ANL/LRP-3

MICROBIAL ECOLOGY STUDIES AT TWO COAL MINE REFUSE SITES IN ILLINOIS

LAND RECLAMATION PROGRAM

ARGONNE NATIONAL LABORATORY

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MICROBIAL ECOLOGY STUDIES AT TWO COAL MINE REFUSE SITES IN ILLINOIS

by

R. Michael Miller and Roy E. Cameron

Land Reclamation Program

Prepared for the U. S. Department of Energy as part of the Argonne Land Reclamation Program, a joint effort of the Energy and Environmental Systems Division and the Division of Environmental Impact Studies.

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PREFACE

This study was performed as a part of the Argonne National Laboratory Land Reclamation Program, which is sponsored by the Department of Energy, Assistant Secretary for Environment and Safety, Division of Biomedical and Environmental Research. The Program is a joint effort conducted by Argonne's Energy and Environmental Systems Division and Environmental Impact Studies Division.

The Land Reclamation Program is addressing the need for coordinated applied and basic research into the physical and ecological problems of land reclamation related to the surface mining of coal and the development of cost-effective techniques for reclaiming/rehabilitating mined coal land to productive end uses. The program is conducting integrated research and development projects focused on near- and long-term reclamation problems in all major U.S. coal resource regions including Alaska, and is coordinating, evaluating, and disseminating the results of related studies conducted at other research institutions. The activities of the Land Reclamation Program involve close cooperation with industry and the academic community, and focus on establishing a comprehensive field and laboratory effort. The program has developed working arrangements with eight coal companies at nine research sites throughout the U.S.

Conducted by R. Michael Miller and Roy E. Cameron of the Environmental Impact Studies Division, this research project involved the investigation of microflora in two abandoned Illinois coal refuse piles. Data from this study will be valuable in determining the soil amendments and reclamation techniques necessary to reestablish a viable below-ground ecosystem in such disturbed areas.

Ralph P. Carter, Director
Land Reclamation Program

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by

R. Michael Miller and Roy E. Cameron

ABSTRACT

An investigation was made of the microflora associated with coal refuse at two abandoned mines in the midwestern United States. Information was gathered for both the edaphic and the biotic composition of the refuse material. Emphasis was placed on heterotrophic and autotrophic components as to numbers, kinds, and physiological groups. The presence of chemolithotrophs was also investigated. The relationship between abiotic and biotic components in regard to distribution of bacteria, fungi, and algae is discussed. Information presented in this report will be utilized in assessing trends and changes in microbial numbers and composition related to manipulations of the edaphic and biotic ecosystem components associated with reclamation of the refuse piles.

1 INTRODUCTION

Aquatic ecosystem impacts associated with acid mine drainage have been extensively documented (18). Coal refuse, resulting from various coal cleaning procedures, often contains enhanced concentrations of acid-forming pyrite. However, little attention has been given to studying the microflora at a coal mine refuse site (4), and the role of these organisms in the refuse before and after reclamation is poorly understood. This report will present "baseline" information on microbial abundance, kinds, and physiological groups, as well as on some of the physical and chemical factors controlling the distribution of these organisms.

* This paper was presented in part at the International Symposium on Microbial Ecology at Dunedin, New Zealand, August 22-26, 1977.

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2 ABANDONED COAL REFUSE

The disposal of coal refuse generated in the coal cleaning process has been a major problem in the United States and other countries for nearly 100 years. The problem is intensified by the current emphasis on increased coal production to meet growing energy demands. Refuse disposal sites are usually adjacent to a coal preparation plant and range in area from a few acres to several hundred acres (1 acre = 0.4047 ha). There are usually two distinct materials comprising mine refuse: (a) gob - consisting of coal fines, associated clays, sand, and associated minerals, as well as some large pieces of consolidated material separated from the coal during the primary cleaning process, and (b) slurry - consisting of fine to very fine particles of coal, clay, and other materials pumped from the coal cleaning plant in a slurry. The slurry, as it comes from the cleaning plant, is approximately 80-90% water. At many sites, an embankment or dike is constructed of gob to contain the slurry effluent until the water and solids separate by sedimentation. The water is then discharged when the impounded area is filled with water, and the slurry is allowed to dry. As the slurry accumulates, a high embankment is built thus enlarging the slurry pond.

Most coal refuse sites in Illinois are unsightly dark heaps with slopes ranging from gentle to very steep; the refuse is often a source of dust, and may contain smoldering material beneath the surface. Most sites also have an associated slurry holding pond. These sites are made even more unsightly when they are used as a dumping ground for miscellaneous trash.

Generally, the refuse sites have remained almost devoid of macrovegetation because of the adverse chemical and physical characteristics of the materials. Plant establishment is severely inhibited by a pH range of 2.0 to 5.0 (with the lower values being common), the presence of water-soluble toxic ions, and nutrient imbalances. The dark color of the refuse can result in solar heating of the surface to temperatures greater than 70°C. The slopes of the gob pile impede infiltration and penetration of precipitation, and promote erosion and particle transport by water and wind.

Acid-mine drainage problems are intensified in areas where pyritic shale overlies the coal seam, since much of this acid-forming mineral becomes deposited on the refuse site during the coal-cleaning process. The refuse areas then become prime sites for the establishment of iron- and sulfur-oxidizing organisms (*Thiobacillus* spp.) and their associated biogeochemical cycles. One of the major products of these cycles is sulfuric acid, which subsequently leaches into local groundwater and surface water, polluting the surrounding aquatic and terrestrial ecosystems.

The extent of acid formation at a particular refuse pile is directly related to the type of mining utilized at that site. Mining operations occurring during the early part of this century normally entailed hand-picking, and thus the cleaning, of coal below ground. Current mining practices, utilizing continuous mining methods, involve the cleaning of coal above ground. Therefore, much more pyrite is associated with current day refuse than with refuse materials from earlier mining practices.

This report presents detailed information on an investigation undertaken to study the microflora associated with orphaned coal refuse piles in Illinois. The purpose of this investigation was to monitor shifts in kinds and abundances of specific populations and physiological groups of soil microbes and relate these shifts to changes in soil properties brought about by reclamation practices, i.e., refuse/soil manipulations and amendments. Also being examined in this effort are the establishment and changes and/or adaptation of microbial groups that occurred because of changes in macroplant establishment and nutrient-availability relationships. Microbial groups were utilized as "indicators" of the suitability of the amended substrate to plant establishment on both a long- and short-term basis.

3 DESCRIPTION OF COAL REFUSE SITES

3.1 STAUNTON REFUSE RECLAMATION SITE

The Land Reclamation Program (LRP) at Argonne National Laboratory (ANL) has completed the reclamation of a coal refuse site near the city of Staunton in Macoupin County, Illinois, and is currently monitoring the long-term success of the project (37). The study site was carefully selected following a survey of abandoned deep mines in several Illinois counties. The ultimate land use for the site was determined, baseline environmental data were collected, engineering plans for the reclamation effort were developed and implemented, and post-construction monitoring and evaluation of reclamation effort has been implemented. The general goals of the overall project were: (a) to reduce or eliminate the pollutants entering the environment from the site; (b) to increase the usefulness and economic potential of the area; (c) to improve the aesthetic value of the area; and (d) to develop, demonstrate, and evaluate the most practicable methods and technologies for reclaiming refuse areas so that the greatest benefit is obtained at the lowest cost.

The Staunton site includes about 34 ac (14 ha), of which 23 ac (9.3 ha) required reclamation. It is located adjacent to Staunton, some 60 mi (100 km) southwest of Springfield (Fig. 1). The mine was open from 1904 to 1923. Prior to the recent reclamation efforts by LRP/ANL, waste material from the above-ground coal cleaning operation was very evident at the site in the form of a large gob pile. The pile covered approximately 4.5 ac (1.8 ha) and rose 80 ft (24 m) above the surrounding terrain (Fig. 2).

Natural drainage is to the north and into Cahokia Creek. At the onset of the study, acid drainage was prevalent at the site and the watercourse was filled with acidic materials from the gob pile. The slurry pond dam had been broken, resulting in erosion that created gullies as deep as 14.7 ft (4.5 m) near the dam at the north end of the slurry area (Fig. 3).

The land prior to mining had been used for row crop production. Vegetation on the site prior to reclamation consisted of volunteer herbaceous shrubs, grasses, and trees,

Fig. 1. Location of the Study Area

with cover concentrated and overlooking the west and southwest sides where it was not affected by acid runoff. Essentially, no vegetation was evident on the gob pile or on the fine slurry materials at the old pond site. The area adjacent to the gob pile on the east was being used for row crop production. Details of reclamation, including some baseline analyses of groundwater, surface water, and surface materials of the gob pile, slurry areas, and adjacent farm field have been presented previously (27,36,37). The gob pile had developed severe erosion gullies due to steep slopes of 1:1 or greater. Gob and slurry pH values were 2.2, runoff pH values were 2.6, water quality was poor, and there was sedimentation of slurry material in the tributaries of Cahokia Creek draining the site. Observations made at the site prior to reclamation indicated extreme environmental degradation from physical and chemical properties of the refuse material.

At present, recontouring of the gob pile has been completed, with all slopes reduced to 5:1 or less, and a retention pond has been constructed. Agricultural limestone and "Code L Alkali" (a mixture of calcium oxide and calcium carbonate) were applied to neutralize and stabilize the gob and slurry, and the entire site covered with 1 ft (0.3 m) of cover material (obtained from an on-site borrow pit). The cover material was then limed, fertilized, and seeded.

Fig. 2. The Staunton Gob Pile Before Reclamation

Fig. 3. Slurry Area at the Staunton Site

3.2 DESCRIPTION OF LITTLE DOG SITE

The second site chosen for this study is that of the Little Dog Mine. This site is an abandoned underground mine located on the north edge of the town of Gillespie, approximately 8 mi (5 km) north of Staunton. The mine opened in 1919 and was operated continuously by four successive independent coal companies until it was abandoned in 1968. The mine extended underground approximately 350 ft (105 m) to extract the Illinois No. 6 coal, which has an average seam thickness of 93 in (236 cm). The average sulfur content was 5 percent. The mine site occupies approximately 20 ac (8 ha), with half the site sloping toward a residential area of Gillespie. The site consists principally of undulating mounds of gob ranging in elevation from 10 to 40 ft (3 to 12 m). The northwestern corner contains a 2000 ft^2 (185 m^2) slurry area which is a remnant of the old slurry pond.

Row crop farmland adjoins the site on two sides. Off-site drainage has affected as much as 10,000 ft^2 (930 m^2) of crop land. Several furrows, drainage ditches, and dikes have been constructed to divert the flow of runoff from the site away from the adjoining cropland.

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4 MATERIALS AND METHODS

4.1 SOIL/REFUSE SAMPLING, HANDLING, AND STORAGE

At both the Staunton and Little Dog sites, samples were collected - using aseptic techniques - from surface materials and from specific depths within the refuse material. Care was taken to clean all collecting instruments before each sample was obtained, and the instruments were then sterilized with a propane torch. This sterilization procedure was used to reduce both chemical and microbial contamination. A portion of material from each sampling location was placed in a plastic "whirl-pak" bag. These aliquots were returned to the laboratory for subsequent microbial analysis. Aliquots obtained for chemical analysis were each placed in a plastic-lined canvas soil bag for return to the laboratory. Undisturbed cores of each sample were also taken with a soil tin sampler for subsequent determination of soil moisture, porosity, and bulk density.

After collection, samples were kept out of direct sunlight and returned to the laboratory as quickly as possible. At the laboratory, soils were kept at -10°C until analyses could be run. For most analytical purposes, each sample was homogenized by passing it through a sterile stainless steel $\leq$2 mm sieve (No. 10 mesh). For physical and chemical analyses, a portion of each sample was air-dried, and ground with a mortar and pestle when necessary.

4.2 PHYSICAL AND CHEMICAL ANALYSES

The chemical and physical analyses of gob, slurry, and field soils were conducted according to the procedures given in Table 1. Detection limits are also given.

4.3 MICROBIAL ANALYSIS

Soils to be analyzed for bacteria were prepared by the following procedures. To each vial containing 0.5 gram of air-dry soil, 9.5 mL of sterile water was added; the mixture was then shaken on a Vortex mixer. Appropriate serial dilutions were made from this mixture, and 0.5 mL aliquots were plated on prepoured trypticase soy agar (TSA) plates. Plates were incubated 48-72 hours at 25°C and colonies counted. The number of bacteria was expressed on a per gram dry weight basis for each soil sample analyzed.

Replicates, using velveteen replicators, were made on plates containing 5% and 10% NaCl in TSA; additional TSA plates were incubated at 45°C overnight and at 6°C for three weeks; MacConkeys agar plates were incubated at 37°C, and Desulfvibrio medium plates (American-type culture collection medium number 42) anaerobically incubated at 25°C for 3-5 days in BBL "gas-pac" apparatus. Direct plating of soil dilutions on TSA and subsequent incubation at 65°C were done to obtain obligate thermophilic bacteria. In cases where soil dilutions failed to yield colonies, the 10 mL soil suspension was plated directly to yield bacterial isolates. If none occurred, the number of isolates was recorded as zero.

Table 1. Chemical and Physical Analyses Conducted on Refuse and Field Soils

Parameter	Detection Limit	Analytical Procedure	Reference
Particle Size (%)	1.0	Hydrometer and sand sieves	7
Bulk Density (g/cm^3)	0.01	Volumetric	6
Porosity (%)	0.1	Volumetric	33
Saturation Point (%)	0.1	Gravimetric	26
Water Retaining Capacity (%)	0.1	Gravimetric	24
pH_s	0.1	Saturated paste	26
pH_1	0.1	1:1 dist. H_2O	28
Redox Potential (mv)	1.0	Saturated paste	25
Conductivity (mmhos/cm)	0.1	Saturated paste	26
Cation Exchange Capacity (meq/100g)	0.01	NH_4OAc	10
Exchangeable Cations Ca^{+2}, Mg^{+2}, Na^{+}, K^{+} (meq/100g)	0.01	1N NH_4OAc	28
Zinc, Copper (ppm)	0.01	0.1N HCl	28
Manganese (ppm)	0.01	0.1N H_3PO_4	28
Boron (ppm)	0.01	Hot dist. H_2O	16
Phosphorus (ppm)	0.1	Bray-1	20
Ammonium-Nitrogen (ppm)	0.1	Steam distillation	28
Nitrate-Nitrogen (ppm)	0.1	Steam distillation	28
Sulfate-Sulfur (ppm)	0.1	2N HOAc	28
Chloride (ppm)	0.1	Dist. H_2O	26
Loss on Ignition (%)	0.1	Gravimetric	28
Organic Carbon (%)	0.1	Wet oxidation-spontaneous heat	34
Organic Nitrogen (%)	0.1	Macro-kjeldahl	8

Colonies from all media used were transferred to TSA slants for stock cultures. These cultures were subsequently gram-stained, and appropriate biochemical reactions done to establish genera and species of all isolates. Counts were recorded from all plates following incubation to provide an index of the physiological response of originally isolated cells.

Numbers of bacteria, actinomycetes, and fungi were determined in pour plates utilizing Czapek-Dox agar in which 1 g of the soil or refuse sample was serially diluted. These plates were incubated at room temperature for one week prior to counts and isolation of fungal colonies.

A straw burial technique was utilized for colonization studies. Sterile alfalfa (*Medicago sativa*) straws were buried in each soil sample and removed at four and seven days. Upon removal each straw was washed thoroughly, clipped into one-inch (2.5 cm) pieces, and placed on carrot-dissection agar plates. Representative colonies were removed daily for further study.

Ten-g duplicates of soil and refuse samples were diluted serially to 10^{-7} and analyzed for their algal and protozoan composition. All soil and refuse samples were diluted and incubated in an inorganic nutrient solution in milk dilution bottles and dilution tubes. As determined from previous studies on many diverse samples of soil and other geologic materials (13), an inorganic basal salt medium derived from a formula used for the culture and enumeration of soil bacteria (2) has given excellent results. The formula for this medium, per liter of solution, is as follows: K_2HOP_4, 1.0 g; $MgSO_4$, $7H_2O$, 0.2 g; $CaCl_2$, 0.1 g; NaCl, 0.1 g; KNO_3, 0.5 g; $FeCl_3$, 0.002 g, and EDTA, trace. Incubations were conducted at approximately 28°C, under fluorescent tubes (Sylvania Gro-Lux) and usually at 200-250 ft-cd light intensity. All cultures were incubated for up to six months and examined periodically; many cultures were maintained under incubation for a year or more to determine if growth would occur. Date of first growth was noted and aliquots of all cultures were examined microscopically for determinations of species.

Gob and slurry samples were also analyzed for their sulfur-oxidizing capability. After sieving, each of the samples was divided into three 50-g (dry weight) portions. As a control, one of the portions was transferred directly to a one-pint bottle, and brought to a 50% water-retaining capacity (WRC), and capped with para-film. To the other two portions, 50 mg of sulfur was added prior to their being placed into pint bottles. All treatments were incubated at 28°C and at 50% WRC. All samples were analyzed for pH and sulfate at the end of 30 days by the method of Halversen and Bollen (17).

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5 RESULTS AND DISCUSSION

5.1 ABIOTIC PROPERTIES

Table 2 shows sampling site locations, dates of collections, and depth of sampling. Samples ST-2 and ST-3 were collected in an adjacent wheat field to obtain information on potential invaders from an existing and marginally disturbed row crop area. Vegetation was not evident on gob at the Staunton or Little Dog sites (Fig. 4). For the most part, the slurry areas were also devoid of vegetation; this was especially evident at the Little Dog Mine. At the Staunton site, an edge effect was evident along the slurry and gob area, with invaders being broom-sedge (*Andropogaon virginicus*), wild rose (*Rosa* sp.), and brambles (*Rubus* spp.) (Fig. 5). Lichens and mosses were also evident, especially at the treeline-slurry interface along the northeast edge of the slurry area. The trees were mainly blackjack oak (*Quercus marilandica*) and willow oak (*Q. phellos*).

Physical properties of the samples are given in Table 3. Most of the sampled materials were dark in color, and were various shades of black to gray if collected from gob and slurry areas. Some showed mottling with sulfur and pyritic materials. Soils were pale brown. Soil colors were due primarily to the mineral composition, and not to the presence of nonfossil organic materials. The darker-colored soils are disadvantageous for certain higher plants, as well as microorganisms, because of their tendency to heat under solar insolation and to favor rapid evaporation and moisture loss.

Bulk density values for the refuse samples are generally indicative of fine textured materials with high total porosity and very little compaction. The two field soils, which were silt loam, had the highest bulk densities. In general, the bulk density of the refuse material increased with depth, and total porosity decreased accordingly. Low porosity values indicated a tendency toward anaerobic conditions for both ST-2 and ST-3, as well as for some of the slurry samples.

Field moisture values were not too different from the saturation percentage, and lower than values obtained for the water holding capacity. This suggests that normal precipitation for the area should provide adequate moisture regimes, however, low oxygen tension (poor aeration conditions) should be expected.

Fig. 4. No Evidence of Vegetation Was Seen on Gob Piles at Either Site

Table 4 lists the physicochemical properties of the samples. All but three had saturated pH values of < 3.0. One slurry sample was at pH 1.80, and the gob sample from Little Dog Mine was 1.30. All of the pH values indicate a substrate that is restrictive for most organisms.

Eh values were also determined because they are valuable in interpreting the milieu in which various physiological groups of microorganisms can grow or adapt. The Eh values obtained on all the samples in this study are indicative of oxidized and aerated materials. Wide Eh variations in soils can be caused by climate, influxes and outfluxes of the ecosystem, and the activities of microorganisms (22). For materials collected for this study, it would appear that the most important factors determining Eh potential are aeration and oxygen content, oxidized condition of minerals (and salts) and the resultant low pH values, and variations in soil structure.

It is useful to plot the pH-Eh conditions for various materials to indicate biotic limits of the system (3). Milieu relevant to our study are shown in Fig. 6. A similar plot for our samples, Fig. 7, indicates that they are all outside the range of normal or undisturbed soils, but some fall within the boundary of oxidized mine water. The two Little Dog samples lie at the upper limit of the acid-oxidized range of the pH-Eh milieu, thereby indicating the very restrictive nature of these materials for biotic activity. The rH values, calculated from pH and Eh data, are also indicative of oxidized soils (Table 4). Values of rH 20 or lower, not encountered in our samples, would indicate a tendency toward a reducing system (19). The rH values for drier, oxidized soils are around 30 to 35, and approximately 25 to 30 for desert clays (12). Higher values are reported for those of normal and inundated arable soils (25). The rH milieu of our samples would not be favorable for anaerobic processes (19).

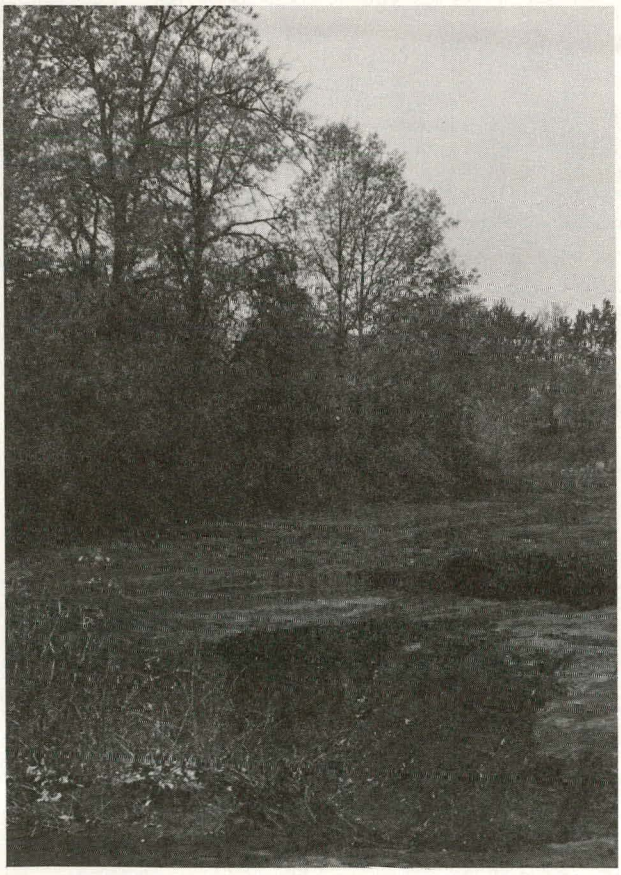

Fig. 5. Edge-Effect Vegetation at Staunton

Electrical conductivity values encountered for gob samples indicate potential problems for those organisms not able to adapt to the osmotic stresses exerted by this matrix (Table 4). Except for the Little Dog gob sample, electrical conductivity values indicate that salt concentrations should not be detrimental to most organisms. However, only salt-tolerant organisms should be active in the LD-1 sample. Slurry samples comparatively had much lower electrical conductivity; only samples ST-5 and LD-2 appear to be osmotically detrimental. Calcium and, secondarily,

Table 2. Coal Refuse Sampling Sites

Sample No.[a]	Date	Location[b]	Associated Vegetation
ST-1	12-9-75	Surface sample of gob from S. side of refuse pile.	None
ST-2	12-9-75	Surface sample of soil collected directly E. of refuse pile in wheat field.	Wheat
ST-3	12-9-75	Sample obtained directly below ST-2 at 6 in. (plow depth) in field (soybean stubble evident).	Wheat
ST-4	12-9-75	Surface sample of slurry obtained from site N. of refuse pile on E. side of drainage and 100 ft N. of new county road.	None
ST-5	12-9-75	Sample of slurry taken directly below ST-4 at 7-8 in. level.	None
ST-6	3-8-76	Surface sample (0-1 in.) of gob from N. central side of refuse pile, 33 ft from old county road.	None
ST-7	3-8-76	Gob sample taken directly below ST-6 at 8 in. level.	None
ST-8	3-8-76	Slurry sample taken at surface of slurry-treeline interface at N. corner of site.	Mosses and lichen on slurry; treeline composed of *Quercus* spp.
ST-9	4-23-76	Slurry sample taken at 2 in. depth at N.E. corner of slurry area.	Brambles
ST-10	4-23-76	Sample taken at 4 in. depth directly below ST-9 sample.	Brambles
ST-11	4-23-76	Sample taken at 6 in. depth directly below ST-10 sample.	Brambles
LD-1	10-12-76	Gob sample taken on surface, S. edge of gob pile.	None
LD-2	10-12-76	Slurry sample taken from surface, W. side of slurry area.	None

[a] ST = Staunton Site
LD = Little Dog Site

[b] One in. = 2.54 cm; 1 ft = 0.3048 m.

Table 3. Physical Properties of Coal Mine Refuse and Adjacent Field Sites

Sample No.	Depth (in)[a]	Munsell Color (dry)	Soil Color[b]	Texture[c]	Porosity (percent)	Bulk Density (g/cm^3)	Field H_2O (percent)	Saturation (percent)	Water Retaining Capacity (mL/100 g)
Soil									
ST-2	1	10YR 6/3	pale brown	silt loam	51	1.29	32.7	29.0	47.7
ST-3	1	2.5YR 6/2	light brownish gray	silt loam	46	1.42	28.6	35.1	41.4
Gob									
ST-1	1	5Y 5/1	gray	sandy loam	74	0.69	20.6	33.8	54.1
ST-6	1	10YR 5/1	gray	sandy loam	68	0.84	32.8	38.0	65.8
ST-7	8	10YR 4/1	dark gray	sandy loam	61	1.04	41.3	46.9	101.8
LD-1	1	5Y 5/1	gray	sand	70	0.80	28.1	35.0	69.8
Slurry									
ST-4	1	N 31	very dark gray	sandy loam	71	0.76	46.4	39.8	66.9
ST-5	8	5Y 2.5/1	black	loam	73	0.71	64.6	69.3	119.6
ST-8	1	10YR 2/1	black	sandy loam	75	0.67	70.1	64.4	98.1
ST-9	2	5YR 2.5/1	black	silt loam	75	0.66	49.5	61.2	111.3
ST-10	4	5YR 2.5/1	black	loam	72	0.75	64.0	72.1	107.4
ST-11	6	N 2.1	black	loam	73	0.71	58.2	66.8	38.2
LD-2	1	5Y 2.5/1	black	loam	72	0.73	32.7	54.9	37.0

[a]One inch = 2.54 cm.

[b]Munsell Soil Charts, 1973 edition.

[c]USDA Textural Triangle.

Table 4. Physicochemical Properties of Coal Mine Refuse and Adjacent Field Sites

Sample No.	pH_s	Eh + mv Saturated Paste	rH[a]	Electrical Conductivity (mmhos/cm @ 25°C)	Osmotic Pressure[b] (atm)
Soil					
ST-2	3.95	566	27.4	0.28	0.10
ST-3	3.95	611	29.0	0.40	0.14
Gob					
ST-1	1.80	716	28.3	3.61	1.30
ST-6	2.60	656	28.9	1.09	0.40
ST-7	2.30	706	28.9	1.55	0.56
LD-1	1.30	741	28.2	9.35	3.37
Slurry					
ST-4	2.00	611	27.8	1.06	0.38
ST-5	1.80	701	37.8	6.07	2.19
ST-8	3.40	651	29.2	0.18	0.07
ST-9	2.90	766	32.2	0.40	0.15
ST-10	2.65	661	28.1	0.76	0.27
ST-11	2.50	701	29.2	0.79	0.29
LD-2	2.05	696	28.1	3.55	1.28

[a] $rH = (Eh/0.029) + 2\ pH$.

[b] Osmotic Pressure = $0.360 \times EC \times 10^3$ at 25°C.

magnesium, were probably the contributing cations responsible for the high conductivity values, whereas sulfate appears to be the contributing anion (Tables 5 and 6). In the LD-1 sample, sodium is the contributing cation. Detrimental levels appear to be only prevelant in gob samples. The majority of ions appear not be be adsorbed, as shown by the discrepancy between CEC values and exchangeable base levels encountered for the gob and slurry samples.

The cations on the exchange complex of the samples were dominated by the monovalent and divalent ions listed in Table 5. A high CEC can be expected for soils high in organic matter; clays contribute almost all the CEC in sandy soils, but because clay concentration is low, the CEC of these soils are low. Weathered shale, slate, and coal have a higher CEC because the

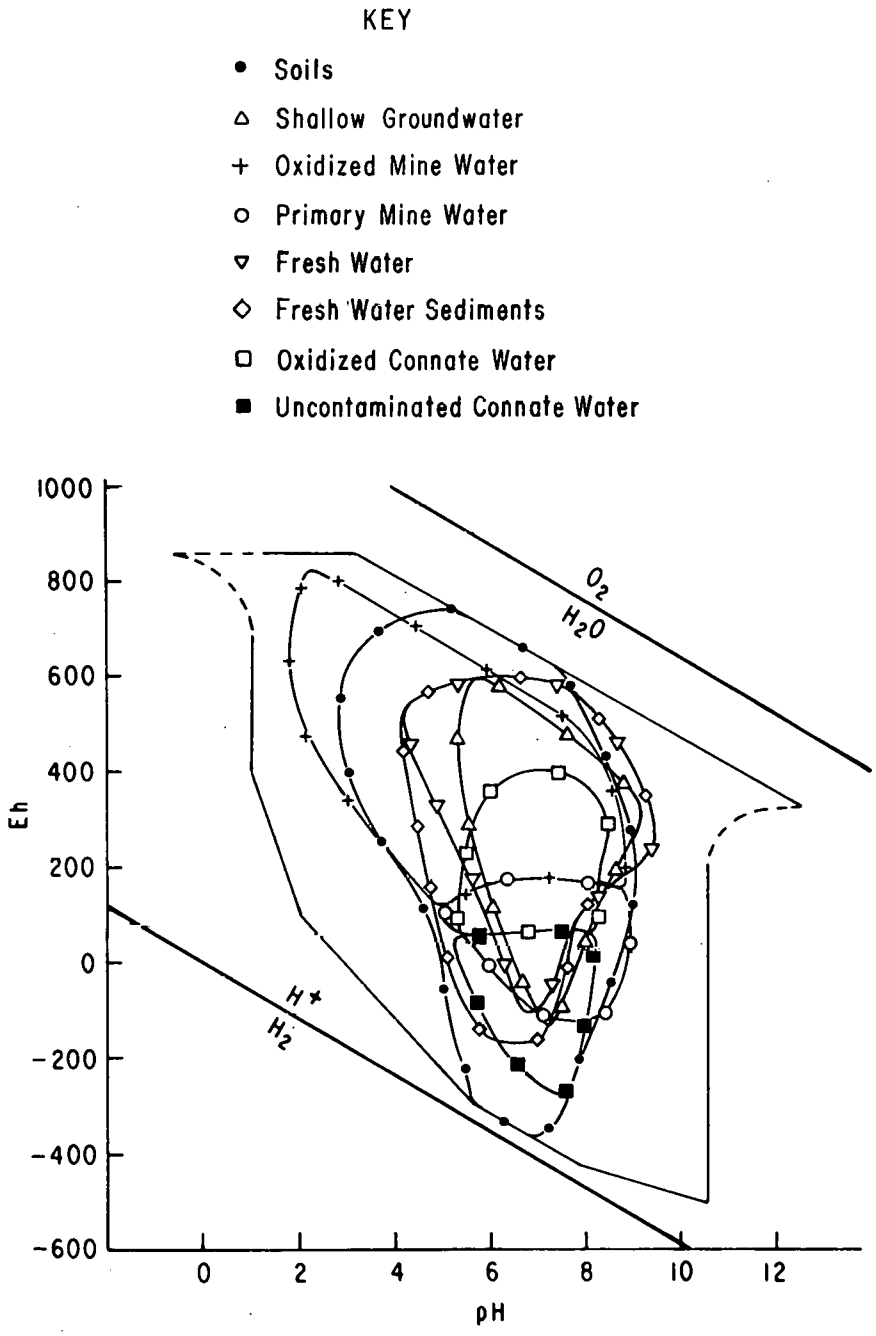

Fig. 6. Approximate "Areas" of Eh and pH for Some Natural Environments. (From L. G. M. Baas Becking, et al., Limits of the Natural Environment in Terms of pH and Oxidation-Reduction Potentials, J. Geol. 68:243-284, 1960. Reproduced by permission)

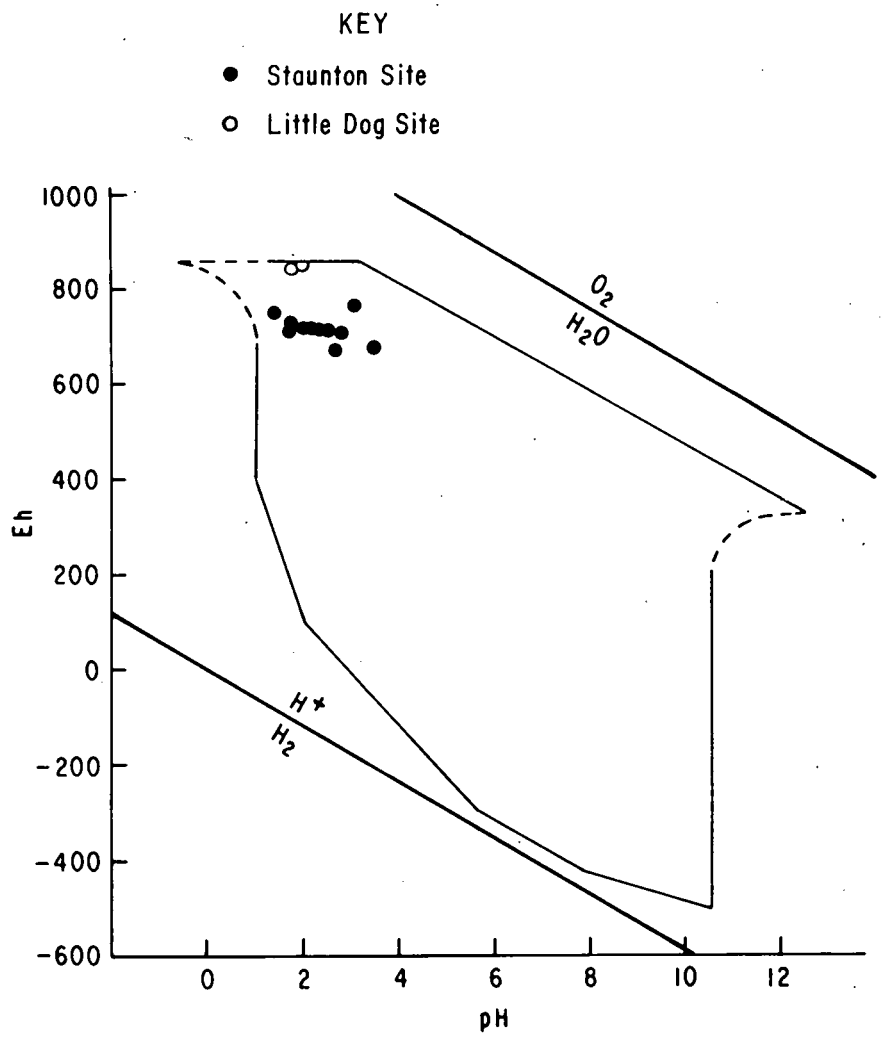

Fig. 7. "Areas" of Eh and pH for the Refuse Study Sites

Table 5. Chemical Concentrations and Related Data for Some Exchangeable Cations in Coal Mine Refuse and Field Soils

Sample No.	Na^+	Ca^{++}	Mg^{++}	K^+	Sum of Exchangeable Bases (meq/100 g)	pH_1	Cation Exchange Capacity (meq/100 g)	Clay (Percent)
	(meq/100 g)							
Soil								
ST-2	1.7	10.6	5.4	5.0	22.7	4.0	8.2	14
ST-3	2.0	8.5	2.8	2.9	16.2	4.0	12.8	12
Gob								
ST-1	2.4	197.0	23.8	2.1	225.3	2.2	31.1	9
ST-6	1.2	302.9	11.8	1.5	317.4	2.0	27.3	13
ST-7	4.3	579.3	35.1	1.5	620.2	1.8	25.7	0
LD-1	96.7	130.9	121.9	4.7	354.2	1.7	11.8	2
Slurry								
ST-4	1.3	61.5	3.1	1.4	67.3	2.5	40.6	6
ST-5	1.2	31.8	5.0	2.4	40.4	2.0	27.4	18
ST-8	0.4	57.4	2.2	3.9	61.5	2.4	32.2	9
ST-9	0.4	5.8	5.8	1.4	13.4	3.1	17.4	21
ST-10	0.5	4.1	2.2	1.7	8.5	2.8	17.5	7
ST-11	0.2	2.4	1.2	1.8	5.6	2.7	24.1	9
LD-2	5.4	70.1	15.7	1.8	93.0	1.9	30.5	14

Table 6. Available Ion Concentrations in Coal Refuse and Adjacent Field Soils (ppm)

Sample No.	Mn^{+2}	Zn^{+2}	Cu^{+2}	BO_3^{-3}	SO_4^{-2}	Cl^-	PO_4^{-3}
Soil							
ST-2	47	9	2	2	284	35	46
ST-3	4	7	3	2	188	28	27
Gob							
ST-1	5	80	3	32	3,553	26	78
ST-6	5	7	2	9	479	12	39
ST-7	2	5	2	6	564	8	29
LD-1	9	31	5	13	13,881	74	31
Slurry							
ST-4	4	8	2	14	3,337	18	95
ST-5	8	98	5	57	3,487	38	377
ST-8	27	10	3	4	849	12	164
ST-9	14	14	7	8	263	5	165
ST-10	1	16	8	9	283	8	273
ST-11	1	5	5	10	296	12	139
LD-2	10	14	6	11	3,715	54	119

sand-sized particles are actually stable aggregates of clay-like substances (31). This would certainly seem to be applicable to our samples. Only ST-2, the field surface soil, had a relatively low CEC value. If ion exchange is considered to be one of the more important soil processes (25), then CEC is not a limiting factor in the samples obtained from both coal mine sites.

Concentrations of ions must be considered from the viewpoint of their solubility, and whether they are available and exchangeable, all of which is pH-dependent. Except for LD-1, none of the samples could be considered detrimental in terms of Na^+, which is a commonly restrictive ion in many temperate soils. Levels of Ca^{+2}, Mg^{+2}, and K^+ also should not be restrictive. Concentrations of PO_4^{-3}, NH_4^+, and NO_3^- appear adequate for plant and microbial growth, although concentrations of 20 ppm NO_3^- or higher would be more desirable (21). The higher levels of SO_4^{-2} encountered in some of the samples are associated with pyritic coals and are not particularly beneficial to the microorganisms in refuse areas with appreciable water available. The concentrations of Mn^{+2}, Zn^{+2}, Cu^{+2}, and BO_3^{-3} are also not conducive to the most favorable conditions for growth of both microorganisms and macrophytes at the concentrations reported here.

Loss on ignition is quite high for all but the two wheat field samples, as shown in Table 7. The high ignition losses are attributed to the combustibles in the gob and slurry samples. High ignition losses for ST-4 and ST-11 can be attributed entirely to coal fines. These values are supported by the high organic carbon percentages which were also obtained for the samples. From the viewpoint of the soil organisms, unfortunately, the organic matter present is primarily of fossil origin and therefore is not available as a nutrient source. Graphic carbon may be oxidized by microorganisms in certain soils (29), but it may be necessary to sulfomethylate or sulfonate coal before it can be used by these organisms (11). Ammoniated coal does not appear to undergo any chemical change by soil microorganisms (5).

It also should be noted that the C:N ratios, except for samples ST-1 and ST-2, are wider than is desired for plant growth. An organic C:N ratio <10:1 is "narrow." The C:N ratio for microorganisms is usually between 4:1 and 9:1. A critical ratio of organic C:N between 20:1 and 25:1 would indicate that nitrogen mineralization would occur, and wider ratios (as displayed by most of the refuse samples) would favor almost complete immobilization of the available (leachable) nitrogen as microbial protoplasm (30). Nitrogen must therefore be added as an amendment to the substrates for macroplant growth.

Table 7. Carbon and Nitrogen in Coal Mine Refuse and Adjacent Field Site Soils

Sample No.	Loss on Ignition %	Organic Carbon %	Organic Nitrogen %	NH_4-N ppm	NO_3-N ppm	C:N
Soil						
ST-2	7.6	2.03	0.13	43	11	16.1
ST-3	8.0	2.21	0.15	11	9	14.8
Gob						
ST-1	47.6	11.07	0.48	53	8	22.3
ST-6	39.2	13.10	0.40	49	11	32.8
ST-7	37.2	12.10	0.35	26	5	34.6
LD-1	36.4	6.40	0.34	20	14	18.4
Slurry						
ST-4	77.8	15.07	0.88	46	7	17.2
ST-5	66.2	13.94	0.50	59	5	28.1
ST-8	70.7	24.50	0.54	39	5	45.4
ST-9	59.1	19.68	0.50	21	15	33.5
ST-10	65.9	22.41	0.44	35	6	38.1
ST-11	80.5	28.63	0.60	38	8	48.7
LD-2	70.6	28.02	0.60	41	4	46.1

5.2 MICROORGANISMS

Numbers of bacteria, actinomycetes, and fungi for gob, slurry, and field samples are shown in Table 8. All samples, under the designated conditions of incubation, culture, and isolation methods, showed the presence of microorganisms. This does not imply, however, that the isolates were indigenous and growing in the aliquot collected from a given site; quite possibly some of the microorganisms were transients, temporarily deposited in the area by wind, water, or animals. As expected, the two wheat-field samples contained by far the largest abundance of bacteria, 1.3 to 4.0 x 10^6/g dry wt.; low to moderate numbers (0.3 to 300 x 10^3/g dry wt.) of fungi were isolated from all the samples. These microorganisms would therefore be considered ubiquitous to both refuse sites. However, algae were almost as ubiquitous (Table 9). Due to the low pH, gob samples ST-1 and LD-1 were essentially negative for any significant numbers of heterotrophic bacteria, as were slurry samples ST-4, ST-5, and LD-2, and gob sample ST-7. For the algae, ST-5 was unfavorable for growth, and gob sample ST-6 may have had a very few unhealthy, misshapen, and unidentifiable coccoid green algae.

Table 8. Numbers (1000/g dry wt) and Percentage of Microorganisms in Refuse and Adjacent Field Soils

Sample No.	Total Microbes	Bacteria	Actino-mycetes	Fungi	% Bact.	% Act.	% Fungi
Gob							
ST-1	20.0	0	--	20	0	0	100
ST-6	103.6	3.6	--	100	3	0	97
ST-7	300.4	0.4	--	300	<1	0	100
LD-1	0.3	0	--	0.3	0	0	100
Slurry							
ST-4	96	0	--	96	0	0	100
ST-5	31.2	0.2	--	31	<1	0	99
ST-8	232	100	2	130	43	1	56
ST-9	224	14	10	200	6	5	89
ST-10	112	22	--	90	20	0	80
ST-11	95.1	94	--	1.1	99	0	1
LD-2	100.3	0.3	--	100	<1	0	100
Field Soils							
ST-2	4600	4040	160	160	88	6	6
ST-3	1380	1300	10	70	94	1	5

Table 9. Abundance of Algae and Protozoa in Coal Mine Refuse and Adjacent Sites

Sample No.	Sample Location	Positives at Highest Dilution	
		Algae	Protozoa
ST-1	Gob	10^4	0
ST-2	Field	10^4	10^4
ST-3	Field	10^3	10^3
ST-4	Slurry	10	0
ST-5	Slurry	0	0
ST-6	Gob	10(?)	0
ST-7	Gob	10	0
ST-8	Slurry	10^3	10^2
ST-9	Slurry	10^4	10
ST-10	Slurry	10^5	0
ST-11	Slurry	10^4	0
LD-1	Gob	10	0
LD-2	Slurry	10	0

Abundance of algae and protozoa in the samples was quite low, despite the lengthy incubation period. Considering the low pH levels and other unfavorable substrate characteristics, it is worth noting that the algae and some protozoa did grow. It should be noted that the algae grew in the slurry samples with lengthy incubation periods -- up to one year. Protozoa occurred in four of the eleven algal cultures. For the two wheat field samples, protozoa were as abundant as the algae. In slurry and gob samples, protozoa concentrations were commonly one to two logarithm units less than the algal numbers.

The percentage of bacterial types (physiological groups) cultured from some of the samples from the field and slurry samples are shown in Table 10. As shown, the wheat-field bacteria had the capability to grow in a 5% added NaCl, but only the bacteria from slurry sample ST-10 had this capability. Of the samples examined, ST-9, 10, and 11 had the lowest concentration of Na^+. The percentage of osmophils was nil or low, as shown by essentially negative growth obtained with 10% NaCl added to the culture medium.

A high number of thermotolerant isolates has been encountered for the slurry samples as indicated by the ability of the isolates to grow at 45°C. This trend was especially evident for subsurface slurry samples ST-9 and ST-11. It was significant that all of the wheat-field isolates from surface sample ST-2 could grow at 6°C, but subsurface sample ST-3, taken directly below ST-2, did not yield any isolates capable of growth at 6°C.

Table 10. Percent Distribution of Bacterial Types from Field and Slurry Samples

Sample No.	Count g/dry wt	5% NaCl	10% NaCl	6°C	45°C	Mac-Conkey	Facultative Anaerobes
Field							
ST-2	4,040,000	100	0	100	48	100	-[a]
ST-3	1,300,000	34	2	0	15	0	-[a]
Slurry							
ST-9	2,800	0	0	33	66	0	66
ST-10	20,000	100	0	0	20	0	100
ST-11	94,000	0	0	0	77	0	31

[a] Not done.

Sample ST-2 also was the only sample capable of yielding isolates that could grow on MacConkeys agar. Some additional tests were performed for facultative anaerobes and for growth at 55°C. Two of the slurry samples yielded thermophiles capable of growing at 55°C, and all three contained a high percentage of facultative anaerobes, these being of the genus *Bacillus*.

Species of heterotrophic bacteria isolated from the samples are given in Table 11. *Bacillus* spp., usually found in soils, were the most common, although there does not appear to be any particular species that is more prevalent over the others. Spore formation appears to be of benefit to survival under the adverse conditions encountered in these samples. It is also obvious that cultivated fields yielded not only a greater abundance of microorganisms, but also a greater diversity. Wheat field samples ST-2 and ST-3, respectively, yielded six and seven identifiable bacterial species. For the algae and protozoa, this trend was even more prevalent where sample ST-2 yielded 13 identifiable algae and six protozoa (Tables 12 and 14). For ST-3, there were seven species of algae and nine protozoans identified.

At the pH levels encountered for gob and slurry samples, the predominance of fungi is not unexpected. Though most fungi prefer pH levels higher than 4, it is not unusual to encounter activity of fungi at pH 3 and below (14,15). Fungi were isolated from all of the samples studied, with isolates of *Penicillium* spp. being the most frequently encountered (Table 13). *P. janthinellium* and *Aspergillus fumagatus* isolates were encountered from five of the samples, including both of the wheat-field samples and the vegetated slurry-area samples. An unidentified black yeast-like form was isolated from seven of the samples; it appeared to be Geotrichum-like in sample LD-1, since it showed mycelial fragmentation. When cultured, the greatest amount of growth occurred at pH 3-4, with abrupt cessation of growth at pH 5. Growth in the culture solution occurred down to pH 1.0. *Geotrichum* has previously been reported to have an optimum pH for growth

Table 11. Heterotrophic Bacterial Isolates from Field and Refuse Samples

Isolates	Samples
Bacillus coagulans	ST-9
Bacillus firmis	ST-2, ST-10, ST-8
Bacillus laterosporus	ST-10
Bacillus licheniformis	ST-2, ST-3
Bacillus megaterium	ST-2, ST-3
Bacillus stereothermophilus	ST-10
Bacillus subtilis	ST-3, ST-9, ST-10, ST-11
Brevibacterium linins	ST-3, ST-8, ST-9
Citrobacter frueuendi	ST-2
Flavobacterium breve	ST-2
Flavobacterium sp.	ST-9
Micrococcus sp.	ST-8
Pseudomonas alcaligenes	ST-3
Pseudomonas fluorescens	ST-2
Pseudomonas stutzeri	ST-3
Pseudomonas Group 3	ST-3

of 3.0 (14). The greatest diversity in species occurred for samples obtained in the vegetated slurry area, where samples ST-8 and ST-9 each yielded ten identifiable isolates. Samples ST-10 and ST-11 each had seven identifiable isolates.

Cooke, in his study of geo-fungi occurring in acid mine drainage, found a similar distribution for fungal species isolated from water impacted with acid mine runoff (15). In that study a predominance of fungal forms similar to those occurring in our study was evident. Many of these same fungi are ubiquitous, being able to adapt to a wide variety of habitats. As long as an appropriate substrate is available, a fungus capable of utilizing that substrate will also be present.

Since fungi are heterotrophic, their population levels and activity should correspond to the availability of a colonizable substrate as well as the mileu in which that substrate occurs. The high percentage of *Penicillium* spp. isolates is highly indicative of a disturbed matrix. Within gob and slurry, the mileu is quite harsh. This was especially evident for samples LD-1, ST-1, and ST-5, where low population levels were encountered for fungi. These low numbers were probably due to the high levels of soluble

Table 12. Algae Identified in Refuse and Field Samples

Species	Samples
Agmenellum quadruplicatum	ST-7
Anacystis marina	ST-2, ST-9, ST-10
Chlamydomonas globosa	ST-2
Chlamydomonas sp.	ST-3
Chlorella vulgaris	ST-2, LD-1, LD-2
Chlorococcum minutum	ST-2, ST-3, ST-8, ST-9, ST-10
Chlorogonium elongatum	ST-3
Chlosterium intermedium	ST-2, ST-3
Coccochloris peniocystis	ST-2
Cyanidium caldarium	ST-4, LD-2
Gloeocystis gigas	ST-2, ST-3, ST-7
Navicula sp.	ST-2
Neochloris aquatica	ST-7, ST-8
Schizothrix arenaria	ST-2
Schizothrix calcicola	ST-2, ST-8
Stichococcus bacillaris	ST-2, ST-8, ST-9, ST-10, ST-11
Stichococcus subtilis	ST-2, ST-3, ST-9, ST-10, ST-11
Surirella ovalis	ST-2
Tetracystis sp.	ST-3

salts present in these samples. The electrical conductivity values encountered, especially for the gob samples, indicate an osmotic regime detrimental to most fungi (32). Also, the levels of zinc encountered for these samples indicate potential problems for fungal growth.

Of the algae identified in the cultures, green algae - both coccoid and filamentous species - were predominant. *Chlorococcum minutum, Stichococcus subtilus* (usually few-celled and/or coccoid) and *S. bacillaris* were the most commonly observed. These organisms were encountered frequently in both soil and refuse material. Blue-green algae, including the common small-diameter *Anacystis marina* and *Schizothrix calcicola,* were also observed. This is unusual considering the acidic nature of the samples (9). *Agmenellum quadruplicatum,* a planktonic blue-green alga, was observed in ST-7. A single isolate, appearing similar to Allen's "blue-green *Chlorella,*" now known to be *Cyanidium caldarium* and a member of the Rhodophyta (9), was observed in cultures of LD-2 and ST-4, at a pH value of 2.0. As reported by Allen (1), her isolate, which was obtained from a hot spring containing 0.1 N H_2SO_4, would

Table 13. Fungi Isolated and Identified From Refuse and Field Samples

Species	Samples
Ascomycete sp.	ST-8
Aspergillus fumigatus	ST-2, ST-3, ST-9, ST-10, ST-11
Black myceloid species	ST-9, ST-10, ST-11
Black yeast	ST-1, ST-2, ST-4, ST-5, ST-6, ST-11, LD-2
Candida sp.	ST-4
Cephalosporium sp.	ST-4
Chaetomium fusiforme	ST-3
Chaetomium gangligerum	ST-3
Chaetomium murorum	ST-8, ST-9, ST-10, ST-11
Fusarium sp.	ST-2, ST-3, ST-5
Geotrichum sp.	ST-5, LD-1
Gliocladium virens	ST-2, ST-3
Isarium sp.	LD-2
Metarrhizium brunneum	ST-2, ST-3
Mucor sp.	ST-3
Penicillium bacillosporum	ST-4, ST-5
Penicillium citrinum	ST-8
Penicillium cyclopium	ST-9
Penicillium decumbens	ST-10
Penicillium fellutanum	ST-8
Penicillium frequentans	ST-9, ST-10, ST-11
Penicillium implicatum	ST-1, ST-5
Penicillium janthinellum	ST-6, ST-7, ST-8, ST-9, ST-10
Penicillium levitum	ST-2, ST-3
Penicillium luteum	ST-3, ST-9
Penicillium purpurogenum	ST-2, ST-3, ST-4, ST-5
Penicillium raistrickii	ST-9, ST-11
Penicillium rugulosum	ST-2, ST-8
Penicillium thomii	ST-4
Penicillium viridicatum	ST-8
Penicillium sp. 1	ST-3
Penicillium sp. 2	ST-8
Penicillium sp. 3	ST-10
Penicillium sp. 4	ST-10
Phyllostica sp.	ST-8
Rhizopus sp.	ST-2, ST-9
Trichoderma koningi	ST-2
Trichoderma viride	ST-2, ST-3, ST-8, ST-9
Trichoderma sp.	ST-10, ST-11
Yellow brown myceloid species	ST-9
Zygomycete species	ST-3, ST-9

Table 14. Protozoans, Rotifers, Aquatic Fungi Imperfecti, and Phycomycetes Identified in Refuse and Field Samples

Organisms	Samples
Protozoa	
Acanthamoeba polyphaga	ST-8
Amoeba limnicola	ST-2
Amoeba gorgonia	ST-8
Amoeba spumosa	ST-3
Bodo edax	ST-3
Bodo minimus	ST-2, ST-3, ST-9
Bresslaua vorax	ST-8
Cercomastrix parva	ST-3, ST-8
Chlorogonium elongatum	ST-3
Monas elongata	ST-2, ST-3, ST-8
Monas socialis	ST-2, ST-3, ST-9
Nebela militaris	ST-2
Phyllomitus amylophagus	ST-3
Spiromonas angusta	ST-2
Vahlkamptia limax	ST-8
Rotifers	
Cephalodella gibba	ST-3
Eosphora anthadis	ST-2
Squatinella mutica	ST-2
Aquatic Fungi Imperfecti	
Anguillospora longissima	ST-7
Anguillospora sp.	ST-3
Piricularia sp.	ST-3
Tricladium caudatum	ST-7
Varicosporium elodeae	ST-9
Aquatic Phycomycetes	
Apodachlya sp.	ST-3
Rhizidium sp.	ST-3

grow in 1 N H_2SO_4. This species may be a "first-invader," capable of reproducing in the acidic and toxic conditions of refuse areas. Under the culture conditions utilized in our study, this organism was reproducing at a pH of 1.93 and an Eh of +834. This oxidation potential is much higher than previously reported for algae and may be approaching the limit for life as defined by these parameters (3).

Protozoa were observed only as they occurred in algal samples (Tables 9 and 14); they may have been more numerous, and more species may have been evident if special measures had been taken to culture them. Flagellates were the most frequently observed. These included the small diameter *Bodo minimus, Monas elongata,* and *M. socialis.* All three species occurred in wheat-field samples. The largest abundance of protozoa also occurred in these samples, but ST-8, a sample containing moss and lichen crusts at the tree-line of *Quercus* sp., also contained a relatively large abundance of protozoa. The wheat-field samples, ST-2 and ST-3, contained an additional diversity of nonprotozoan species, including a community of rotifers, aquatic hyphomycetes, and aquatic phycomycetes. Normally, aquatic hyphomycetes are not associated with soils.

Attempts were not made to quantitate the iron- and sulfur-oxidizing bacteria (*Thiobacillus* spp.) in gob and slurry. However, a bioassay for sulfur-oxidizing capacity was undertaken for the refuse samples. Several trends were evident from the data collected. Activity seems to be governed by the depth at which the sample was collected; also of importance is the age or length of time at which the sample has been exposed to the surface environment. In Table 15, a lowering of activity was evident for samples as the depth of acquisition increased; this was evident for both slurry and gob. Since the Thiobacilli are strict aerobes, this finding is not surprising. The time of exposure to the surface environment for refuse is also important because for samples collected from older mining operations, sulfur-oxidizing capacity decreased as age of the refuse increases. This explains the very high levels of activity for Little Dog samples (8 yrs) and lower activity levels for Staunton samples (55 yrs). The 444% sulfur oxidation for Little Dog gob is explained by the high amounts of pyrite within the gob, which along with the sulfur is also being oxidized. Also, the lower oxidation values encountered for Little Dog slurry samples is due to the presence of little or no pyrite within the samples, since most pyrite remains in the gob during the cleaning process.

These trends support the findings of Belly and Brock (4), who found a correlation between $^{14}CO_2$ uptake and most probable numbers (MPN) of iron-oxidizing bacteria, but not with acid-tolerant heterotrophes encountered in refuse. They found maximal incorporation of $^{14}CO_2$ in two-to-three year old coal refuse, with little incorporation in fresh or older material (40 years). They also found the maximum uptake was from samples acquired from the surface level down to depths of 4 in (10 cm).

The problems of toxicants and nutrient imbalance may remain problems of concern, despite ameliorative measures, if untreated refuse below the amended layer is exposed or leached with time. Response to these toxicants will depend upon (a) the nature of the toxicant, (b) concentration, (c) exposure time, (d) environmental characteristics of the receiving stream, (e) age and condition of the exposed organisms, and (f) the presence of other toxicants (9).

Table 15. Sulfur-Oxidizing Capacity of Refuse Material

Site	Depth[a] (in)	Control pH[b]	Treatment pH[c]	% S Oxidized
Little Dog				
Gob	1	2.1	2.0	444.0
Slurry	1	2.4	2.4	56.3
Staunton				
Gob	1	2.9	2.6	45.7
	8	2.8	2.6	26.9
Slurry	2	3.3	2.8	40.4
	4	2.9	2.6	25.8
	6	2.9	2.7	10.1

[a]One in. = 2.54 cm.

[b]Refuse pH without addition of sulfur.

[c]pH after 30 days incubation with sulfur.

Finally, the role of microbial populations, responses, and interactions in acid mine wastes must be put into larger perspective. Acid mine drainage amounts to over 4 million tons per year of acid from active and abandoned mines (23). Microorganisms appear to be significantly responsible for this problem, but they also can play a beneficial and primary role in the amelioration or alleviation of this detrimental effect. As abandoned mines are reclaimed and returned to productive uses, reestablishment of the necessary biogeochemical cycles will contribute to a healthy below-ground and above-ground ecosystem.

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6 CONCLUSIONS

Baseline microbial and ecological studies of samples obtained from two abandoned coal mine refuse sites in Illinois indicate that the nature of refuse materials is unfavorable for survival and growth of soil organisms. Despite the pioneer populations established by some microorganisms, especially acidophilic fungi and some acid-tolerant algae, the refuse materials should be amended or ameliorated to raise the pH and provide needed nutrients (especially nitrogen). Biodegradable organic matter should also be added for its physical and biological effects. Nature has the capacity to ameliorate the refuse material, but at its own pace, however; although 60 years have passed at the Staunton site since the last coal was mined, little invasion has occurred from the periphery. The invasion that was evident had occurred on the protected northern exposures along the treeline-slurry interface where mosses, lichens, and a diverse microflora had gained a foothold. With an increase in the number of established organisms, both macrophytes and cryptogams, an increase in the flow of nutrients into the system will take place. A buildup of organic matter and a concomitant increase in cation exchange capacity and buffering capacity will follow, thus giving way to different seres.

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The Land Reclamation Program is addressing the need for coordinated applied and basic research into the physical and ecological problems of land reclamation, and advancing the development of cost-effective techniques for reclaiming land mined for coal. This program is conducting integrated research and development projects focused on near- and long-term reclamation problems in all major U.S. coal resource regions, and is evaluating and disseminating the results of related studies conducted at other research institutions. These activities involve close cooperation with the mining industry. Regional and site-specific reclamation problems are being addressed at research demonstration sites throughout the country, and through laboratory and greenhouse experiments.

Principal Investigators for *Microbial Ecology Studies at Two Coal Mine Refuse Sites in Illinois:* R. Michael Miller and Roy E. Cameron, Environmental Impact Studies Division.

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