to termination; provided, however, that this period may be extended for additional periods by written agreement of the parties.

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ARTICLE III - OBLIGATION OF FUNDS, ESTIMATE OF COST

A. Obligation of Funds. The amount currently obligated by the Contractor to this subcontract is \$61,000. Such amount may be increased unilaterally by the Contractor by written notice to the Subcontractor and may be increased or decreased by written agreement of the parties. Payments by the Contractor on account of allowable costs shall not in the aggregate exceed the amount obligated with respect to this contract.

B. Estimate of Cost. The total estimated cost of the work under this subcontract is Two Hundred Nine Thousand Eight Hundred Fifty-Four dollars (\$209,854). Fixed- Fee: None.

ARTICLE IV - REPORTS

The Subcontractor shall prepare and submit (postage prepaid) the Reports to the Contractor as delineated in the attached Appendix E. Preparation of the specified reports shall be in accordance with the "DOE Uniform Contractor Reporting System Guidelines", or as agreed to by the Contractor.

ARTICLE V - KEY PERSONNEL

The key personnel referred to in the article of Appendix B entitled "Key Personnel" are as follows:

Dr. Mary M. Brown

ARTICLE VI - NEGOTIATED OVERHEAD RATES

Pursuant to FAR Subpart 31.3 and OMB Circular A-21, Section G- Paragraph 4, Predetermined Fixed-rate for Indirect Costs, the following predetermined rate for on-campus shall apply for the period specified below:

October 1, 1985 to September 30, 1986 - 25% of modified total direct costs.

ARTICLE VII - TITLE TO EQUIPMENT

Title to equipment and property shall vest in accordance with the provisions of Appendix B (DOE Set 304) Contract Clauses- Paragraph 46. DEAR 952.24505 GOVERNMENT PROPERTY COST REIMBURSEMENT, TIME AND MATERIAL, OR LABOR HOUR CONTRACT) APR 1984.

ARTICLE VIII- TECHNICAL DIRECTION

The work to be performed by the Subcontractor under this subcontract is subject to the surveillance and written technical direction of Dr. Nathaniel Revis, and/or his designee.

ARTICLE IX - PAYMENT DUE DATES AND INVOICE REQUIREMENTS

A. Payments pursuant to the provisions of paragraph (c) of the allowable cost, and payment clause of this contract shall be due thirty (30) calendar days after receipt of a proper invoice or billing by the Subcontractor provided the amounts claimed are reimbursable in accordance with other provisions of that clause.

B. Upon submission by the Subcontractor of a "completion invoice" or "completion voucher" to the Contractor, payment of otherwise allowable costs determined to be reimbursable pursuant to the allowable cost, and payment provisions of this subcontract shall be thirty (30) calendar days after receipt of the completion invoice or voucher for payment.

C. Notwithstanding any other directions pursuant to paragraph (b) of the allowable cost and payment provision (DOE Set 304), Subcontractor invoices or public voucher shall include the Subcontractor's name and invoice date; subcontract number; cost amounts by elements of cost, claimed currently; cumulative amounts of costs by element of cost claimed from inception of the subcontract through the current period; total estimated subcontract cost amount not subject to contract specified withholding, as appropriate; other substantiating documentation or information required by the subcontract; and name (where practicable), title, phone number, office name and complete mailing address of responsible official to whom payment is to be sent.

ARTICLE X - CONTENTS OF CONTRACT

The parties executing this contract agree that effective with this subcontract, the contractual instrument in its entirety consists of the following which, if not heretofore, are hereby incorporated herein and made a part of this subcontract:

- a. This basic document entitled "Schedule",
- b. Appendix A - Statement of Work
- c. Appendix B - PART II - CONTRACT CLAUSES(DOE Set 304) AND PATENT SUPPLEMENT
- d. Appendix C - Alterations to Contract General Provisions
- e. Appendix D - Requirements Relating to Exchange of Information;
- f. Appendix E - Reporting Requirements; and
- g. Other provisions of the subcontract whether incorporated by reference or otherwise made a part of this subcontract.

ARTICLE X - ORDER OF PRECEDENCE

In the event of an inconsistency between the provisions of this subcontract,

SUBCONTRACT NO. OHR1-85-3

the inconsistency shall be resolved by giving precedence as follows:

- (a) SCHEDULE; (b) Appendix A; (c) Appendix B; (d) Appendix C; (e) Appendix D; (f) Appendix E; and (g) other provisions of this subcontract, whether incorporated by reference or otherwise.

It is understood that the words "Government/ DOE/ Contracting Officer/ and Technical Project Officer" as may be used in Appendixes B & D attached hereto, and as may otherwise be used elsewhere in this subcontract, shall mean to refer to the Contractor, ORRI, and/or Dr. Nathaniel Revis, whichever terminology is appropriate. In addition, the word "Contract" as may be used in the above appendixes shall mean to refer to "Subcontract".

WHEREFORE, the parties have caused this agreement to be executed as set forth below.

CONTRACTOR

OAK RIDGE RESEARCH INSTITUTE

By:
Dr. Nathaniel Revis
Director

Date: 9/27/85

SUBCONTRACTOR

KNOXVILLE COLLEGE

By:
Dr. Robert Shepherd
President

Dr. Mary M. Brown
Director of the Division of
Natural Sciences and
Mathematics

Date: September 18, 1985

Tax I.D.# 1-620475689-A1

APPENDIX A

STATEMENT OF WORK

The principal investigator will study the environmental impact of the introduction of waste-degrading microorganisms into simulated ecosystems. The effects monitored would be those on the survival of resident microflora, on energy flow and nutrient cycling, on the persistence of the introduced waste-degraders, and on genetic changes among the introduced and resident microflora. Controls would include both unstressed microcosms and those containing waste materials without introduced waste degraders. Long-term studies will include those concerned with the effects of environmental parameters on the reestablishment of self-sustaining conditions. The data generated should provide a source for predicting the effects of wastes and waste-degraders on natural ecosystems. The principal investigator will be assisted by students and a technician.