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Environmental Effects of Western Coal Surface Mining

Part IV—Chemical and Microbiological Investigations of a Surface Coal Mine Settling Pond

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ENVIRONMENTAL EFFECTS OF WESTERN COAL SURFACE MINING PART IV - CHEMICAL AND MICROBIOLOGICAL INVESTIGATIONS OF A SURFACE COAL MINE SETTLING POND

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FOREWORD

This report is one of a series that characterizes the impact of surface mining coal in the western U.S. In this study chemical-microbial changes in the settling pond receiving mine runoff are described. Since the form of compounds do change substantially as a result of physical conditions and microbial activity, such information is useful in design of such settling ponds.

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ABSTRACT

Chemical and microbiological investigations of the settling pond system at the West Decker Coal Mine in southeastern Montana were undertaken during an 18-month period from November 1975 to April 1977. West Decker is one of the largest surface coal mines in the United States. Extensive chemical analysis of pond water samples during a portion of this period revealed that coal mining in the Decker area may be impacting groundwater quality since the pond water differed chemically from surrounding groundwaters. Concentrations of total dissolved solids, bicarbonate, sodium, sulfate, and nitrogen species in pond water were elevated in comparison to those in other nearby surface waters; however, it was concluded that these would not significantly impact the Tongue River or Tongue River Reservoir. Constituents of the mine drainage waters underwent chemical changes within the pond which were at least in part due to the activities of microorganisms. The increase in pH value and in concentration of dissolved oxygen, partially attributed to photosynthesis within the pond, oxidized reduced chemical species such as sulfide and ferrous iron. The high numbers and activity measurements of sulfate reducing bacteria as well as the significant amount of metal-bound sulfides in pond sediments made a strong case for the contributions of these organisms to metal precipitation in, and possible detoxification of, pond waters. Acidophilic iron and sulfur bacteria, responsible for the production of acid mine drainage, were consistently detected within the pond system and other locations at Decker. However, no acidic conditions were observed, most likely because relatively high concentrations of mineral carbonate in the overburden neutralized acid formed from pyritic materials present.

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SECTION I

INTRODUCTION

Approximately one-half of the nation's known coal reserves are located in a six-state sector of the West which includes Colorado, Montana, North Dakota, South Dakota, Utah, and Wyoming. This geographical area is designated by geologists as the Northern Great Plains and Rocky Mountain Coal Provinces and contains coal deposits ranking from lignite to bituminous (U.S. Environmental Protection Agency 1976). Major scale efforts to remove these resources by surface extraction methods are ongoing and proposed.

Both the quality of the coal and the effects of land disturbance caused by surface coal mining differ in the western reserves compared to those in the East and Midwest. Coal from the Northern Great Plains has a lower sulfur content (by weight) than eastern and midwestern coal, averaging 0.6 percent and ranging from 0.1 to 4.0 percent. In these low sulfur coals, the relative percentage of organic sulfur tends to be higher; thus, there is a comparatively lower amount of inorganic sulfur such as that found in the minerals pyrite and marcasite. The potential for acid production via pyrite dissolution by subsurface waters exists in both the East and West. Because of the lower pyritic content of overburden materials and coal and the high buffering capacity of surface waters in coal-rich regions of the West, acid mine drainage is not widespread. In addition, rubblized overburden is not subjected to high annual precipitation in the semi-arid western states. Lower potentials for water-initiated erosion may also exist; however the sparseness of vegetation decreases the stabilization of western soils so that sedimentation of watersheds in mining-impacted areas may prove problematic (U.S. Environmental Protection Agency 1976). The soluble salts content of overburden materials in western strip mines such as those in Colorado (McWhorter et al. 1975) may have the most significant pollution impact on water quality.

Although information on the quality of coal and overburden, and the quantity of precipitation, has permitted speculation on the nature of environmental perturbations from surface coal mining in the West, detailed studies are needed to assess their magnitude. Specifically, a paucity of information exists on the quality of waters originating from surface mined areas (Gilley et al. 1976).

In November 1975 investigations were initiated on water quality alterations associated with activities at the West Decker Mine, a sizeable open pit mine in the Fort Union Formation of the Northern Great Plains Coal Province. Specifically, an evaluation of the role of a coal mine sedimentation pond in determining the chemical composition of mine discharge waters

was made so that recommendations for improvements in pond design may be applied to the operation of future mines.

The Decker Mine, operated by Decker Coal Company, a subsidiary of Peter Kiewit Sons' Company of Sheridan, Wyoming, and Pacific Power and Light Company of Portland, Oregon, is located in southeastern Montana immediately adjacent to the Tongue River Reservoir (Figure 1). The area of the actively mined pit (effective July 1977) was 193 hectares. Mining operations are currently expanding to include areas to the north and east of the present mine site (Figure 2). When expansion is complete, Decker Coal Company will be the largest subbituminous coal producer in the world. The present mine at Decker discharges an effluent into the Tongue River flood plain at the upper end of the reservoir. The two additional proposed mines will also discharge mine waters directly or indirectly into the reservoir.

The primary function of the settling or sedimentation pond at the West Decker Mine is to reduce the turbidity of the mine drainage (Decker Coal Company personnel, personal communication). However, with the anticipated intensification of coal mining in the vicinity of the Tongue River Reservoir, additional functions such as nutrient transformations, heavy metal accumulation, and general water quality alterations should be studied.

The present investigation was integrated with a separate study on the limnology of the Tongue River Reservoir (Garrison et al. 1975; Whalen et al. 1976; Whalen and Leathe 1976; Gregory 1977) as well as other research projects. The settling pond effluent is projected to be used as a source of water to fill and maintain a proposed northern pike spawning marsh and waterfowl nesting site (Gregory 1976). It is desirable that the quality of the effluent be acceptable for this purpose as well as for irrigation of Decker's reclamation sites, if needed in times of drought.

The function of the settling pond in altering the quality of mine discharge water was examined by three approaches. First, water samples from the pond were analyzed for a wide spectrum of chemicals in an attempt to determine how the pond water differed from groundwater and surface water of the same region. Second, the algal productivity of the pond was estimated. Third, methods for enumeration and indices of biological activity were used to study the function of selected microbial populations in the water column and surface sediment of the settling pond. Through these approaches, the role of the pond in the retention of chemicals from coal and overburden and the roles of microbial populations in the transformation of chemical substances were elucidated.

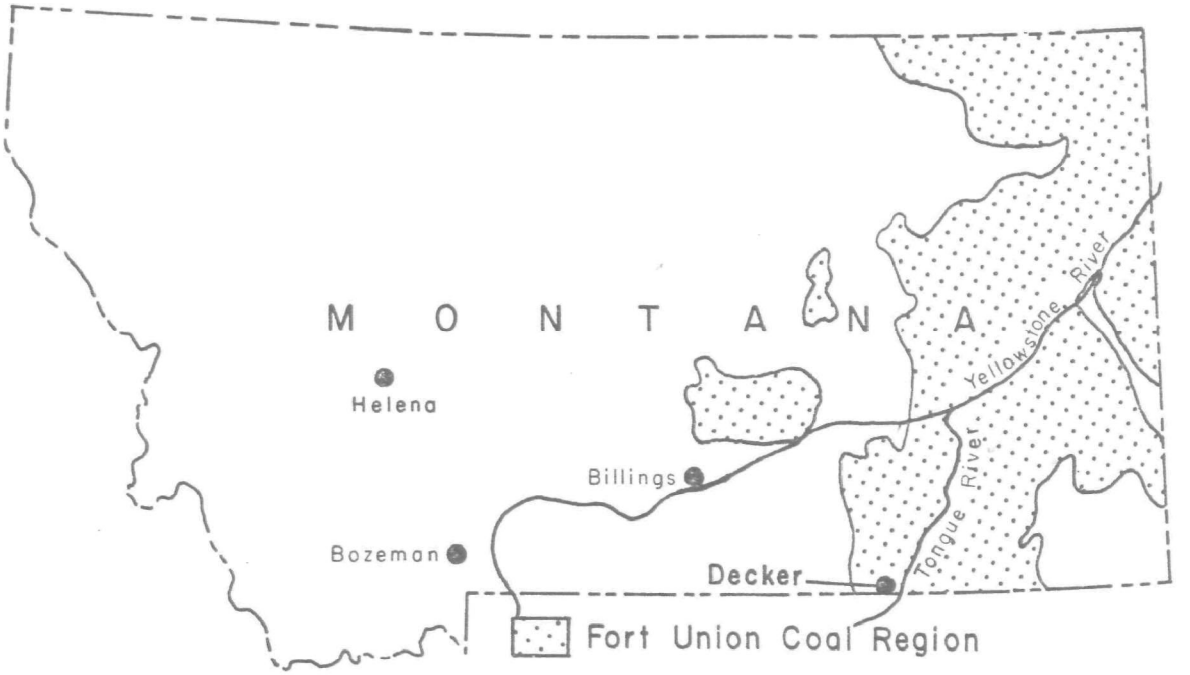


Figure 1. Map of Montana showing location of study area.

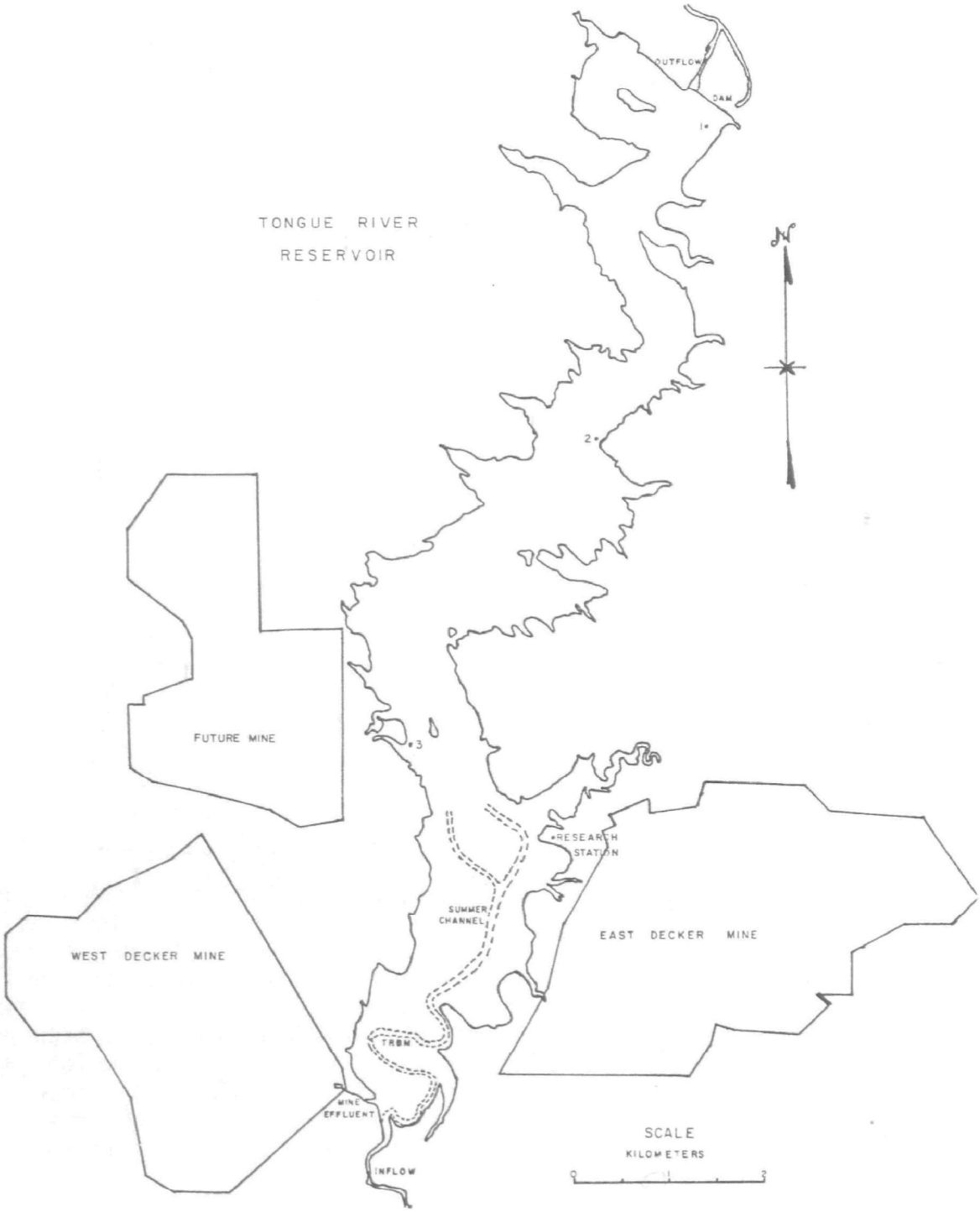


Figure 2. Map of the Tongue River Reservoir showing the locations of the present and future mines operated by Decker Coal Company.

SECTION II

CONCLUSIONS

1. The settling pond effluent from the West Decker Mine discharges into the Tongue River flood plain. The quality of this discharge water was sufficient to meet Montana state standards for coal mine effluents. Although the evaluation of microbial and chemical processes within the settling pond was hampered by its irregular operation, the pond altered the quality of inflowing mine drainage water with respect to a limited number of chemical parameters.

2. Although acidophilic iron and sulfur oxidizing bacteria, which contribute to the formation of acid mine drainage in the East and Midwest, were consistently detected in the settling pond and at other locations near and within the West Decker Mine, acid mine water conditions were not observed. The activities of these bacteria may be restricted to microzones in the overburden or coal in which acid production is neutralized by the presence of carbonate and bicarbonate minerals.

3. The presence of considerable activity of sulfate-reducing bacteria along with significant amounts of metal-bound sulfides in the settling pond sediments make a strong case for the contribution by these organisms to the precipitation of heavy metals and possible detoxification of the pond water. However, average concentrations of mercury in pond samples collected on nine occasions during this study ranged from 0.09 to 0.81 μg/liter; these concentrations are higher than 0.05 μg/liter, the maximum recommended for freshwater aquatic life and wildlife by the U.S. Environmental Protection Agency (1976b). A laboratory bioassay test alga, inoculated into pond water, did not show growth inhibition characteristic of that caused by heavy metals.

4. Maximum algal productivity within the mine pond, even though moderate, improved the quality of the pond water and therefore improved the effluent discharge from the pond onto the Tongue River flood plain, primarily by contributing to the oxidation of sulfide, a chemical toxic in very low concentrations.

5. Laboratory algal bioassays, testing water collected from the settling pond, indicated that the pond was nutrient limited and phosphorus was most frequently the limiting factor. The pond was periodically enriched with nitrogen, apparently by water seepages from an area used for storage of ammonium nitrate explosives located directly adjacent to the pond. In water samples taken after or during nitrogen enrichment, growth of the bioassay alga was never nitrogen limited.

SECTION III

RECOMMENDATIONS

The principal recommendations are listed below; these are discussed in Section VII.

1. A consideration of Hill's (1976) discussion of design parameters such as "(1) capacity and size to meet water quality criteria, (2) storage volume, (3) number of ponds, (4) location of pond, (5) shape of pond, (6) inlet design, (7) outlet design, (8) cleanout procedures, and (9) close down" would be helpful in improvement of the present operation at West Decker or in the initial design of sedimentation basins for Decker Coal Company's future mines. The physical aspects of sedimentation pond design should be strongly interfaced with a consideration of biological activity within the pond system.

2. A two-pond system may be more efficient for improving the quality of coal mine drainage waters. A deep primary pond could serve two purposes, one of which would be to facilitate sedimentation since it would be less subject to wind-driven mixing. Zones of anaerobiosis could develop in deeper waters and promote bacterial sulfate reduction which would remove heavy metals and other elements from solution. Design considerations should be such that adequate contact between metals and bacterially-generated sulfide is assured. A shallow secondary pond would permit the biological uptake of nutrients such as those contributed from nitrogen explosives and carbonaceous materials in the coal.

3. Concentrations of methylmercury should be monitored in coal mine sedimentation ponds expecially when pond water is to be utilized directly as a source for fish culture, or when pond effluent may present a source of mercury pollution to receiving waters outside the mine. Forms of this element are readily accumulated and retained in biological systems.

SECTION IV

DESCRIPTION OF THE STUDY AREA

The settling pond system at the West Decker Mine (Figure 3) is located within T9S, R40E, S16. Mining activities have interrupted the normal flow of groundwater toward the Tongue River at the Decker Mine site. The most important aquifers to be intercepted by mining are the coal seams (Van Voast and Hedges 1975). As a result, groundwater flows into the mine pit and is pumped out so that it will not interfere with mining operations. This "altered" groundwater from a section of the mine was collected in a sump from which it was pumped into the settling pond. In addition, the settling pond received runoff from a relatively small drainage area around it. The water level in the settling pond was controlled by a standpipe at its southern end. Overflow water, referred to as the mine effluent, was discharged into the Tongue River flood plain.

At the time of this study, the settling pond was approximately one meter in depth with an area of 1.2 hectares. Retention time was approximately 6.2 days. From July 1975 to July 1976 the outflow averaged 1.5×10^6 liter per day (Decker Mine personnel, personal communication). During the course of this study, water moved through the settling pond system on an interrupted basis. The pond underwent changes in morphometry as well. These deviations from initial conditions, summarized in Table 1, undoubtedly influenced the chemical and biological parameters and are considered in interpretation of the data.

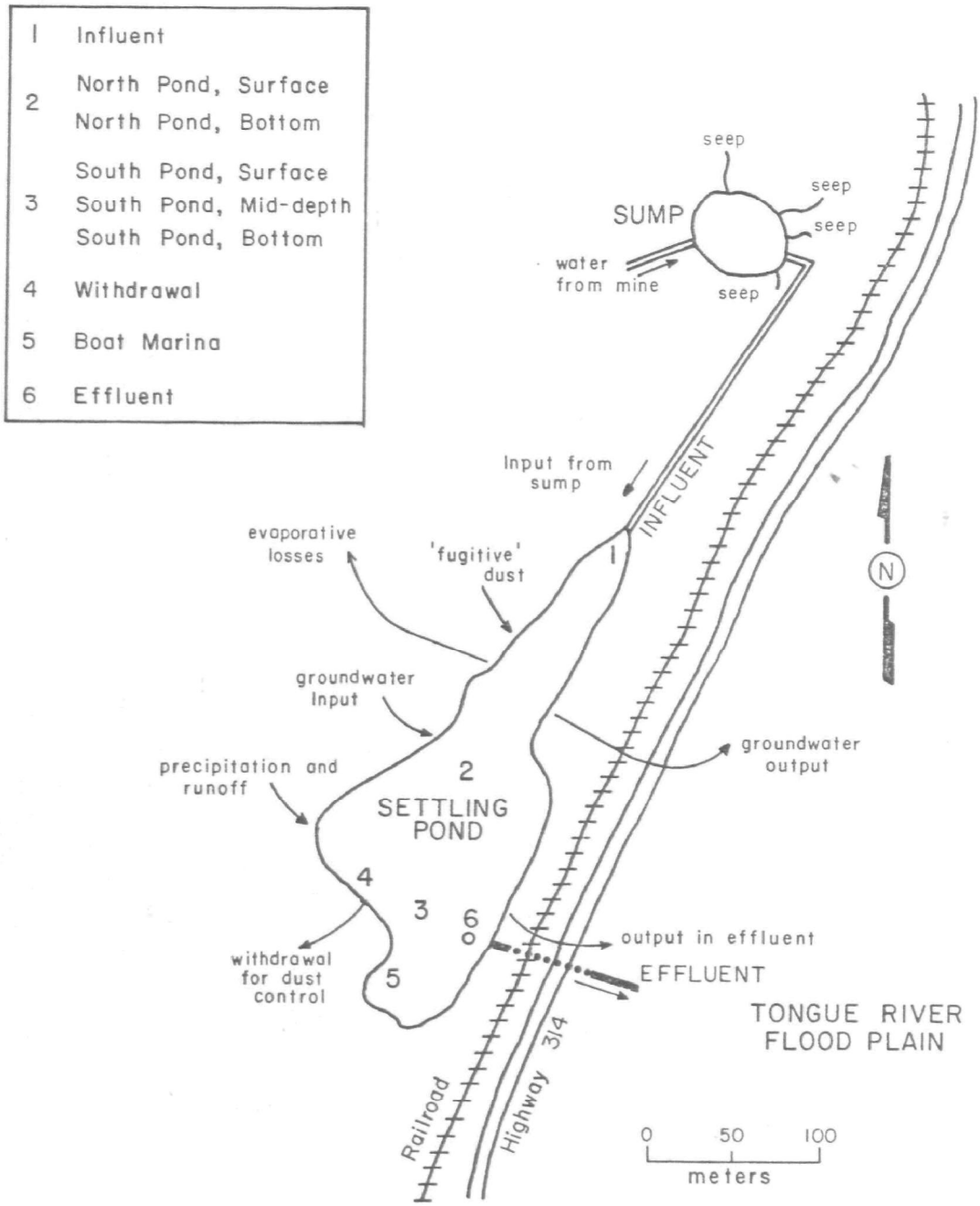


Figure 3. Map of the settling pond system at the West Decker Mine showing sources of chemical input and output, and locations of sampling sites.

TABLE 1. MONTHLY DESCRIPTION OF THE SETTLING POND SYSTEM AT THE WEST DECKER MINE

Date of Sample Collection	Pond Description
29 June 1976	Sump pond and northern arm of settling pond buried; pond system no longer completely resembling illustration in Figure 3; increased mining activity immediately adjacent to pond.
28 July 1976	Two sources of influent piped to pond, one from buried sump and other from mine pit; effluent continuous.
24 August 1976	Influent and effluent intermittent.
19 September 1976	Influent and effluent intermittent.
21 October 1976	No influent except for one observed influx of very turbid water; effluent continuous; water in pond full of suspended particulates.
18 November 1976	No influent or effluent; pond stagnant; thin ice cover over pond surface; water very turbid.
14 December 1976	Northern end of pond dredged, resulting in deepening of part of pond and lowering of water level; pond stagnant but clear; ice cover from 15 to 25 cm thick.
12 January 1977	Pond still stagnant but clear; water level approximately the same as in December 1976; ice cover 25 cm thick.
9 February 1977	Pond stagnant; water level lower than in January; ice cover from 20 to 40 cm thick; evidence of turbid runoff on top of ice.
6 April 1977	No ice cover; influent intermittent; effluent restricted but continuous.

SECTION V

MATERIALS AND METHODS

SAMPLE COLLECTION

Between November 1975 and June 1976 ten collections of water and sediment samples were made at the West Decker Mine settling pond system. Water samples for algal bioassays were collected in acid-cleaned, sterile, two-liter plastic containers from the sump pond (source of influent to the settling pond) and the settling pond effluent. Selected bacterial populations were estimated in surface sediment samples collected from the sump and settling ponds. The most efficient and aseptic instrument for sediment collection was a Phleger core sampler with removable, autoclavable, butyrate liners (Hydro Products, San Diego, California). All samples were kept well-chilled while in transit to Montana State University (MSU) in Bozeman, where they were processed within 24 hours.

More intensive studies of the settling pond were initiated in July 1976 and continued through April 1977. During that time bacteriological studies and most water analyses were performed at a field research station located immediately adjacent to the Tongue River Reservoir. Water samples for chemical analyses were collected in appropriate acid-cleaned glass or plastic bottles at 3 to 6 sites within the pond system. Locations of the sampling stations (Figure 3) were chosen to represent sources of inflowing, intermediate, and outflowing water. Site 1 corresponded to the major source of inflowing water, whereas Site 4, the withdrawal, and Site 6, the effluent, were both sources of outflowing water. The location of Site 5, designated "boat marina" because our small sampling vessel was moored there, was selected to represent a portion of the pond possibly enriched by the ammonium nitrate explosives stored in vats on land adjacent to the pond. The north and south portions of the pond were sampled at Sites 2 and 3, respectively. The settling pond, in addition to being a sedimentation basin, served as a reservoir of water for dust control. Water for this purpose was removed by pumping at Site 4.

Both surface and bottom water samples were taken at Site 3 from July through November 1976, and thereafter only from Site 2. Fewer sites were sampled when the pond stagnated, and there were no sources of inflowing and outflowing waters. The use of a homemade Van Dorn-type apparatus facilitated the collection of bottom samples. Measurements of temperature and dissolved oxygen were taken simultaneously with sample collection. All samples were kept chilled while in transit to the Tongue River Reservoir Research Station.

Collection of water samples for productivity estimates was made at Site 3. Samples for the construction of diurnal oxygen curves were taken by a suction and flushing system similar to that of Welch (1968). Chlorophyll a concentrations and ^{14}C productivity were estimated on mid-depth samples taken with the Van Dorn apparatus. Test water for algal bioassays was also collected from this site except when the influent and effluent were continuous.

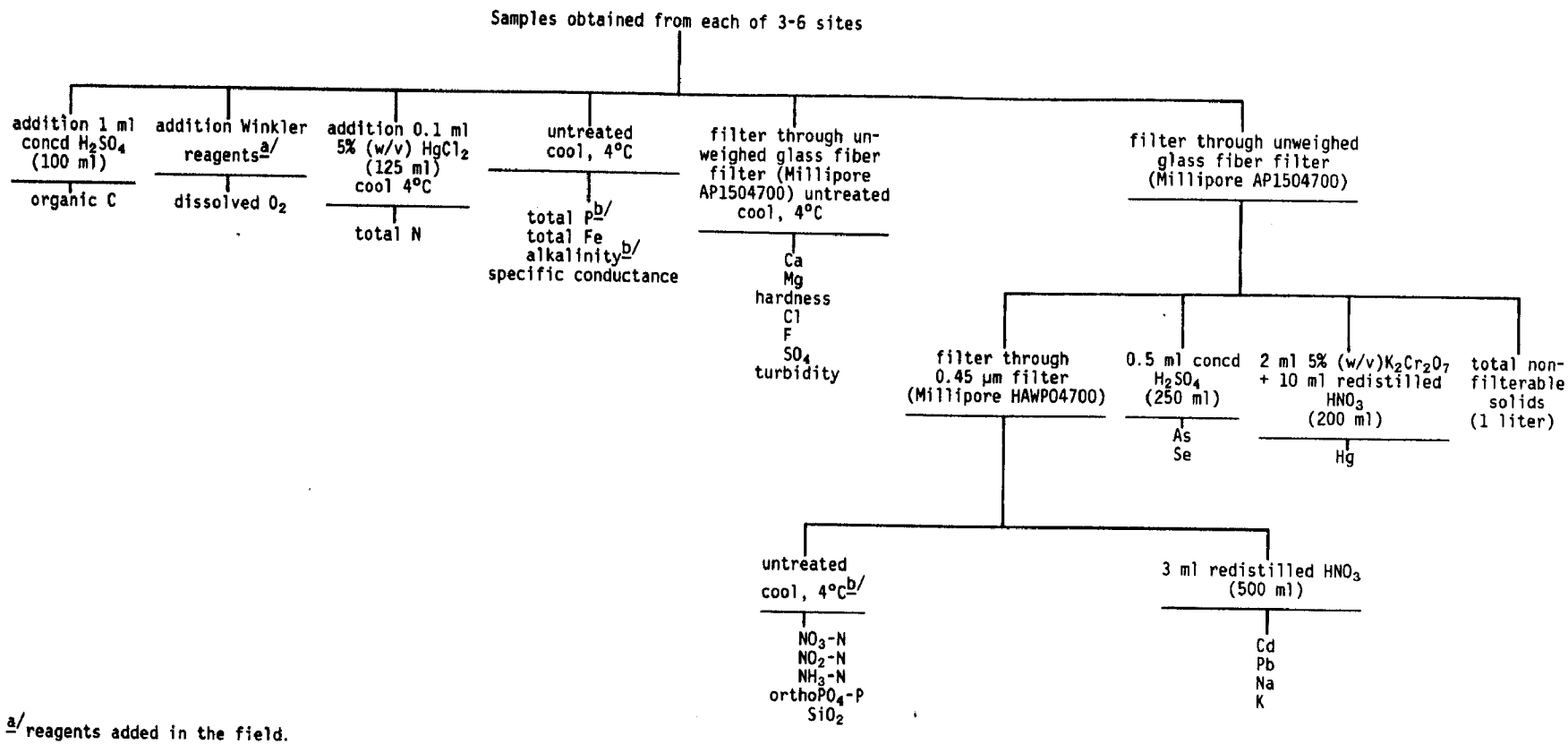
Water and sediment samples for microbial studies were collected from a wide variety of locations within the pond system, the mine, and areas outside the mine. These locations are described in subsequent sections of this report. Sediment samples were taken either by the Phleger core sampler or an Ekman dredge (Wildlife Supply Company, Saginaw, Michigan). Sterile disposable syringes of 1-, 3-, and 10-ml capacity (Becton, Dickinson and Company, Rutherford, New Jersey) were used to subsample from the sediment collected. Sterile plastic containers served as collection vessels for water samples.

In November and December 1976, pond sediments were collected for elemental analysis by the Montana State Board of Health in Billings. One sample was collected at Site 3 in November, and Sites 2 and 3 were both sampled in December. Grab samples, taken with an Ekman dredge, were placed in plastic bags and kept well-chilled in transit to Billings for analysis.

MEASUREMENT OF CHEMICAL PARAMETERS

Immediately after collection, water samples were taken to the Tongue River Reservoir Research Station where they were either preserved or prepared for analysis. Methods for sample preservation or pretreatment are outlined in the flow chart in Figure 4. Alkalinity determinations and nutrient (nitrogen and phosphorus species) analyses were performed in the laboratory at the research station as soon as possible after sample collection. The analyses for silica as silicate, total iron, dissolved oxygen, sulfate, and sulfide were generally completed within 24 hours of sample collection. Sulfate concentrations were determined on samples delivered to MSU Fisheries Bioassay Laboratory. In addition, levels of both sulfate and sulfide were measured on a restricted number of sites involved in the microbial studies. Because a wide spectrum of chemicals were analyzed by different groups of individuals in this study, specific methods used and groups are described in abbreviated form in Table 2.

Some chemical data from a single site (Site 4) had been collected on samples of settling pond water prior to the initiation in July 1976 of more intensive studies of the West Decker Mine pond. These data were provided through the courtesy of the Tongue River Reservoir limnology study undertaken by the Montana Cooperative Fishery Research Unit at MSU. Chemical parameters were quantified according to the methods outlined in Garrison et al. (1975). Sediment samples submitted to the State Board of Health were analyzed by atomic absorption spectrophotometry.



a/ reagents added in the field.

b/ analyses performed as soon as possible after sample collection.

Figure 4. Description of the treatment of water samples obtained from the West Decker Mine settling pond system prior to performing chemical analyses.

TABLE 2. DESCRIPTION OF THE METHODS USED FOR THE CHEMICAL ANALYSES OF WATER SAMPLES FROM THE SETTLING POND SYSTEM AT THE WEST DECKER MINE

Parameter	Method of analysis	Instrumentation	Laboratory performing analysis	Reference
Ca	EDTA titrimetric method	Buret	Fisheries Bioassay Lab, MSU	Am. Publ. Health Assoc. et al. 1976
Mg	Computation from Ca & hardness	--	Fisheries Bioassay Lab, MSU	Am. Publ. Health Assoc. et al. 1976
Hardness	EDTA titrimetric method	Buret	Fisheries Bioassay Lab, MSU	Am. Publ. Health Assoc. et al. 1976
Na, K	(July, August) flame emission	Instrumentation Lab AA/AE Model 151 spectrophotometer	Dep. Microbiology, MSU	--
	(Sept-Feb) atomic absorption (flame)	Varian Techtron AA-5 spectrophotometer	Dep. Chemistry, CSU	Varian AA-5 Instrument Manual
Alkalinity	Potentiometric	Buret	Dep. Microbiology, MSU	Am. Publ. Health Assoc. et al. 1971
HCO_3, CO_3	Computation from alkalinity	--	Dep. Microbiology, MSU	Am. Publ. Health Assoc. et al. 1971
F	Electrode method	Fluoride ion electrode, Orion 94-09	Fisheries Bioassay Lab, MSU	Am. Publ. Health Assoc. et al. 1976
Cl	Mercuric nitrate method	Buret	Fisheries Bioassay Lab, MSU	Am. Publ. Health Assoc. et al. 1976
SO_4	Turbidimetric method	Varian UV-Vis spectrophotometer	Fisheries Bioassay Lab, MSU	Am. Publ. Health Assoc. et al. 1976
$S^=$	Phenylenediamine	Bausch & Lomb "Spectronic 20"	Dep. Microbiology, MSU	Strickland and Parsons 1972
Silica as SiO_2	Molybdosilicate complex	Klett-Summerson colorimeter, Model 800-3	Dep. Microbiology, MSU	Am. Publ. Health Assoc. et al. 1971
O_2	Meter (July-Sept)	Yellow Springs D.O. meter, Model 54	Dep. Microbiology, MSU	--
	Azide modification of iodometric (Oct-Feb)	Buret	Dep. Microbiology, MSU	Am. Publ. Health Assoc. et al. 1971
NO_3-N	Mullin & Riley reduction	Bausch & Lomb "Spectronic 20"	Dep. Microbiology, MSU	Barnes 1959
NO_2-N	Nitriver	Bausch & Lomb "Spectronic 20"	Dep. Microbiology, MSU	Hach Chemical Company 1973
NH_3-N	Hypochlorite (July-Oct)	Klett-Summerson colorimeter, Model 800-3	Dep. Microbiology, MSU	Strickland and Parsons 1972
	Modification of phenol hypochlorite (Nov-Feb)	Klett Summerson colorimeter, Model 800-3		
Total N	Acid digestion & hypochlorite (July-Oct)	Klett-Summerson colorimeter, Model 800-3	Dep. Microbiology, MSU	Strickland and Parsons 1972
	Acid digestion & modification of phenol-hypochlorite (Nov-Feb)	Klett-Summerson colorimeter, Model 800-3		

TABLE 2. Continued.

Parameter	Method of analysis	Instrumentation	Laboratory performing analysis	Reference
Organic N	Computation of difference	--	Dep. Microbiology, MSU	U.S. Environ. Protection Agency 1974
Ortho PO_4-P	Single reagent	Klett-Summerson colorimeter, Model 800-3	Dep. Microbiology, MSU	U.S. Environ. Protection Agency 1974
Total P	Acid persulfate digestion and single reagent	Klett-Summerson colorimeter, Model 800-3	Dep. Microbiology, MSU	U.S. Environ. Protection Agency 1974
Organic P	Computation of difference	--	Dep. Microbiology, MSU	U.S. Environ. Protection Agency 1974
Organic C	Combustion infrared method	Beckman Infrared Analyzer, Model IR315	Dep. Microbiology, MSU	Am. Publ. Health Assoc. et al. 1971
Total Fe	Ferrozine reagent	Klett-Summerson colorimeter, Model 800-3	Dep. Microbiology, MSU	Hach Chemical Company 1973
Total nonfilterable solids	Gravimetric after filtration	Mettler analytical balance, type H16	Dep. Microbiology, MSU	U.S. Environ. Protection Agency 1974
Total dissolved solids	Computation from specific conductance	--	Dep. Microbiology, MSU	Garrison et al. 1975
Specific conductance	Meter	Labline mho meter model mc-1, Mark IV	Dep. Microbiology, MSU	--
pH	Meter	Beckman Expanded Scale pH meter, Model 76	Dep. Microbiology, MSU	--
Turbidity	Nephelometric method	Fisher DR-200 turbidimeter	Fisheries Bioassay Lab, MSU	Am. Publ. Health Assoc. et al. 1976
Hg	Cold vapor technique	Varian Techtron AA-5 spectrophotometer	Dep. Chemistry, CSU	Varian AA-5 Instrument Manual
As	Automated hydride generation	Varian 1200AA spectrophotometer, Technicon Autoanalyzer I	Dep. Chemistry, CSU	Goulden and Brooksbank 1974
Se	Automated hydride generation	Varian 1200AA Spectrophotometer, Technicon Autoanalyzer I	Dep. Chemistry, CSU	Goulden and Brooksbank 1974
Pb	Atomic absorption (flame)	Varian Techtron AA-5 spectrophotometer	Dep. Chemistry, CSU	Varian AA-5 Instrument Manual
Cd	Atomic absorption (flame)	Varian Techtron AA-5 spectrophotometer	Dep. Chemistry, CSU	Varian AA-5 Instrument Manual

ALGAL PRODUCTIVITY

Productivity Estimates.

Productivity at Site 3 was estimated by measuring both diurnal changes in the concentration of oxygen and the *in situ* uptake of labeled inorganic carbon (as $NaH^{14}CO_3$ in 10 μCi ampoules from New England Nuclear Corporation, Boston, Mass.) on a monthly basis from July 1976 through December 1976. The details of diurnal measurements and calculations are given in Odum and Hoskin (1958). Dissolved oxygen concentrations were determined by the azide modification of the iodometric method (American Public Health Association *et al*. 1971). The radioactive carbon method was basically that first described by Steeman-Nielsen (1952) and modified by others (Gurtz and Weiss 1972; Vollenweider 1974). The suggestions of limnologists working on the Tongue River Reservoir were particularly helpful for adapting this technique to the settling pond at West Decker (Whalen and Leathe 1976; Stephen Whalen and Paul Garrison, personal communication). Radioactivity was quantified by a Beckman Model LS-100C liquid scintillation counter.

Chlorophyll Measurements.

Chlorophyll *a*, *b*, and *c* measurements of phytoplankton populations within the settling pond were undertaken on nine water samples collected between July 1976 and April 1977. Collection and extraction methods were those of Whalen and Leathe (1976). Pigment concentrations were quantified by measuring absorbance at 665, 645, and 630 nm on a Varian Techtron Model 635 ultraviolet-visible spectrophotometer, and calculated as described in Strickland and Parsons (1972).

In May and September 1976, artificial substrates (glass slides) to sample periphyton were placed both in the settling pond effluent (below the flume) and within the pond proper. Chlorophyll concentrations of periphytic populations developing on the slides after suitable periods were measured as described above. Biomass accumulation on these same substrates was quantified by the methods of Marcus (1977).

Algal Bioassays.

Algal bioassays were conducted in the laboratory at MSU according to the specifications of the Environmental Protection Agency (U.S. Environmental Protection Agency 1971) on samples collected from the settling pond system. The green alga, *Selenastrum capricornutum* Printz served as the test organism. Relative fluorescence, measured with a Turner Associates Model 111 Fluorometer, was used as an indirect measurement of algal biomass. Nutrient limitation was determined by the production of significantly larger populations of cells with the addition of one or more spikes. Spikes added to the test waters included 1.0 mg/liter of N as $NaNO_3$, 0.05 mg/liter of P as K_2HPO_4, and 1.0 mg/liter of EDTA as $Na_2C_{10}H_{14}O_8N_2 \cdot 2H_2O$.

BACTERIOLOGY

Estimation of Selected Bacterial Populations.

A wide variety of physiological groups of bacteria potentially functional in the nitrogen, sulfur, and carbon cycles were enumerated in sediment samples and, to a lesser extent, water samples collected within the settling pond system at West Decker and other locations. Estimation of these populations was accomplished by the five-tube most probable number (MPN) method for the nitrogen and sulfur cycle organisms and the surface plate count technique for aerobic heterotrophs which function in the carbon cycle. Numbers of sulfur and nitrogen organisms were derived from standard tables giving numerical estimates and their 95% confidence intervals; a full discussion of the MPN technique is given in Alexander (1965a) and Meynell and Meynell (1970). In performing surface plate counts, the suggestions of Clark (1971) were valuable for optimizing results. The different media, methods of detection used, and references to methods used for the estimation of selected bacterial populations are summarized in Table 3. Samples were diluted in sterilized water from site of sample collection.

Enrichment of Selected Bacterial Populations.

Fresh samples of coal from the two principal seams mined at Decker (Dietz-1 and Dietz-2) were obtained from drilling operations. Chunks were crushed with a sterilized mortar and pestle. Approximately 10 grams of crushed sample were inoculated into 90 ml of 9K salts plus ferrous iron (Silverman and Lundgren 1959). After one month, attempts were made to subculture bacteria from the coal-inoculated flasks into test tubes containing the same medium.

Sediment samples were collected from each of the following locations: Tongue River upstream from railroad bridge above the reservoir, Tongue River near rivulet believed to be from swampy area below West Decker Mine effluent, Tongue River downstream from this rivulet, directly below flume, and branch of rivulet resulting from effluent. Triplicate one-gram subsamples were inoculated into 50 ml of 9K salts plus ferrous iron or elemental sulfur. After 16 days, contents of enrichment flasks were subcultured to tubes containing the same medium.

Purification, Tentative Identification, Growth and Survival of Acidophilic Iron Oxidizing Bacteria

Most probable number tubes positive for the oxidation of iron were purified by streaking on the ISP medium of Manning (1975). If these isolates also produced acid when inoculated into 9K salts plus elemental sulfur (Silverman and Lundgren 1959), they were tentatively identified as *Thiobacillus ferrooxidans* (Buchanan and Gibbons 1974).

The survival of two such isolates, TF-1 and TF-2, was evaluated by the following method: cells were grown in 9K salts plus ferrous iron at pH 3, harvested, washed (Silverman and Lundgren 1959), and inoculated into sterile

TABLE 3. METHODS USED FOR THE DETECTION AND ENUMERATION OF SELECTED BACTERIAL POPULATIONS

Physiological Process	Reaction Detected	Method of Enumeration	Culture Medium	Method of Detection	Reference
NITROGEN CYCLE					
Ammonification	$R\text{-}NH_2 \rightarrow NH_3$	most probable number (MPN)	2.0% (w/v) peptone broth	yellow color w/ addition of Nessler's reagent	Seeley & Vandemark (1972)
Nitrification Step 1	$NH_3 \rightarrow NO_2$	MPN	ammonium-calcium carbonate broth	pink color w/addition of Griess-Ilosvay reagent; pink color w/addition of both Devarda's alloy & Griess-Ilosvay reagent	Alexander & Clark (1965) and Matulewich et al. (1975)
Step 2	$NO_2 \rightarrow NO_3$	MPN	nitrite-calcium carbonate broth	no pink color w/addition of Griess-Ilosvay reagent; pink color upon addition of Devarda's alloy	
Denitrification	$NO_2, NO_3 \rightarrow N_2, N_2O$	MPN	asparagine-citrate broth	green to blue color change (increase alkalinity), presence of gas	Alexander (1965b)
Nitrogen fixation (Azotobacter)	$N_2 \rightarrow$ cellular N	MPN	sucrose-mineral salts broth	pellicle formation on surface of broth	Clark (1965)
SULFUR CYCLE					
Sulfate reduction	$SO_4 \rightarrow H_2S$	MPN	Medium E	blackening due to formation of ferrous sulfide	Postgate (1966)
Elemental sulfur oxidation	$S^0 \rightarrow SO_4$	MPN	acidified 9K salts w/elemental sulfur	red upon addition of thymol blue (increase acidity)	Silverman and Lundgren (1959)
Thiosulfate oxidation	$S_2O_3 \rightarrow SO_4$	MPN	neutral thiosulfate broth	macroscopic examination for pellicle formation; yellow upon addition of brom thymol blue (increase acidity)	Vishniac and Santer (1957)
IRON CYCLE					
Iron oxidation	$Fe^{++} \rightarrow Fe^{+++}$	MPN	acidified 9K salts w/ferrous sulfate	orange-brown precipitate of ferric iron	Silverman and Lundgren (1959)
CARBON CYCLE					
Aerobic, heterotrophic growth	growth on carbonaceous medium	plate count	1/2-strength iron peptone agar	colonial growth	Ferrer et al. (1963)

West Decker pond water (pH 8). The count of viable organisms at different times was determined by the MPN technique.

TF-1 and TF-2 were also grown in batch culture (10 liters and 4 liters, respectively) in a Microferm Model MF-214 fermenter to test the correlation between cell number and ferrous iron concentration. Cell numbers were determined by direct microscopic counts in a Petroff-Hauser chamber. The concentration of ferrous iron was measured by titration with potassium permanganate (Skoog and West 1976). The initial ferric iron concentration was taken as zero, and differences between the initial and subsequent ferrous iron concentrations were taken as the amount of ferric iron present.

Leaching Experiments with *Thiobacillus ferrooxidans*

Ten-gram quantities of 28- to 80-mesh rider seam coal obtained from West Decker were added to 250-ml Erlenmeyer flasks containing 100 ml of 9K salts. Sodium azide (0.03g) and iron (4.42g $FeSO_4 \cdot 7H_2O$) were added to some of the flasks. All flasks were inoculated with 0.2 ml of a washed suspension of T. ferrooxidans containing 10^8 cells per ml. After four hours to permit equilibration, pH was measured (Beckman Model SS-3 Zeromatic pH meter) and the amount of acidity was determined titrimetrically (American Public Health Association et al. 1976). Acidity and pH values were redetermined after a 33-day incubation period.

Physiological Studies of *Thiobacillus ferrooxidans* using Respirometry

Oxygen consumption by T. ferrooxidans was measured in a Gilson Differential Respirometer. Cells were grown in batch culture as previously described. Each respirometer vessel contained 0.2 ml of washed cells (approximately 5×10^9 cells) and 2.8 ml of basal salts plus an energy source. Energy sources were the following: iron, 100 μmoles (as $FeSO_4 \cdot 7H_2O$); thiosulfate, 100 μmoles (as $Na_2S_2O_3 \cdot 5H_2O$); elemental sulfur, 50 mg; or glucose plus yeast extract (0.1% w/v and 0.5% w/v, respectively). The center well of each vessel contained 0.2 ml of 20% (w/v) KOH and a filter paper wick as a CO_2 trap. The experiments were performed at 28 C, with an agitation rate of 140 oscillations per minute. The gas phase in the vessels was air. All results were corrected for endogenous respiration.

Sulfate Reduction in Pond Sediments

In situ rates of sulfate reduction were measured in the West Decker settling pond sediments on three occasions (September, October, and December 1976) using the technique of Ivanov (1964). Radioactive sulfate as $H_2{}^{35}SO_4$ (New England Nuclear Corporation) was incubated with samples of settling pond sediment. After a suitable incubation period, biological reactions were terminated and evolved sulfide was fixed with a solution of 3.5% w/v cadmium acetate in 4% v/v acetic acid. Upon return to the laboratory at MSU, the radioactive cadmium sulfide precipitate was subjected to acidification and heat which drove off the sulfide. Evolved sulfide was trapped in an absorption column containing cadmium acetate. The radioactivity of this precipitate was then quantified with a liquid scintillation counter (Beckman

LS-100C). A lactate spike (300 mg/liter) was added to one set of experimental vessels in both October and December. The rate of sulfate reduction was calculated by the following equation (Ivanov 1964):

$$\text{mg } H_2S \text{ per liter per day} = \frac{(\text{concentration of } SO_4^{=})\ (\text{radioactivity of } H_2{}^{35}S)\ (24 \text{ hr})\ (1.06)}{(\text{radioactivity of } H_2{}^{35}SO_4)\ (\text{duration of experiment in hours})}$$

SECTION VI

RESULTS AND DISCUSSION

CHEMICAL PARAMETERS

Nutrients, Cations, Anions, and Other Parameters

Results of chemical analyses performed on samples taken within the pond are presented in Table 4. In the data collected from July 1976 to February 1977, it should be noted that the values listed as highest and lowest in the ranges are actually means of concentrations of chemical parameters for three to five sites sampled within the pond on a particular sampling trip. Averaging the data from different pond sites collected on the same occasion was deemed justifiable because these values did not differ markedly. Chemical data from the pond are presented in summary form in Table 4 and are given in more detail in the Appendix, Tables A-1 through A-9. Chemical data provided through the courtesy of the Montana Cooperative Fishery Research Unit (November 1975 to June 1976) are included in Table 4 because they essentially characterized the pond prior to increased nearby mining activity and to changes in operation and morphometry. They will be referred to in the discussion only in comparison to our data.

Some concentrations, particularly those of nitrogen species, were highly variable from month to month as evidenced by the wide ranges in Table 4. This may indicate that monthly sampling was too infrequent to characterize a heterogeneous system such as the settling pond, which was subjected to random changes in mining activity.

Concentrations of dissolved oxygen, bicarbonate, sulfate, nitrogen species, and total dissolved solids (specific conductance) were markedly different in the November 1976 through February 1977 samples compared to previous data. These parameters, with the exception of dissolved oxygen, showed increased levels in response to several factors. Notably higher concentrations of nitrogen reflected the influence of overburden blasting with ammonium nitrate explosives. These materials were stored in vats approximately 50 meters from the pond, and it is very likely that they also periodically enriched the pond waters. Van Voast and Hedges (1975) also observed sporadic abnormally high concentrations of nitrate in the pond effluent and attributed them to overburden blasting or to dissolved nitrate-containing substances from disturbed materials. The higher concentrations of dissolved nitrogen species in the settling pond, compared to those in the Tongue River as determined by Whalen and Leathe (1976), were an indication of the more intensive mining activities in the vicinity of the pond.

TABLE 4. RESULTS OF CHEMICAL ANALYSES PERFORMED ON SAMPLES FROM THE WEST DECKER MINE SETTLING POND

	Nov 1975-Jun 1976 Average (Range) [No. of analyses]	Jul 1976-Feb 1977 Average (Range) [No. of analyses]
Temperature (°C)	13.3 (3.0-25.3) [11]	9.9 (0.3-22.9) [7]
Specific conductance (μmho/cm)	1528 (1129-1728) [11]	1865 (1254-2831) [8]
pH	8.29 (7.65-8.98) [11]	8.14 (7.85-8.50) [8]
Turbidity (NTU)	21.4 (3.2-44.0) [10]	12.1 (4.4-34.0) [4]
Calcium (me/liter)	1.96606 (0.98802-2.87424) [10]	2.66396 (1.37225-3.92713) [7]
Magnesium (me/liter)	4.11300 (2.96136-4.39263) [10]	3.37924 (1.62875-5.06722) [7]
Sodium (me/liter)	10.87500 (7.39500-13.70250) [10]	14.43500 (9.27860-22.76360) [7]
Potassium (me/liter)	0.21735 (0.18155-0.28127) [10]	0.14140 (0.13475-0.22169) [6]
Chloride (me/liter)	0.23414 (0.02257-0.30467) [10]	0.08328 (0.00282-0.16221) [5]
Fluoride (me/liter)	0.05790 (0.04738-0.07422) [10]	0.11212 (0.05632-0.18582) [6]
Sulfate (me/liter)	6.45522 (3.81189-9.85259) [11]	6.12131 (4.62430-8.74860) [6]

TABLE 4. Continued.

	Nov 1975-Jun 1976 Average (Range) [No. of analyses]	Jul 1976-Feb 1977 Average (Range) [No. of analyses]
Total alkalinity (mg/liter $CaCO_3$)	518.6 (382.5-595.5) [11]	703.0 (431.4-1223.0) [8]
Total hardness (mg/liter $CaCO_3$)	--	273 (194-431) [7]
Silica (mg/liter SiO_2)	13.5 (6.8-23.0) [10]	10.8 (7.4-14.9) [5]
Dissolved oxygen (mg/liter)	12.3 (10.1-14.4) [11]	7.0 (1.3-11.1) [8]
Dissolved iron (mg/liter)	0.019 (0-0.037) [10]	0.015 (0.013-0.016) [2]
Total iron (mg/liter)	--	0.099 (0.057-0.187) [8]
Total dissolved solids (mg/liter)	1024 (756-1158) [11]	1250 (840-1897) [8]
Total non-filterable solids (mg/liter)	--	11.6 (5.6-20.5) [8]
Organic carbon (mg/liter)	5.9 (3.0-8.8) [9]	6.9 (4.7-9.1) [2]
Nitrate-Nitrogen (mg/liter)	0.378 (0.020-1.133) [10]	5.8 (0.098-21.8) [8]
Nitrite-Nitrogen (mg/liter)	0.032 (0-0.108) [11]	0.107 (0.002-0.279) [8]

TABLE 4. Continued.

	Nov 1975-Jun 1976 Average (Range) [No. of analyses]	Jul 1976-Feb 1977 Average (Range) [No. of analyses]
Ammonia-Nitrogen (mg/liter)	0.329 (0.030-0.952) [9]	1.40 (0.109-3.82) [8]
Total nitrogen (mg/liter)	--	8.6 (0.406-25.2) [8]
Inorganic nitrogen (mg/liter)	0.793 (0.065-2.028) [9]	7.3 (0.267-23.0) [8]
Organic nitrogen (mg/liter)	--	1.250 (0.032-3.344) [8]
Orthophosphate phosphorus (mg/liter)	0.032 (0.003-0.078) [11]	0.005 (0.001-0.008) [8]
Total phosphorus (mg/liter)	0.013 (0.001-0.042) [11]	0.046 (0.021-0.063) [8]
Organic phosphorus (mg/liter)	0.029 (0.005-0.048) [11]	0.036 (0.020-0.055) [8]

From December 1976 through February 1977 the pond was stagnant and ice-covered. Concentrations of dissolved oxygen were much lower than those recorded by the Tongue River Reservoir limnologists (see Table 4). Stagnation and ice cover prevented atmospheric exchanges and allowed processes which consume oxygen to exceed those which release it.

The chemical oxygen demand may have been high at this time because in early December the northern end of the pond was dredged, and reduced chemicals in the sediments were released into the water column. The combination of increased mining activity, pond stagnation, dredging, and ice cover appear to have greatly influenced the concentrations of certain chemicals in the water column, and were responsible for the differences in pond water chemistry between the summer and winter sampling periods.

Comparison of the influent and effluent was only possible for a few months (July to October 1976). Although a new pumphouse was completed in the early spring of 1977 for pumping drainage waters from an underground pipe system into the pond, the flow into the pond was still not continuous. Dredging the pond in early December deepened it. Because the water was then below the standpipe, there was very little discharge into the Tongue River flood plain.

Based on comparison of the analyses performed, the influent to the mine settling pond was chemically different from the effluent. Inflowing waters were characterized by relatively low dissolved oxygen, lower pH values, and higher concentrations of iron. Specific conductance and total non-filterable solids were greater in the effluent samples. Values for selected parameters are given in Table 5. Waters entering the pond underwent oxygenation with the concomitant loss of sulfide. Photosynthetic activity may have increased the pH values within the pond resulting in a more alkaline discharge.

The West Decker Mine settling pond was designed to function as a sedimentation basin. Greater quantities of suspended particulate matter were detected in the settling pond than in the influent. This observation suggested the importance of fugitive dust and runoff as contributors to the levels of particulate materials within the pond. Continuous and heavy traffic of trucks was observed on the roads immediately adjacent to the pond. In addition, nearby overburden blasting produced noticeable clouds of dust which eventually drifted over the pond. The pond was very shallow and was subjected to considerable wind-driven mixing which may account for relatively high concentrations of particulates. The effect of wind in reducing the efficiency of sedimentation ponds has been discussed by Janiak (1975). Suspended materials were largely abiotic in nature. Sampling may have been too infrequent to collect data on intermittent, very turbid inflowing water (referred to as secondary influents) which contained significantly higher concentrations of suspended materials. On 28 July 1976, a very turbid secondary influent was sampled. It contained 115.6 mg/liter of total non-filterable solids (total suspended solids) compared to 3.2 and 11.6 mg/liter of the primary influent and the settling pond, respectively.

Although the federal government (U.S. Environmental Protection Agency 1977b) has advanced guidelines for the water quality of effluents from

TABLE 5. SELECTED CHEMICAL PARAMETERS MEASURED ON WATER SAMPLES COLLECTED FROM INFLUENT AND EFFLUENT WATERS OF THE SETTLING POND SYSTEM AT THE WEST DECKER MINE (DATA ARE PRESENTED AS AVERAGE VALUES OF FOUR MONTHLY DETERMINATIONS)

Parameters	Influent	Effluent
Dissolved oxygen (mg/liter)	4.4	12.3
Specific conductance (μmho/cm)	1338	1471
Total non-filterable solids (mg/liter)	3.2	15.1
pH	7.46	8.36
Sulfate (mg/liter)	256.0	283.0
Sulfide (mg/liter)	0.23	not detectable
Sodium (mg/liter)	224.0	268.0
Total Iron (mg/liter)	0.196	0.096
SAR values[a]	5.094	7.037

[a] SAR is mathematically expressed as $\dfrac{\text{me/liter } Na^{+}}{\left(\dfrac{\text{me/liter } Ca^{2+} + \text{me/liter } Mg^{2+}}{2}\right)^{\frac{1}{2}}}$

surface coal mines, individual standards for each mine may be modified at the region or state level based on local conditions (rate of effluent discharge, soil conditions of the receiving area, etc.). As of June 1977, the effluent from the West Decker Mine settling pond has to meet the following criteria (Montana Department of Health and Environmental Sciences 1977): (1) total suspended solids of 30 mg/liter for a daily maximum; (2) the pH value shall not be less than 6.0 standard units nor greater than 9.0 standard units; (3) total iron is limited to 3.5 mg/liter for a daily average and 7.0 mg/liter for a daily maximum; (4) the concentration of oil and grease shall not exceed 10 mg/liter.

The first three criteria are those suggested by the U.S. Environmental Protection Agency (1977b). The level of total suspended solids for the West Decker Mine has been modified by EPA Region VIII requirements. The fourth criterion has been set by the State of Montana.

Water samples were not analyzed for oil and grease as part of the present study, but the data (Table 5) indicate that the other criteria were met. The chemical characteristics of the pond effluent also have been reported in detail in a limnological study of the Tongue River Reservoir (Whalen and Leathe 1976). This study found that the concentrations of sodium, sulfate, ammonia-nitrogen, nitrate-nitrogen, and bicarbonate were elevated in the discharge from the settling pond, but that these chemicals had virtually no measurable impact on the surface water quality of the Tongue River based on theoretical loading calculations. Researchers investigating the quality of other mine effluents entering the Tongue River reached similar conclusions (Dettman and Olsen 1977). They reported that discharge from the Big Horn Mine, approximately 50 river kilometers upstream from Decker, also contained concentrations of these same chemicals which were greater than those in the Tongue River; the concentrations of dissolved conservative parameters downstream from the Big Horn Mine were not significantly higher than above the mine.

Cations and anions are expressed in milliequivalents per liter in Table 4 to facilitate comparison. The ionic composition of inland surface waters consists of four major cations, Ca^{++}, Mg^{++}, Na^{+}, and K^{+}, and four major anions, $SO_4^{=}$, HCO_3^{-}, $CO_3^{=}$, and Cl^{-} (Wetzel 1975). The relative abundance of cations and anions in the West Decker Mine settling pond compared to the Tongue River above the West Decker Mine and to inland surface waters is as follows:

	West Decker Mine settling pond	Tongue River above mine effluent (Garrison et al. 1975; Whalen et al. 1976; Whalen and Leathe 1976)	Inland Surface Waters (Wetzel 1975)
Cations	Na>Mg>Ca>K	Ca≥Mg>Na>K	Ca>Mg≥Na>K
Anions	HCO_3>SO_4>Cl	HCO_3>SO_4>Cl	CO_3>SO_4>Cl (all bicarbonate considered as carbonate)

The water of the settling pond showed a different predominance of cationic species with the equivalent proportion of sodium exceeding proportions of other cations by an order of magnitude. In addition, magnesium was greater than calcium. Both of these cations exceeded potassium by an order of magnitude. The anionic composition of the pond water followed the general tendency for proportions of bicarbonate to dominate, with sulfate equivalents exceeding those of chloride. Within the pH ranges measured, bicarbonate accounted for almost all of the total alkalinity. These results were similar to the order of anion predominance in samples of Tongue River water and in the generalized scheme for inland surface waters. In the pond samples collected from November 1976 through February 1977, the equivalent portions of nitrate were greater than those of chloride and fluoride combined. This was somewhat unusual because, although nitrate is of immense biological importance, it does not contribute significantly to the anionic components of natural waters unless they are highly enriched (Wetzel 1975).

In typical inland waters of temperate regions, total hardness is approximately equal to total alkalinity when both are expressed as mg per liter of calcium carbonate. It is evident from Table 4 that total alkalinity exceeded total hardness and that significant amounts of another cation, obviously sodium, were present. The settling pond can be characterized as containing bicarbonate water with sodium as the predominant cation and, in this respect, it was like the groundwaters of the same region. A similarity between the two waters was not unexpected because groundwaters from the overburden of the Dietz-1 coal seam, and those from both the Dietz-1 and Dietz-2 coal were eventually piped into the settling pond (Van Voast 1974). This similarity, however, was limited; differences between the groundwaters and surface water of the pond are best illustrated by Table 6 which is taken from Whalen and Gregory (1977). Groundwater data for the construction of this table were obtained from Van Voast (1974); the chemical parameters of the mine effluent have been analyzed in the Tongue River Reservoir limnology study (Garrison et al. 1975; Whalen et al. 1976; Whalen and Leathe 1976).

The data in Table 6 indicate that the equivalent proportion (me/liter) of sodium in the groundwater was greater than that in the settling pond water. Van Voast and Hedges (1975) have reported that in aquifers within the coal seams, groundwaters under pressure are supersaturated with sodium bicarbonate. High levels of sodium are the result of dissolution of sodium-rich feldspars and other minerals and, to some extent, base exchange reactions (Roger Lee, U.S. Geological Survey, personal communication, 1978).

Water from the settling pond contained higher proportions of magnesium and calcium in comparison to those in groundwater from the coal-bearing aquifers. This observation agrees with the contention that minerals (such as those containing magnesium and calcium) are more easily dissolved in rubblized overburden than they are in the undisturbed materials. McWhorter et al. (1975) suggested that the magnesium and calcium in runoff from spoils sampled at the Edna Mine in Colorado resulted from the exposure of the minerals gypsum, epsomite, dolomite, and calcite to leaching action. This phenomenon may be operable at the West Decker Mine.

Perhaps the best reflection of the relative concentrations of sodium, calcium, and magnesium in the two types of water is the sodium absorption ratio (SAR). This ratio was derived to predict the magnitude to which irrigation water potentially undergoes cation exchange reactions in soil (Hem 1971). Water with values less than 4 will not disturb the nutritional balance in crop plants, while SAR values less than 8 will not damage soil structure (Bernstein 1967 in Whalen and Leathe 1976). The average SAR reported by Whalen and Gregory (1977), given in Table 6, was similar to that calculated for the influent and effluent of the settling pond (Table 5). The value for groundwaters in the West Decker area was high, but that of the pond water was below 8. Although not ideal for irrigation of mine spoil reclamation sites, water from the settling pond would not cause adverse effects if diluted sufficiently. The degree of dilution necessary depends, of course, on the nature of the dilution water. If Tongue River water were used, settling pond water would have to be diluted greater than two-fold to produce an SAR value below 4.

TABLE 6. COMPARISON OF CHEMICAL PARAMETERS OF GROUNDWATER SAMPLES OBTAINED FROM AQUIFERS IN THE COAL SEAMS AT DECKER AND THE WATER OF THE MINE SETTLING POND EFFLUENT[a]

Parameter[b]	Groundwater	Mine effluent
Ca^{++}	0.17	1.65
Mg^{++}	0.08	3.49
Na^{+}	18.56	11.04
HCO_3^{-}	18.50	9.78
$SO_4^{=}$	0.09	6.15
Hardness (mg/liter as $CaCO_3$)	12 (925)[c]	257 (489)[c]
SAR	54.6	6.4
Dissolved Fe (mg/liter)	0.10	0.02
pH	8.3	8.3

[a] Whalen and Gregory 1977.

[b] Concentrations reported in milliequivalents/liter unless otherwise indicated.

[c] Numbers in parentheses represent theoretical hardness, which is calculated by assuming that (a) the total alkalinity is due to bicarbonate, and (b) in a "typical" natural water, predominantly a calcium or calcium-magnesium bicarbonate system, the total hardness (chiefly due to Ca^{++} and Mg^{++}) is approximately equal to total alkalinity when both are expressed as mg $CaCO_3$ per liter.

Another parameter showing differences between the groundwater and the settling pond water was sulfate; Table 6 indicates that sulfate concentrations were much greater in the settling pond water than in groundwater. Van Voast and Hedges (1975) reported that high sulfate concentrations in the settling pond effluent, relative to those in the groundwaters, cannot be explained solely by mixing of the groundwaters. They attributed this difference to the dissolution of sulfate from freshly crushed coal. However, coal is not the primary source of the sulfur present in surface and groundwaters in coal-bearing regions. Pyritic materials are also present in the overburden and, because large amounts of overburden must be disrupted during mining, overburden may contribute much of the sulfur found in these surface waters and groundwaters. Sulfate, like magnesium and calcium, may be leached from the minerals gypsum and epsomite (McWhorter et al. 1975). It is possible that reduced inorganic sulfur such as that in pyrite was being oxidized with the concomitant increase in concentrations of sulfate. This oxidation process is responsible for acid mine drainage problems in mining areas of the eastern and midwestern United States. Not all of the coal beds in those areas, however, produce acid waters. For example, alkaline mine waters associated with the Sewickley coal seam in Pennsylvania and West Virginia are attributed to neutralization of acid by mineral carbonate present (Temple and Koehler 1954).

Acidic drainage has not been reported at West Decker; the pH values of the settling pond influent and effluent were always above neutral (Table 5). The absence of acidic conditions has been attributed primarily to the high buffering capacity of the regional waters, particularly the surface waters. The presence of less pyritic material in western overburden and coal strata may also reduce the potential for acid production. Acid mine drainage is discussed in more detail in subsequent sections of this report.

Potentially Toxic Elements

Levels of possible toxicants in waters of the West Decker Mine settling pond system are given in Table 7. According to the water quality criteria of the U.S. Environmental Protection Agency (1977a), the levels of arsenic, selenium, cadmium, and lead did not exceed those reported to be hazardous to public water supplies or exceed that recommended for the protection of aquatic life. Concentrations of mercury, however, exceeded the recommended values for the protection of freshwater aquatic life and wildlife by an order of magnitude. Joensuu (1971) and Klein (1973) also reported that relatively high concentrations of mercury are found associated with coal-bearing strata. Because of negligible loading to, and dilution by, the Tongue River, mercury was not detected in the river at concentrations above that recommended by EPA (1977a). However, a spawning marsh for northern pike, to be located in the flood plain in the region of the settling pond discharge, is currently planned (R. Gregory, Montana Cooperative Fishery Unit, personal communication, 1978). Chemical monitoring of the settling pond discharge into this projected spawning region is highly recommended.

TABLE 7. LEVELS OF POSSIBLE TOXICANTS IN WATERS OF THE WEST DECKER MINE SETTLING POND SYSTEM[a] (RESULTS OF POND SAMPLES REPRESENT MEANS OF VALUES RECORDED FOR TWO OR MORE LOCATIONS WITHIN THE POND)

Site and date of sample collection	Mercury µg/liter	Recommendations	Arsenic µg/liter	Recommendations	Selenium µg/liter	Recommendations	Cadmium mg/liter	Recommendations	Lead mg/liter	Recommendations
Pond										
7-27-76	0.81	Should not	1.4	Data not	0.5	Should not	<0.001	Should not	<0.01	Should not
8-24-76	0.38[b]	exceed 0.05	2.1	sufficient for	0.5	exceed 0.01 of	<0.002	exceed 12.0	<0.01	exceed 0.01
9-19-76	0.38	µg/liter[d]	1.4	aquatic life	<0.3	the 96-hour LC50	<0.005	µg/liter[d]	<0.1	times the 96-
10-21-76	0.17		1.7		0.4	as determined	<0.005		<0.1	hour LC50[d]
11-18-76	0.09		1.8	Should not	1.4	through bioassay	<0.005		<0.1	
12-14-76	0.28		1.2	exceed 50	2.3	using a sensi-	<0.005		<0.1	Should not
1-12-77	0.26		1.1	µg/liter for	2.2	tive resident	<0.005		<0.2	exceed 50[e]
2-09-77	0.24		1.2	other purposes[e]	2.6	species[d]	<0.005		<0.1	µg/liter for
4-06-77	0.27		1.2		0.3	Should not exceed 10.0 µg/liter for other purposes[e]	<0.005		<0.1	other purposes
Influent										
7-27-76	0.85		1.6		0.4		<0.001		<0.01	
8-24-76	0.55		3.7		0.6		<0.002		<0.01	
9-19-76	0.11		3.3		<0.3		<0.005		<0.1	
10-21-76	<0.05[c]		1.5		<0.3		<0.005		<0.1	
4-06-77	0.58		1.6		<0.3		<0.005		<0.1	
Effluent										
7-27-76	0.87		1.3		0.4		<0.001		<0.01	
8-24-76	0.42		1.9		0.4		<0.002		<0.01	
9-19-76	0.42		1.2		<0.3		<0.005		<0.1	
10-21-76	0.11		1.9		0.5		<0.005		<0.1	
4-06-77	0.38		1.1		0.5		<0.005		<0.1	

[a] Approximate values (for example, < a certain number) could not be averaged; however, estimations for different sites within the pond were always recorded as < the same value on any one sampling date.

[b] Spuriously high value of 90 µg/liter disregarded in calculation of mean.

[c] Taken from well whose water was source of influent.

[d] For freshwater aquatic life and wildlife (U.S. Environmental Protection Agency 1977a).

[e] For domestic water supply (U.S. Environmental Protection Agency 1977a).

Sediment Chemistry

Elemental analyses performed by the Montana State Board of Health on sediment samples obtained from the settling pond are presented in Table 8. Values are similar for the different locations and sampling dates. Although it is not possible from such a limited number of samples to make detailed comments on these results, several elements, especially boron, iron, and aluminum, were present in relatively high concentrations. The settling pond may be acting as a trap or sink for some elements; this subject is discussed more fully in the bacteriology section on sulfate reduction.

ALGAL PRODUCTIVITY

Data on all estimations of algal productivity are included in the Appendix, Tables A-10 through A-13.

Productivity Estimates

When ice cover and high loading of suspended particulates were absent, the settling pond was sufficiently shallow to allow light penetration throughout the water column. The construction of diurnal oxygen curves indicated that gross primary productivity of the settling pond community averaged 6.88 grams of oxygen per square meter per day for July through September 1976. Welch (1968) recorded an annual average very similar to this for a small, fertile farm pond in Georgia. Our estimates of gross primary productivity for the summer months (July through September 1976) ranged from 4.57 to 8.44 grams of oxygen per square meter per day; these values are within the ranges Odum and Hoskin (1958) gave for systems with both plankton and bottom mud components and systems with dominant bottom plant and animal communities and less important planktonic components. Estimates of pond metabolism for October through December 1976 were very low, and no net primary productivity occurred. These observations were the result of decreasing metabolic activity with lower water temperatures as well as high turbidity and ice cover reducing the amount of light available for photosynthesis.

Productivity data from labeled carbon uptake experiments indicated that fixation of inorganic carbon in the water column was generally low from July through December 1976, ranging from 0 to 0.12 g carbon fixed per square meter per day. Wetzel (1975) presented comparative rates of phytoplankton primary productivity in selected fresh waters of different fertilities. The range of the limited number of measurements performed as part of the present study was similar to ranges in the productivity values reported by Wetzel for oligotrophic waters.

A direct comparison of planktonic and community primary productivity was not possible. Both gross and net values may be calculated from diurnal oxygen data while values somewhere between gross and net results from carbon-14 fixation measurements. However, in comparison to values reported in the literature for both diurnal oxygen and carbon-14 estimates of primary productivity, community productivity was moderate and planktonic productivity

TABLE 8. ELEMENTAL ANALYSES OF SEDIMENT SAMPLES COLLECTED AT THE WEST DECKER MINE SETTLING POND (CONCENTRATIONS ARE GIVEN IN μg PER GRAM OF SEDIMENT)

	Sample Location		
Parameter	North End (12/16/76)	South End (8/24/76)	South End (12/16/76)
Arsenic	0.025	0.059	0.001
Lead	<0.05	<0.05	<0.05
Lithium	0.25	0.29	0.28
Zinc	0.84	0.6	0.89
Manganese	4.0	4.3	4.3
Strontium	1.1	1.7	1.4
Boron	100	64	71
Aluminum	292	310	345
Cadmium	<0.001	0.003	0.003
Copper	0.35	0.34	0.28
Iron	225	240	251
Selenium	0.005	0.007	0.008
Mercury	0.0005	0.001	0.0002

was low during the summer. Indirectly, it appeared that the major contribution of primary production was from benthic populations which probably included plankton that had settled out.

Chlorophyll Measurements

Chlorophyll a measurements were made on water samples obtained from the settling pond to provide monthly estimates of phytoplankton standing crop. Pigment concentrations, calculated by the monochromatic method, were relatively low within the water column during the summer (0 to 1.20 mg per cubic meter), higher during the autumn (3.16 to 6.78 mg per cubic meter), and markedly higher during the winter (35.82 to 521.18 mg per cubic meter). These values were not averaged because of significant month to month

variation. Explanations may be advanced for the increase in autumnal standing crop although these considerations are somewhat in contradiction to the results of diurnal oxygen measurements. Garrison (P. J. Garrison, Montana State University, personal communication) suggested that stagnation of the pond may have allowed phytoplankton populations, normally flushed out in the effluent, to increase substantially within the water column.

The extremely high pigment concentrations recorded from the January and February samples remain unexplained. High phytoplankton standing crop is usually accompanied with oxygen production due to photosynthesis. Although oxygen concentrations within the pond were low (1.3 to 2.2 mg per liter) and the thick ice cover prevented solar radiation from penetrating into the pond waters, pigment concentrations were considerably higher than in the summer and autumn samples. Attempts made to correlate levels of chlorophyll a with those of inorganic nitrogen (which were also higher in the November through February pond samples) were unsuccessful. Cell counts and volumes would be helpful in interpreting these data, but are not currently available. Productivity measurements were not performed during these two months; carbon-14 uptake rates would have provided corroborative data on the phytoplankton populations.

Artificial substrate (glass slides) were placed in the settling pond effluent (below the flume) to sample periphyton. The slides were allowed to incubate approximately one month before periphytic populations were removed for estimations of biomass and the concentration of chlorophyll a. Average rates of bioaccumulation were 100.2, 300.5, and 17.2 mg of carbon per square meter per day for the May-June 1976, June-July 1976, and July-August 1976 incubation periods, respectively. These values, when compared to those from a variety of habitats given in Stockner and Shortreed (1976) would be considered relatively low. The May-June interval showed higher chlorophyll a values relative to the amount of bioaccumulation, indicating that populations of photosynthetic organisms were higher at this time. Very little periphyton development was noted during the autumn and winter, probably as a result of discontinuous to nonexistent discharge from the pond, and lower water temperatures.

A periphyton sampler, similar to the one for the effluent, was placed within the settling pond near the standpipe. Data were obtained for the September-October 1976 incubation period only; the average rate of bioaccumulation was 439.0 mg of carbon per square meter per day, and average chlorophyll a concentration was 24.7 mg per square meter.

Algal Bioassays

In the Algal Assay Procedure: Bottle Test performed on samples of settling pond water, there was a tendency for both nitrogen and phosphorus limitation. This observation was most prominent in test waters collected from November 1975 to September 1976. Pond samples collected in October and November 1976 were phosphorus limited for the growth of the test alga. Chemical assessment of the sample waters supported this finding; orthophosphate-phosphorus ranged from 0.001 to 0.010 mg per liter and total phosphorus from 0.012 to 0.013 mg per liter. Nitrogen levels during these months,

particularly November, were high, probably as a result of overburden blasting and increased mining activities in the vicinity of the pond. Phosphorus limitation was not surprising considering the findings of Chiaudani and Vighi (1976). These researchers observed nitrogen limitation of the growth of Selenastrum capricornutum only when N:P ratios in the culture medium were 6 or less. An N:P ratio of 8 or greater resulted in phosphorus limitation. In the settling pond, the autumn and winter nitrogen levels exceeded those of phosphorus by two orders of magnitude and algal growth was phosphorus limited.

Stimulation of algal growth with the EDTA spike was rarely observed, indicating that either micronutrients were not limiting or toxic concentrations of elements, capable of being chelated, were not present.

BACTERIOLOGY

Estimation of Selected Bacterial Populations

Most probable number results for organisms potentially functional in the nitrogen cycle are given in Table 9 as ranges in the order of magnitude of these determinations. No seasonal trends were evident in the distribution of these data. The ratio of dry weight to wet weight was computed for each sediment sample that was diluted and inoculated into MPN tubes as a check on the moisture content of samples collected at different times.

TABLE 9. MOST PROBABLE NUMBER (MPN) ESTIMATIONS OF ORGANISMS POTENTIALLY FUNCTIONAL IN THE NITROGEN CYCLE OF SEDIMENT SAMPLES TAKEN FROM THE WEST DECKER MINE SETTLING POND

Physiological process	Number of MPN estimations	Number of organisms per 100 grams (wet weight) of sediment	
		Mine sump	Settling pond
Ammonification	7	10^6-10^9	10^6-10^9
Nitrification, Step 1	7	10^5-10^8	10^5-10^8
Nitrification, Step 2	7	10^4-10^6	10^4-10^7
Denitrification	7	10^5-10^8	10^5-10^8
Nitrogen fixation (Azotobacter)	4	10^2-10^4	10^1 -10^4

It is difficult to judge whether the numbers of organisms detected in sediment samples from the West Decker Mine settling pond were high relative to counts of similar groups of bacteria recovered elsewhere. Comparisons between our study and the limited number of similar studies found in the literature should be made with caution because of differences in methods of collection and treatment of sediment samples. In general, however, our MPN estimations of bacteria potentially functional in the nitrogen cycles exceeded those of similar groups of bacteria in Lake Erie sediments (Dutka et al. 1974). Most probable numbers of nitrifying bacteria in New Jersey river sediments (Matulewich et al. 1975) were also somewhat lower than those obtained from samples taken at the West Decker Mine.

Plate counts of aerobic heterotrophic bacteria present in the settling pond sediments estimated their populations to range from 10^6 to 10^8 colony-forming units per gram (dry weight) of sediment. Again, it is difficult to compare these results with those of other investigators because sample treatment itself may account for differences of two orders of magnitude. However, densities of aerobic heterotrophs in the settling pond sediment samples were approximately 100 times greater than those in Tongue River sediments which were sampled concurrently. According to chemical analysis of both types of sediment, the pond samples were higher in organic carbon and, consequently, in substrates for these organisms. Higher organic carbon may be due to the influence of coal residues.

The organisms potentially functional in the sulfur cycle have been the most intensively studied in the West Decker Mine settling pond. There is a biogeochemical cycle of sulfur (Figure 5), and areas where coal mining has occurred provide a natural environment for sulfur bacteria (Lundgren et al. 1970). Microorganisms are particularly important in the mediation of reactions within the sulfur cycle. Sulfur can exist in six oxidation states, but usually sulfate, elemental sulfur, sulfide, and organic sulfur (shown encircled in Figure 5) are of importance in nature. In brief, the sulfur cycle involves: (1) the oxidation of reduced inorganic sulfur compounds, (2) the reduction of sulfate to sulfide, (3) the assimilation of both reduced and oxidized forms of sulfur by organisms for biosynthesis and other metabolic functions, and (4) the decomposition of organic sulfur-containing substances. The contributions of sulfur cycle organisms to acid mine drainage problems have been studied (Lundgren et al. 1972; Dugan 1972) in the East and Midwest, but little is known about their activity in alkaline situations such as those characterizing most coal mines in the western states. Data on the relative abundances of different groups of sulfur cycle bacteria in water samples from a variety of sites and sediments, primarily from the settling pond, are presented in Table 10. One of the most important findings in these data was the relatively high populations of acidophilic iron oxidizing bacteria in the water and sediments, and acidophilic sulfur oxidizing bacteria in the sediments. It is possible that T. ferrooxidans is responsible for at least part of the MPN values reported for acidophilic sulfur oxidizers, since sulfur can serve as an energy source for this organism. Both types of bacteria are characteristic of acid mine drainage; however, acidic conditions have never been reported at West Decker. The presence of T. ferrooxidans in mine waters and

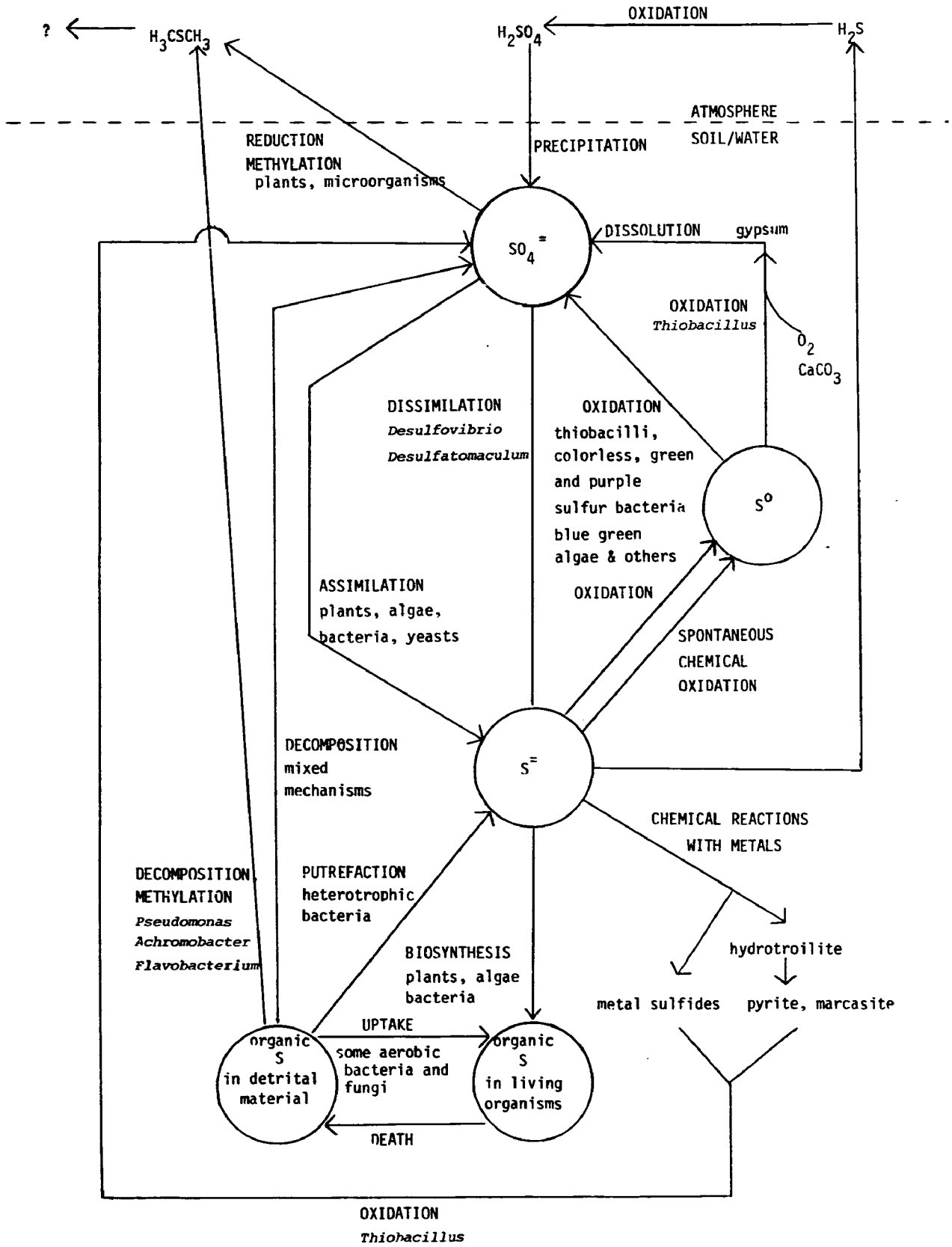


Figure 5. The biological sulfur cycle.

TABLE 10. MOST PROBABLE NUMBER (MPN) ESTIMATIONS OF ORGANISMS POTENTIALLY FUNCTIONAL IN THE SULFUR CYCLE[a/]

A. Samples taken from various locations

Location	Date	Sulfate reduction	Sulfur oxidation (pH 2.5)	Iron oxidation (pH 2.5)	Thiosulfate oxidation (pH 7.5)
Settling pond influent waters (Site 1)	9-21-76	$>2.4 \times 10^4$	2.1×10^3	$>2.4 \times 10^4$	5.4×10^3
	10-21-76	3.5×10^4	7.0×10^3	3.5×10^4	3.5×10^4
	4-05-77	1.6×10^4	4.9×10^2	1.6×10^4	2.2×10^2
Settling pond effluent waters (Site 6)	9-21-76	$>2.4 \times 10^4$	0	2.2×10^3	2.6×10^2
	10-21-76	3.3×10^3	0	0	1.1×10^4
Settling pond surface water (site 3)	11-18-76	1.1×10^3	0	4.9×10^2	$>2.4 \times 10^4$
		3.5×10^3 [b/]	4.0×10^1	5.4×10^3	1.6×10^4
	12-16-76	5.4×10^3	0	7.0×10^1	9.2×10^3
	1-13-77	7.9×10^2	0	5.0×10^1	4.9×10^2
	2-11-77	2.3×10^2	0	0	2.4×10^1
	4-05-77	7.9×10^2	0	2.4×10^3	3.3×10^2
Test pit water	11-18-76	--	0	3.3×10^3	4.6×10^3
Stock well water east of mine	1-13-77	1.7×10^3	2.0×10^1	7.0×10^1	3.3×10^2
	2-11-77	1.3×10^2	0	2.0×10^1	4.9×10^2
Tongue River Reservoir water	12-16-76	2.0×10^1	0	0	0
Tongue River Reservoir sediment	12-16-76	1.3×10^7	0	0	0

TABLE 10. (Continued)

B. Sediment samples taken at Site 3

Date	Sulfate reduction	Sulfur oxidation (pH 2.5)	Iron oxidation (pH 2.5)	Thiosulfate oxidation (pH 7.5)	Dry weight conversion[c]
1-14-76	5.4×10^6	8.0×10^3	2.3×10^4	--	18.32
	5.4×10^6 [b]	1.3×10^4	3.3×10^4	--	27.32
2-20-76	2.4×10^6	2.3×10^4	7.0×10^4	--	12.33
4-20-76	8.0×10^4	3.3×10^4	3.3×10^4	--	31.75
5-20-76	4.9×10^6	2.3×10^4	1.7×10^5	--	38.31
6-29-76	2.3×10^6	1.1×10^5	1.3×10^6	--	33.00
8-19-76 [d]	1.1×10^6	--	--	--	5.08
	3.3×10^5 [e]	--	--	--	1.18
9-21-76	$>2.4 \times 10^6$	4.0×10^3	4.0×10^3	9.2×10^6	27.61
10-21-76	4.9×10^6	3.3×10^4	1.7×10^5	5.4×10^6	29.78
11-18-76	6.3×10^6	2.3×10^4	3.4×10^4	1.4×10^6	37.34
12-16-76	3.5×10^7	1.7×10^4	6.3×10^4	1.4×10^6	26.00
1-13-77	1.3×10^6	7.9×10^4	4.9×10^5	2.2×10^6	30.09
2-11-77	--	3.3×10^4	3.1×10^4	4.3×10^5	36.88
4-05-77	1.3×10^6	2.4×10^5	--	7.9×10^4	45.52

[a] Results are expressed as the number of organisms per 100 ml of water or 100 g (wet weight).

[b] Duplicate determinations.

[c] Numbers are grams dry weight per 100 g wet weight (January-August 1976) or 100 ml (September 1976-April 1977) of sediment.

[d] Values are for sediment diluted 1:1 with sterile distilled water.

[e] Sample taken at Site 2.

sediments indicates that they have oxidized pyrite to acid, the relative numbers of organisms correlating with the amounts of pyrite oxidized (Dugan 1972).

Our data show numbers of T. ferrooxidans comparable to those of an acid mine drainage stream described by Tuttle et al. (1969); thus, there is a high level of pyrite oxidizing activity in water and sediments associated with the West Decker Mine. Unlike results at Decker, Johnson and Bromel (1977) reported very low or non-detectable numbers of iron oxidizing bacteria in sediment samples obtained from impoundments at two coal mines and from flowing waters in the Knife River Basin of North Dakota. Surface waters associated with the Belle Ayr Mine near Gillette, Wyoming, contained higher numbers of sulfur oxidizing bacteria than those in the West Decker samples (John Adams, Department of Microbiology and Veterinary Science, University of Wyoming, personal communication). Numbers of sulfate reducing bacteria detected either in sediments (North Dakota) or in surface water (Wyoming) were similar to estimations of the abundance of this group of bacteria in West Decker samples.

On one occasion (March 1977) the acid mine streams near Sand Coulee, Cascade County, Montana were sampled. A description of this area is given in McArthur (1970). Samples collected from two sites along No Name Creek (Bar Z Ranch and across from the Sand Coulee Fire Department) yielded 1.7 x 10^4 and 4.9 x 10^3 sulfur oxidizing bacteria per 100 ml of sample water, respectively. Iron oxidizing bacteria at both sites exceeded 2.4 x 10^5 organisms per 100 ml of water. Concentrations of ferrous iron and sulfate ranged from 310-563 mg/liter and 5,050-15,000 mg/liter; pH values ranged from 2.7-2.8. These conditions resembled those of acid drainages associated with coal mining practices in the eastern and midwestern states, and provided a sharp contrast to the visible nature and chemical composition of West Decker Mine waters.

Enrichment of Selected Bacterial Populations

Acidophilic iron oxidizing bacteria were not isolated from the West Decker Dietz-1 and Dietz-2 coal samples that were analyzed. These organisms may be found within microzones in the coal or overburden where the existence of oxidizable sources of iron and sulfur are likely. Acidophilic iron oxidizing bacteria were isolated from the exposed coal seam wall near West Decker's sump pond. Pyritic materials in overburden and coal, once exposed to air, will spontaneously oxidize. Regions of low pH, resulting from these localized oxidations, present favorable growth conditions for acidophilic thiobacilli.

Enrichment for iron and sulfur oxidizing bacteria in the variety of locations described in Section V was successfully accomplished. Acidophilic iron oxidizing bacteria (probably T. ferrooxidans) were detected at all locations. It appears that these organisms are much more ubiquitous than previously thought. According to Starkey (1966) acidophilic thiobacilli are not isolated from non-acidic environments. It therefore seems unlikely that the environment in which these organisms were detected would be favorable for their growth; the results reported in the next section have some bearing

upon this. Acidophilic iron and sulfur oxidizing bacteria were originally considered to be associated with areas where mining operations were proceeding, and that it was possible that the effluent from the settling pond was acting as one source of these organisms in the Tongue River flood plain. Their presence in the Tongue River sediments upstream from the West Decker Mine may be due to discharges from other mining operations.

Growth and Survival of Acidophilic Iron Oxidizing Bacteria

Growth curves of the two organisms, TF-1 and TF-2, isolated and purified on ISP medium, are shown in Figure 6. These curves compare very favorably to one of *T. ferrooxidans* given in Silverman and Lundgren (1959). Because these isolates were capable of growth in an inorganic medium (9K salts) supplemented with either ferrous iron or elemental sulfur, and showed similarities to previously studied organisms, they appeared to be typical strains of *T. ferrooxidans*.

Survival of isolates TF-1 and TF-2 in sterile settling pond water is shown in Table 11. These organisms did not survive well in mine water. The presence of acidophilic iron oxidizing bacteria in the waters of the West Decker Mine settling pond may be due to a continuous input in the influent waters; however, they were still detected in the water column when there was no influent. The survival of bacteria in sediments has been documented (Hendricks 1971; Kaneko and Colwell 1973; Van Donsel and Geldreich 1971) and, considering how widely these organisms were distributed in sediments of the Tongue River flood plain, long term survival therein seems likely. It is possible that upwelling from the sediments could have redistributed these organisms in the water column of the pond.

TABLE 11. SURVIVAL OF TWO ACIDOPHILIC IRON OXIDIZING ISOLATES, TF-1 AND TF-2, IN STERILE SETTLING POND WATER[a]

Organism	Initial MPN/100 ml	1 hr	3 hr	17 hr	24 hr
TF-1	1.6×10^6	--	--	--	0
	2.4×10^6	--	--	--	0
TF-2	$>2.4 \times 10^8$	$>2.4 \times 10^8$	1.6×10^8	1.4×10^5	4.9×10^3

[a] Viable organisms were determined by the MPN technique.

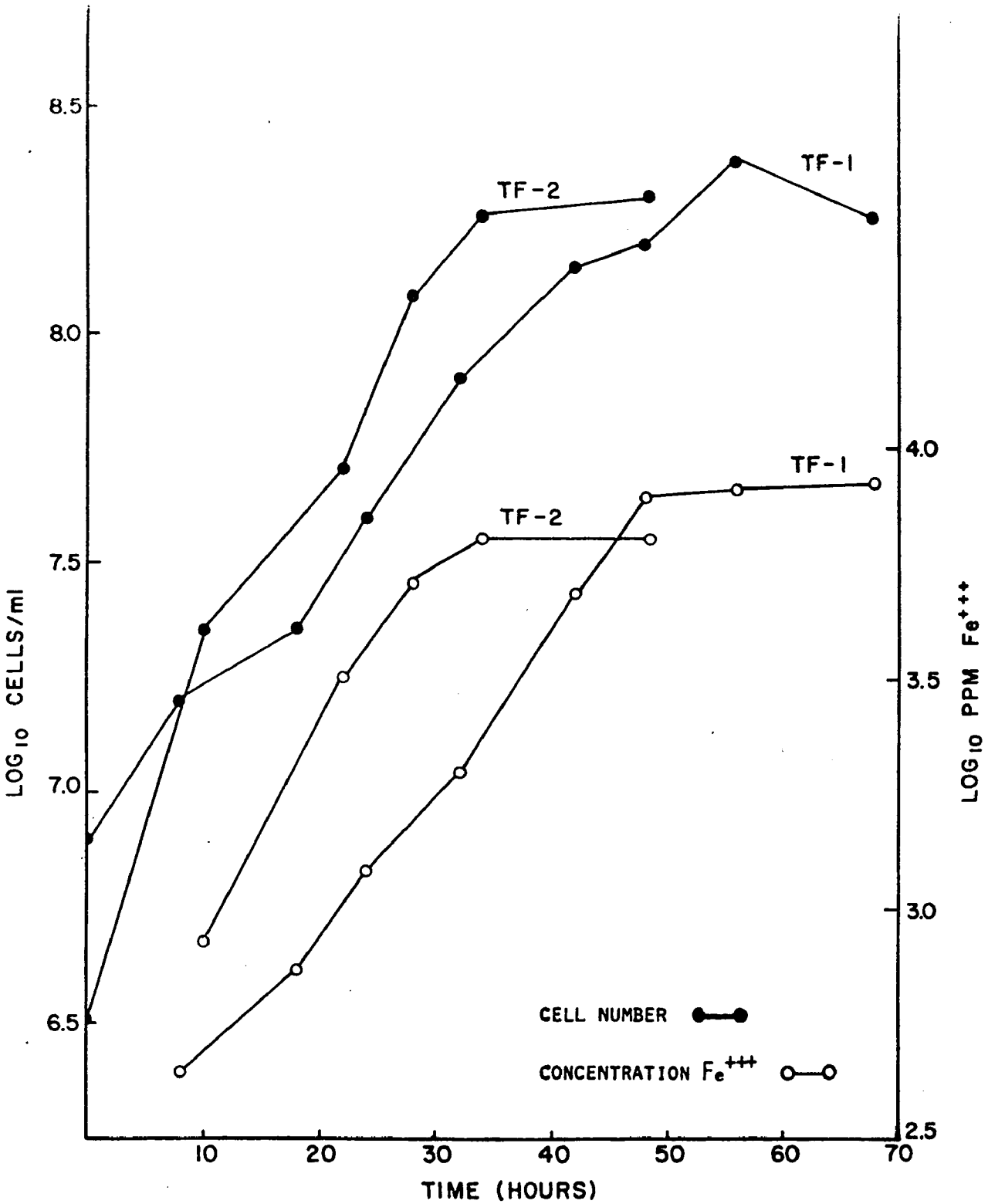


Figure 6. Growth curve of two isolates tentatively identified as Thiobacillus ferrooxidans, TF-1 and TF-2.

Leaching Experiments with *Thiobacillus ferrooxidans*

Results of leaching experiments presented in Table 12 indicate that little pyrite was available for microbial oxidation. The first and second sets of experimental flasks showed insignificant differences between the pH values and titratable acidity of untreated samples and controls (sodium azide added to inhibit biological activity). A decrease in pH values was more significant in the third set of flasks compared to controls. Iron, added to serve as an energy source, appeared to promote the biological production of acid. It was impossible to measure titratable acidity of this set of flasks because the addition of base spontaneously oxidized the ferrous iron. Suspended ferric iron interfered with end-point detection of the titration. This experiment, and the one attempting to enrich for growth of acidophilic iron oxidizing bacteria in coal samples from Decker, indicated that the energy sources in coal may be non-uniform; that is, may be located in specific microzones.

Microzonal activity could have been of significance in connection with the analyses of coal by Chadwick et al. (1975) for sulfur and trace elements in the Rosebud and McKay coal seams (part of the Fort Union formation) near Colstrip. These analyses indicated that sulfur and the heavy metals: antimony, arsenic, beryllium, cadmium, mercury, nickel, and selenium are "markedly enriched" at the base of the coal seams. Chromium, copper, germanium, and zinc also showed concentration tendencies in some of the test cores. These metals may also occur as inclusions, intergrowths, or replacement bodies in pyrite (Chadwick et al. 1975), perhaps subjecting them to direct microbial attack. Pockets of pyrite or other metal sulfides in the coal could create microzones of acid production by thiobacilli, resulting in solubilization of heavy metals. Although analyses of this type have not been carried out on Decker coal, the possibility of a similar situation exists.

Similar leaching experiments with Decker overburden were not performed in the present study. The potential importance of pyritic materials in the overburden when compared to amounts present in the coal itself is recognized. Perhaps the difficulty in demonstrating acid production from T. ferrooxidans incubated in coal further demonstrates the possible significance of pyritic materials in the overburden.

Physiological Studies of *Thiobacillus ferrooxidans* Using Respirometry

Respirometric experiments were undertaken to characterize optimum growth conditions of our isolates from the West Decker Mine which were tentatively identified as T. ferrooxidans. The results, presented in Table 13, indicated that the isolates TF-1 and TF-2 resembled T. ferrooxidans in their preference for iron as an energy source at low pH. Only marginal activity was noted with reduced sulfur compounds at low pH, and virtually no respiration was measured at pH values corresponding to West Decker Mine water environments (pH 8). T. ferrooxidans is capable of oxidizing reduced sulfur compounds but sometimes requires a considerable adaptation period in order to do so (Tuovinen and Kelley 1972). Our data strongly suggested that these isolates were typical T. ferrooxidans, capable of growth only at low pH. Their

TABLE 12. INCUBATION OF THIOBACILLUS FERROOXIDANS WITH COAL

Set	Treatment	Initial		33 Days		Net change	
		pH	Acidity (mg/liter $CaCO_3$)	pH	Acidity (mg/liter $CaCO_3$)	pH	Acidity (mg/liter $CaCO_3$)
1.	None	7.09 ± 0.26	249 ± 6	6.09 ± 0.03	336 ± 69	-1.00	+87
	Sodium azide	7.21 ± 0.27	221 ± 15	6.42 ± 0.12	307 ± 136	-0.79	+86
2.	None	4.47 ± 0.09	397 ± 37	5.32 ± 0.10	425 ± 65	+0.85	+28
	Sodium azide	4.45 ± 0.20	422 ± 61	5.38 ± 0.03	411 ± 86	+0.93	-11
3.	Iron	3.77 ± 0.07	--	2.24 ± 0.13	--	-1.53	--
	Iron + sodium azide	3.93 ± 0.08	--	2.86 ± 0.08	--	-1.07	--

TABLE 13. RESULTS OF RESPIROMETRY STUDIES ON TWO ISOLATES OF THIOBACILLUS FERROOXIDANS

Energy source	pH	$\mu l\ O_2$ taken up $\cdot\ h^{-1} \cdot (5\times10^9)^{-1}$ cells	
		TF_1	TF_2
Fe^{++}	3	1238	1441
Fe^{++} + glucose-yeast extract	3	1668	1455
glucose-yeast extract	3	1.2	1.1
S_2O_3	5	3.4	3.4
S_2O_3	8	2.0	1.3
S^o	3	13.6	6.2
S^o	5	12.8	20.9
S^o	8	0.4	5.3

presence in the West Decker Mine was possibly due to their activity in low pH microzones somewhere in the coal-bearing strata.

Sulfate Reduction in Pond Sediments

The detection of sulfide in water and sediment samples of the settling pond system and in groundwaters from a limited number of wells in areas adjacent to the mine prompted a study of sulfate reduction by bacteria. Sulfate reducing bacteria are responsible for the generation of hydrogen sulfide in environments conducive to their growth. In addition to certain ranges of temperature and pH values, these bacteria require simple organic compounds or hydrogen as sources of energy, inorganic nutrients, and reducing conditions (Eh = -0.2V). Sulfate is also required, not only for assimilation as a nutrient, but because it acts as the terminal electron acceptor for respiration. Dissimilatory (or respiratory) sulfate reduction results in the production of hydrogen sulfide which has extensive industrial, economic, and ecological effects (Postgate 1965). Consequently, a voluminous amount of literature, ranging from technical reports to complete academic treatises, exists on these organisms (see reviews - Postgate 1959, 1960, and 1965).

Results of the *in situ* measurements of sulfate reduction in sediment samples of the West Decker Mine settling pond are presented in Table 14.

TABLE 14. RESULTS OF *IN SITU* MEASUREMENTS OF SULFATE REDUCTION IN SEDIMENTS OF THE WEST DECKER MINE SETTLING POND

Dates experiment initiated	Location within pond	Treatment	Duration (h)	$SO_4^=$ Concn (mg/liter)	Temp °C	Dry weight of 1.0 ml sediment (g)	$H_2{}^{35}SO_4$ input (cpm/liter)	Cd ^{35}S trapped (cpm/liter)[a]	Rate of sulfate reduction (mg H_2S/liter sediment/day)[b]
September 1976	Site 2	None	48	150	16.2	0.288	3.03×10^7	4.84×10^5	1.27
	Site 3	None	48	150	17.1	0.264	3.03×10^7	1.52×10^6	3.98
October 1976	Site 3	None	43.5	180	6.1	0.298	2.93×10^7	2.92×10^6	10.50
	Site 3	Lactate spiked (300 mg/liter)	43.5	180	6.1	0.298	2.93×10^7	9.68×10^6	34.85
December 1976	Site 3	None	145.5	44	1.0	0.443	6.25×10^6	1.49×10^6	1.83
	Site 3	Lactate spiked (300 mg/liter)	--	--	--	--	5.64×10^6	1.12×10^6	1.54

[a] September, 40% counts recovered; October and December, >70% counts recovered.

[b] Calculated by method of Ivanov (1964): mg $H_2S/liter^{-1}/day^{-1}$ = $\frac{(\text{Concentration } SO_4)\ (\text{Radioactivity } H_2{}^{35}S)\ (24\ h)\ (1.06)}{(\text{Radioactivity } H_2{}^{35}SO_4)\ (\text{Duration of experiment})}$

Two locations in the pond were compared in September 1976. Surface sediment at the southern end (Site 3) had a higher rate of bacterial dissimilatory sulfate reduction than that at the northern end (Site 2). All subsequent rate determinations were conducted on samples from the southern end.

Because sulfate reducing bacteria require organic materials for growth, insufficient amounts of these substances limit the process of sulfate reduction. In October and December 1976, experiments were undertaken to test possible carbon limitation in the West Decker settling pond sediments. In the October experiment, addition of a lactate spike (a readily utilizable energy source) of 300 mg/liter resulted in a greater than threefold increase in the rate of sulfate reduction. This stimulation, due to the presence of excess carbonaceous substrate, was not observed in December because another factor, possibly temperature, limited the bacterial reduction of sulfate. Few direct investigations of rates of sulfate reduction appear in the literature (Trudinger et al. 1972). Sand et al. (1975) developed a radioisotope assay for estimating populations of sulfate reducing bacteria in water and sediments sampled in the vicinity of a pulp mill effluent. The technique measured sulfate reduction indirectly, that is, by the disappearance of radioactive sulfate rather than the appearance of labeled hydrogen sulfide. Chebotarev (1974), using an isotope method similar to ours to evaluate the bacterial production of hydrogen sulfide in two karst lakes, considered rates of 6.2 and 13.3 mg H_2S per kilogram of sediment per day to be quite vigorous. October 1976 rates exceeded those reported by Chebotarev, indicating that relatively high rates of sulfate reduction were occurring in the sediments of the West Decker settling pond and, consequently, large amounts of hydrogen sulfide were being generated.

The liberation of hydrogen sulfide has major ecological consequences; however, only those pertaining to water quality alterations will be discussed. The sulfide ion, produced by the dissociation of hydrogen sulfide, reacts with heavy metals and other elements to form insoluble sulfide minerals; possible toxicants are thereby effectively removed from solution. In laboratory experiments, Miller (1950) demonstrated the formation of the sulfides of antimony, bismuth, cobalt, cadmium, iron, lead, nickel, and zinc as a result of the activities of Desulfovibrio desulfuricans, which is the most common and best known species of sulfate reducing bacteria. Laboratory experiments by Baas Becking and Moore (1961) showed that a variety of metal sulfide minerals could be formed by sulfate reduction. Tuttle et al. (1969) showed that a wood dust dam containing an active population of sulfate reducing bacteria increased the pH value of acid mine waters coming into contact with it. In addition, the precipitation of iron (as ferrous sulfide) was promoted.

To test whether the West Decker Mine settling pond was acting as a trap for heavy metals, settling pond sediments were analyzed using the procedures of Vasudevamurthy et al. (1956). We extracted 2.13 mg of metal bound sulfides per g (dry weight) of settling pond sediment. Sorokin (1968) felt that sulfides in concentrations of 410 to 4520 mg per liter of sediment (making up as much as 1% of the dry weight of the sediment) were "enormous" quantities. Thus, the process of bacterial dissimilatory sulfate reduction appears to be important in the precipitation of metals in mine waters flowing into the pond before their discharge into the Tongue River flood plain.

SECTION VII

SUMMARY

The study described herein was designed to elucidate the function of a surface coal mine settling pond in altering water quality. Although three approaches to this investigation were attemped, the erratic operation of the pond during the course of this study limited how completely these approaches could be undertaken. Nonetheless, certain conclusions and tentative recommendations can be made. Clearly, the mine drainage waters underwent alterations within the settling pond. It is important that the settling pond system be maintained and improved so that the effluent from the mine can be nonhazardous to the receiving environment and can serve useful purposes.

CONCLUSIONS

(1) Influent water was not continually supplied to the pond. When it was, however, effluent was discharged into the Tongue River flood plain. Although concentrations of total dissolved solids, bicarbonate, sodium, sulfate, and nitrogen species were higher in the discharge water than in the Tongue River, the quality of the effluent was sufficient to meet Montana state-imposed criteria for discharge water from a surface coal mine.

(2) This study supported the findings of Whalen and Leathe (1976) that, under the conditions operable during the course of this investigation, West Decker Mine produced no measurable impact upon the Tongue River or Tongue River Reservoir. However, coal mining in the West Decker area may be impacting groundwater quality since the settling pond water, considered "altered groundwater," differed chemically from surrounding groundwaters. In ionic composition, pond water was intermediate between the local groundwater and the surface waters of the nearby Tongue River and Tongue River Reservoir.

(3) The West Decker Mine settling pond provided a unique opportunity to study the microbial sulfur cycle. One of the most significant observations to emerge was the consistent detection of the bacterium *Thiobacillus ferrooxidans*. Cultures of this organism were physiologically similar to those associated with acid drainage problems in eastern and midwestern coal mining regions. Acidic conditions, however, were not observed within the pond waters or drainage waters pumped into the West Decker Mine settling pond. This is attributable to the high buffering capacity of waters in this region. It was difficult to demonstrate acid production in the laboratory using samples of West Decker Mine coal. *T. ferrooxidans* may be restricted to pyrite-containing microzones in the overburden and coal strata. It is

possible that either this organism entered the pond via drainage waters and survived in the sediments, or that it is much more ubiquitous than previously reported in the literature.

(4) Other sulfur cycle bacteria are also important from a water quality standpoint. The relatively high numbers and activity measurements of sulfate reducing bacteria, as well as the significant amount of metal-bound sulfides in the settling pond sediments, make a strong case for the contributions of these organisms toward precipitation of toxic metals.

(5) The settling pond water was sampled on nine occasions for mercury, arsenic, selenium, lead, and cadmium. Recommended limits for aquatic life have not been firmly established for all of these potentially toxic elements, but of those that have been, only mercury exceeded the acceptable level. Average mercury concentrations in settling pond samples ranged from 0.09 to 0.81 μg per liter, which are considerably higher than that recommended for freshwater aquatic life and wildlife (0.05 μg/liter) by the U.S. Environmental Protection Agency (1977a).

(6) Blasting of mine overburden with ammonium nitrate explosives, and input of explosives in runoff from storage vats adjacent to the pond, periodically enriched the pond with nitrogen.

(7) Values for the sodium absorption ratio (SAR) calculated from chemical data of water samples collected from the West Decker Mine settling pond averaged below 8, in contrast to reports of relatively high ratios for groundwaters in the Decker area. The average SAR of pond water was not ideal for irrigation of mine spoil reclamation sites but would not cause adverse effects to vegetation and soil structure if sufficiently diluted.

(8) Instability of the settling pond limited the value of primary productivity measurements made from July to December 1976; data were insufficient to assess the trophic status of the pond. The major primary producers appeared to be benthic algal populations in summer 1976. The pond showed a potential for moderate productivity which probably was important in water quality improvements such as removal of inorganic nitrogen in the water column and oxidation of sulfide contributed by the influent.

(9) Algal bioassays indicated that the settling pond water was nutrient limited. Phosphorus limitation was observed in pond samples collected in autumn 1976 when concentrations of nitrogen were particularly high. Laboratory bioassays did not show inhibition of the test alga by heavy metals in these waters.

RECOMMENDATIONS

(1) Convenience, rather than careful planning, has been the major consideration in the design and operation of West Decker's settling pond. Hill (1976) has recently reviewed aspects of the physical function and design of surface mine sedimentation ponds. His discussion of design considerations such as "(1) capacity and size to meet water quality criteria,

(2) storage volume, (3) number of ponds, (4) location of pond, (5) shape of pond, (6) inlet design, (7) outlet design, (8) cleanout procedures, and (9) close down" would be helpful in improvement of the present operation at the West Decker Mine or in the initial design of sedimentation basins for Decker Coal Company's future mines.

(2) A two-pond system may be more efficient for improving water quality. A deeper primary pond could serve two purposes, one of which would be to facilitate sedimentation since it would be less subject to wind-driven mixing. Zones of anaerobiosis could develop in deeper waters and promote bacterial sulfate reduction which would remove heavy metals and other elements from solution. Design considerations should be such that adequate contact between metals and bacterially-generated sulfide is assured. A more shallow secondary pond would permit the biological uptake of nutrients such as those contributed from nitrogen explosives and carbonaceous materials in the coal. This recommendation should be evaluated by experimentation at the pilot plant level. Hill (1976) discussed the advantages of a multiple pond system from an engineering standpoint. The physical aspects of sedimentation pond design should be strongly interfaced with considerations of biological activity within the pond system.

(3) Settling ponds should be located as far from the main focus of mining activity as practical, and blasting material storage vats should not be located where their leachates would drain directly into the settling ponds. Nitrogen enrichment in the ponds, and high turbidity levels from fugitive dust, may be reduced thereby.

(4) In addition to a source of water for possible irrigation of reclamation sites, current plans call for the use of settling pond water to fill and maintain a northern pike spawning marsh. Influent water should not be used for these purposes because of the sulfide present. Immature stages of northern pike are particularly sensitive to hydrogen sulfide. Laboratory bioassays have indicated that 100 percent mortality occurred at 0.063 and 0.020 mg/liter of hydrogen sulfide for northern pike eggs and sac fry, respectively (Adelman and Smith 1970). The average levels of hydrogen sulfide detected in the influent exceeded these lethal limits by one order of magnitude. However, this same water, after being oxygenated in the settling pond, contained sulfide concentrations which were more tolerable for fish culture.

(5) Efforts should be made to maintain normal operation of the pond during those times when water must be supplied to the pike spawning marsh. Dredging of the pond followed by stagnation in winter 1976 resulted in increased amounts of certain chemical parameters and a markedly lower concentration of dissolved oxygen in the water column. Effluent from the settling pond at that time might have produced deleterious effects on a spawning marsh.

(6) The analytical methods used for the detection of mercury did not distinguish between the more toxic and less toxic forms; therefore, it is difficult to assess the potential effect mercury in the pond water may have on fishes. Mercury concentrations in the pond should be monitored because its forms are readily accumulated and retained in biological systems.

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APPENDIX

WATER CHEMISTRY AND ALGAL PRODUCTIVITY DATA, WEST DECKER MINE SETTLING POND, JULY 1976 - APRIL 1977

Table A-1. Water chemistry data for West Decker Mine settling pond, 27 July 1976.

(Chemical parameters are expressed in mg/liter unless otherwise specified.)

Site No.	Temperature °C	Specific Conductance µmho/cm, 25 C	pH	Turbidity NTU	Calcium	Magnesium	Sodium	Potassium
1	16.2	1495	7.42		73	47	185	6.5
2 Surface	not sampled							
3 Surface	22.5	1746	8.14		78	48	275	8.1
3 Bottom	24.2	1750	8.17		75	51	285	7.6
4	22.2	1739	8.18		83	59	245	8.7
5	not sampled							
6	--	1717	8.14		80	61	270	8.1

	Chloride	Fluoride	Sulfate	Total Alkalinity as $CaCO_3$	Total Hardness as $CaCO_3$	Silica as SiO_2	Dissolved Oxygen	Dissolved Iron
1	0.6	1.2	175	389	374	18.0	7.80	
2 Surface								
3 Surface	0.5	1.1	420	457	393	13.8	7.73	
3 Bottom	0.3	1.1	480	463	396	16.0	8.93	
4	0.3	1.0	410	454	449	14.1	8.31	
5								
6	0.8	1.1	180	455	449	15.7	--	

	Total Iron	Total Dissolved Solids	Total Non-Filterable Solids	Organic Carbon	Nitrate-Nitrogen	Nitrite-Nitrogen	Ammonia-Nitrogen	Total Nitrogen
1	0.145	1002	4.2	4.2	0.023	0.004	0.190	0.693
2 Surface								
3 Surface	0.163	1170	13.1	4.2	0.032	0.002	0.108	0.772
3 Bottom	0.190	1172	14.9	4.6	0.030	0.004	0.110	0.830
4	0.198	1165	15.7	18.2	0.339	0.003	0.104	0.779
5								
6	0.186	1150	27.1	4.6	0.402	0.001	0.115	0.832

	Inorganic Nitrogen	Organic Nitrogen	Orthophosphate Phosphorus	Total Phosphorus	Organic Phosphorus	Hg µg/ℓ	As µg/ℓ	Se µg/ℓ	Pb	Cd
1	0.217	0.476	0.025	0.036	0.011	0.85	1.6	0.4	<0.01	<0.001
2 Surface										
3 Surface	0.142	0.630	0.011	0.036	0.025	0.90	1.5	0.4	<0.01	<0.001
3 Bottom	0.144	0.686	0.009	0.057	0.048	0.73	1.2	0.4	<0.01	<0.001
4	0.446	0.333	0.006	0.042	0.036	0.79	<0.3	0.6	<0.01	<0.001
5										
6	0.518	0.314	0.009	0.038	0.029	0.87	1.3	0.4	<0.01	<0.001

Table A-2. Water chemistry data for West Decker Mine settling pond, 24 August 1976.

(Chemical parameters are expressed in mg/liter unless otherwise specified.)

Site No.	Temperature °C	Specific Conductance µmho/cm, 25 C	pH	Turbidity NTU	Calcium	Magnesium	Sodium	Potassium
1	17.1	1292	7.66	1	61.1	33	299	7.7
2 Surface	not sampled							
3 Surface	20.9	1258	8.47	2	39.6	34	273	6.3
3 Bottom	20.4	1260	8.47	1.5	41.3	32	339	7.1
4	20.0	1242	8.51	2	51.2	24	323	7.1
5	21.0	1259	8.52	1	41.3	28	315	7.4
6	20.5	1257	8.49	2	39.6	30	317	7.3

	Chloride	Fluoride	Sulfate	Total Alkalinity as $CaCO_3$	Total Hardness as $CaCO_3$	Silica as SiO_2	Dissolved Oxygen	Dissolved Iron
1	<1	2	262	442	287		5.05	
2 Surface								
3 Surface	<1	2	249	444	237		10.45	
3 Bottom	<1	2	256	444	234		10.23	
4	<1	2	231	444	227		10.05	
5	<1	2	240	447	218		10.20	
6	<1	2	225	447	221		10.30	

	Total Iron	Total Dissolved Solids	Total Non-Filterable Solids	Organic Carbon	Nitrate-Nitrogen	Nitrite-Nitrogen	Ammonia-Nitrogen	Total Nitrogen
1	0.240	866	2.2	4.5	1.190	0.05	0.746	2.367
2 Surface								
3 Surface	0.052	843	4.2	6.1	0.208	0.05	0.726	0.688
3 Bottom	0.038	844	5.3	5.5	0.210	0.04	0.588	1.358
4	0.085	832	10.5	4.7	0.215	0.04	0.436	1.181
5	0.065	844	5.1	3.4	0.205	0.04	0.318	0.967
6	0.051	842	6.7	4.8	0.232	0.05	0.332	1.150

	Inorganic Nitrogen	Organic Nitrogen	Orthophosphate Phosphorus	Total Phosphorus	Organic Phosphorus	Hg µg/ℓ	As µg/ℓ	Se µg/ℓ	Pb	Cd
1	1.986	0.381	0.017	0.034	0.017	0.55	3.7	0.6	<0.01	<0.002
2 Surface										
3 Surface	0.984	undetectable	undetectable	0.017	0.017	0.38	2.0	0.5	<0.01	<0.002
3 Bottom	0.838	0.520	undetectable	0.020	0.020	0.42	2.2	0.4	<0.01	<0.002
4	0.691	0.490	undetectable	0.030	0.030	90.	2.2	0.4	<0.01	<0.002
5	0.536	0.431	0.004	0.018	0.014	0.33	2.0	0.5	<0.01	<0.002
6	0.614	0.536	undetectable	0.017	0.017	0.42	1.9	0.4	<0.01	<0.002

Table A-3. Water chemistry data for West Decker Mine settling pond, 19 September 1976.

(Chemical parameters are expressed in mg/liter unless otherwise specified.)

Site No.	Temperature °C	Specific Conductance µmho/cm, 25 C	pH	Turbidity NTU	Calcium	Magnesium	Sodium	Potassium
1	14.1	1273	7.44	5	68.9	23.4	200	5.2
2 Surface	not sampled							
3 Surface	17.1	1313	8.37	5	68.0	21.5	210	5.2
3 Bottom	16.2	1283	8.38	5	68.0	18.5	220	5.2
4	16.1	1293	8.30	5	66.4	19.5	210	5.4
5	not sampled							
6	15.9	1323	8.34	5	65.2	21.7	210	5.4

	Chloride	Fluoride	Sulfate	Total Alkalinity as $CaCO_3$	Total Hardness as $CaCO_3$	Silica as SiO_2	Dissolved Oxygen	Dissolved Iron
1	5.5	2.0	360	445	268	18.0	6.7	
2 Surface								
3 Surface	5.6	1.9	290	456	258	11.8	10.7	
3 Bottom	5.7	2.0	315	457	246	12.0	10.8	
4	5.6	1.8	300	456	246	12.4	11.0	
5								
6	5.2	1.8	240	459	252	12.4	10.2	

	Total Iron	Total Dissolved Solids	Total Non-Filterable Solids	Organic Carbon	Nitrate-Nitrogen	Nitrite-Nitrogen	Ammonia-Nitrogen	Total Nitrogen
1	0.100	853	1.7		0.194	0.027	0.545	0.587
2 Surface								
3 Surface	0.050	880	5.4		0.118	0.010	0.166	0.426
3 Bottom	0.059	860	6.4		0.126	0.012	0.140	0.533
4	0.056	860	13.1		0.057	0.013	0.169	0.375
5								
6	0.059	886	9.2		0.116	0.012	0.148	0.367

	Inorganic Nitrogen	Organic Nitrogen	Orthophosphate Phosphorus	Total Phosphorus	Organic Phosphorus	Hg µg/ℓ	As µg/ℓ	Se µg/ℓ	Pb	Cd
1	0.766	not detectable	0.020	0.025	0.005	0.11	3.3	<0.3	<0.1	<0.005
2 Surface										
3 Surface	0.294	0.132	0.001	0.021	0.020	0.13	1.5	<0.3	<0.1	<0.005
3 Bottom	0.278	0.255	0.003	0.024	0.021	0.53	1.6	<0.3	<0.1	<0.005
4	0.239	0.136	0.003	0.023	0.020	0.48	1.2	<0.3	<0.1	<0.005
5										
6	0.276	0.091	0.002	0.024	0.022	0.42	1.2	<0.3	<0.1	<0.005

Table A-4. Water chemistry data for West Decker Mine settling pond, 21 October 1976.

(Chemical parameters are expressed in mg/liter unless otherwise specified.)

Site No.	Temperature °C	Specific Conductance µmho/cm, 25 C	pH	Turbidity NTU	Calcium	Magnesium	Sodium	Potassium
1	13.0	1220	7.41	1.4	73.4	26.8	190	4.5
2 Surface	not sampled							
3 Surface	6.1	1395	8.47	4.0	44.1	36.6	250	6.0
3 Bottom	6.4	1386	8.46	4.3	44.1	37.6	250	5.8
4	6.6	1403	8.47	4.4	38.9	39.7	250	5.8
5	6.4	1405	8.46	5.0	36.1	42.4	240	5.6
6	6.1	1407	8.43	4.4	36.1	40.5	250	5.6

	Chloride	Fluoride	Sulfate	Total Alkalinity as $CaCO_3$	Total Hardness as $CaCO_3$	Silica as SiO_2	Dissolved Oxygen	Dissolved Iron
1	4.9	1.5	228	418	294	17.4	0.7	
2 Surface								
3 Surface	5.5	1.9	255	493	260	9.8	11.2	
3 Bottom	6.2	2.0	235	496	264	10.8	11.1	
4	5.8	2.0	280	493	260	8.4	11.3	
5	5.5	1.9	265	498	264	10.6	11.1	
6	5.8	1.9	280	498	256	10.6	11.0	

	Total Iron	Total Dissolved Solids	Total Non-Filterable Solids	Organic Carbon	Nitrate-Nitrogen	Nitrite-Nitrogen	Ammonia-Nitrogen	Total Nitrogen
1	0.334	814	4.63		0.354	0.001	0.244	0.790
2 Surface								
3 Surface	0.125	930	22.43		0.430	0.020	0.180	0.930
3 Bottom	0.125	925	86.93		0.425	0.020	0.170	1.141
4	0.121	936	18.85		0.551	0.019	0.176	1.201
5	0.120	937	23.64		0.443	0.022	0.173	0.764
6	0.126	938	17.20		0.688	0.022	0.198	1.759

	Inorganic Nitrogen	Organic Nitrogen	Orthophosphate Phosphorus	Total Phosphorus	Organic Phosphorus	Hg µg/ℓ	As µg/ℓ	Se µg/ℓ	Pb	Cd
1	0.599	0.191	0.001	0.047	0.046	<0.05	1.5	<0.3	<0.1	<0.005
2 Surface										
3 Surface	0.630	0.300	0.007	0.043	0.036	0.20	1.9	0.4	<0.1	<0.005
3 Bottom	0.615	0.526	0.007	0.046	0.039	--	--	--	<0.1	<0.005
4	0.746	0.455	0.005	0.061	0.056	0.22	1.5	0.3	<0.1	<0.005
5	0.638	0.126	0.005	0.045	0.040	0.09	1.8	<0.3	<0.1	<0.005
6	0.908	0.851	0.007	0.043	0.036	0.11	1.9	0.5	<0.1	<0.005

Table A-5. Water chemistry data for West Decker Mine settling pond, 18 November 1976.

(Chemical parameters are expressed in mg/liter unless otherwise specified.)

Site No.	Temperature °C	Specific Conductance µmho/cm, 25 C	pH	Turbidity NTU	Calcium	Magnesium	Sodium	Potassium
1	not sampled							
2 Surface		1515	8.48	13.2	30.1	38.0	300	6.0
3 Surface		1703	8.43	22.5	29.7	40.8	330	6.5
3 Bottom		2083	8.39	102	24.1	31.7	430	6.8
4		1218	8.47	12.6	25.7	33.0	240	6.5
5		1689	8.41	19.5	28.1	39.5	340	6.5
6								

	Chloride	Fluoride	Sulfate	Total Alkalinity as $CaCO_3$	Total Hardness as $CaCO_3$	Silica as SiO_2	Dissolved Oxygen	Dissolved Iron
1								
2 Surface	5.7	2.0	215	567	186	7.0	10.50	
3 Surface	0.3	2.4	230	633	197	8.2	10.70	
3 Bottom	0.4	2.9	260	846	154	8.4	10.48	
4	7.2	2.0	180	623	161	6.0	9.39	
5	1.1	2.4	225	689	191	8.4	10.16	
6								

	Total Iron	Total Dissolved Solids	Total Non-Filterable Solids	Organic Carbon	Nitrate-Nitrogen	Nitrite-Nitrogen	Ammonia-Nitrogen	Total Nitrogen
1								
2 Surface	0.086	1015	10.92		15.9	0.248	0.92	19.3
3 Surface	0.116	1141	18.89		27.3	0.240	1.13	30.4
3 Bottom	0.131	1396	47.40		30.6	0.243	1.11	35.3
4	0.086	816	10.49		14.5	0.200	0.83	17.4
5	0.114	1132	19.92		27.6	0.260	1.15	31.2
6								

	Inorganic Nitrogen	Organic Nitrogen	Orthophosphate Phosphorus	Total Phosphorus	Organic Phosphorus	Hg µg/ℓ	As µg/ℓ	Se µg/ℓ	Pb	Cd
1										
2 Surface	17.1	2.2	0.006	0.038	0.032	0.08	1.9	1.2	<0.1	<0.005
3 Surface	28.7	1.7	0.005	0.058	0.053	0.09	1.7	1.3	<0.1	<0.005
3 Bottom	32.0	3.3	0.006	0.075	0.069	0.09	2.2	2.1	<0.1	<0.005
4	15.5	1.9	0.005	0.039	0.034	0.09	1.5	1.2	<0.1	<0.005
5	29.0	2.2	0.006	0.067	0.061	<0.05	1.6	1.4	<0.1	<0.005
6										

Table A-6. Water chemistry data for West Decker Mine settling pond, 14 December 1976.
(Chemical parameters are expressed in mg/liter unless otherwise specified.)

Site No.	Temperature °C	Specific Conductance µmho/cm, 25 C	pH	Turbidity NTU	Calcium	Magnesium	Sodium	Potassium
1	not sampled							
2 Surface	1.0	2438	7.83	5.3	42.1	46.8	420	8.5
2 Bottom	1.0	2617	7.83	5.1	42.5	48.0	440	8.6
3 Surface	not sampled							
3 Mid-depth	2.5	2600	7.87	4.5	40.9	50.0	430	8.9
6	not sampled							

	Chloride	Fluoride	Sulfate	Total Alkalinity as $CaCO_3$	Total Hardness as $CaCO_3$	Silica as SiO_2	Dissolved Oxygen	Dissolved Iron
1								
2 Surface	0.1	3.1	310	916	234	8.2	2.28	
2 Bottom	0.1	3.6	335	914	239	9.6	2.33	
3 Surface								
3 Mid-depth	0.1	3.9	305	940	246	10.4	2.07	
6								

	Total Iron	Total Dissolved Solids	Total Non-Filterable Solids	Organic Carbon	Nitrate-Nitrogen	Nitrite-Nitrogen	Ammonia-Nitrogen	Total Nitrogen
1								
2 Surface	0.069	1633	10.36		9.30	0.204	2.24	13.74
2 Bottom	0.092	1753	16.59		8.54	0.260	2.55	13.43
3 Surface								
3 Mid-depth	0.083	1742	8.54		8.98	0.325	2.31	13.79
6								

	Inorganic Nitrogen	Organic Nitrogen	Orthophosphate Phosphorus	Total Phosphorus	Organic Phosphorus	Hg µg/ℓ	As µg/ℓ	Se µg/ℓ	Pb	Cd
1										
2 Surface	14.55	not detectable	0.004	0.026	0.022	0.36	1.4	2.3	<0.1	<0.005
2 Bottom	11.35	2.08	0.004	0.043	0.039	0.30	1.2	2.2	<0.1	<0.005
3 Surface										
3 Mid-depth	11.61	2.18	0.003	0.032	0.029	0.18	1.1	2.5	<0.1	<0.005
6										

Table A-7. Water chemistry data for West Decker Mine settling pond, 12 January 1977.

(Chemical parameters are expressed in mg/liter unless otherwise specified.)

Site No.	Temperature °C	Specific Conductance µmho/cm, 25 C	pH	Turbidity NTU	Calcium	Magnesium	Sodium	Potassium
1	not sampled							
2 Surface	0.5	2889	8.05		72	59.5	490	
2 Bottom	0.5	2958	8.00		74	59.3	530	
3 Surface	not sampled							
3 Mid-depth	0.0	3222	7.97		78	65.9	550	
6	not sampled							

	Chloride	Fluoride	Sulfate	Total Alkalinity as $CaCO_3$	Total Hardness as $CaCO_3$	Silica as SiO_2	Dissolved Oxygen	Dissolved Iron
1								
2 Surface			494	1153	316		2.45	0.019
2 Bottom			494	1188	317		2.42	0.015
3 Surface								
3 Mid-depth			588	1277	348		2.04	0.015
6								

	Total Iron	Total Dissolved Solids	Total Non-Filterable Solids	Organic Carbon	Nitrate-Nitrogen	Nitrite-Nitrogen	Ammonia-Nitrogen	Total Nitrogen
1								
2 Surface	0.056	1936	5.56		10.36	0.245	3.70	14.46
2 Bottom	0.105	1982	18.35		9.78	0.220	3.70	14.10
3 Surface								
3 Mid-depth	0.128	2159	--		10.82	0.178	3.94	15.30
6								

	Inorganic Nitrogen	Organic Nitrogen	Orthophosphate Phosphorus	Total Phosphorus	Organic Phosphorus	Hg µg/ℓ	As µg/ℓ	Se µg/ℓ	Pb	Cd
1										
2 Surface	14.30	0.16	0.009	0.033	0.024	0.20	1.1	2.3	<0.2	<0.005
2 Bottom	13.70	0.40	0.009	0.054	0.045	0.18	0.9	2.0	<0.2	<0.005
3 Surface										
3 Mid-depth	14.94	0.36	0.006	0.063	0.057	0.39	1.4	2.3	<0.2	<0.005
6										

Table A-8. Water chemistry data for West Decker Mine settling pond, 9 February 1977.

(Chemical parameters are expressed in mg/liter unless otherwise specified.)

Site No.	Temperature °C	Specific Conductance µmho/cm, 25 C	pH	Turbidity NTU	Calcium	Magnesium	Sodium	Potassium
1	not sampled							
2 Surface	1.5	2320	7.94				700	9.7
2 Bottom	3.0	3000	7.95				830	11.4
3 Surface	not sampled							
3 Mid-depth	0.5	3120	7.92				830	11.4
6	not sampled							

	Chloride	Fluoride	Sulfate	Total Alkalinity as $CaCO_3$	Total Hardness as $CaCO_3$	Silica as SiO_2	Dissolved Oxygen	Dissolved Iron
1								
2 Surface			455	885			1.19	
2 Bottom			581	1005			1.49	
3 Surface								
3 Mid-depth			612	1086			1.23	
6								

	Total Iron	Total Dissolved Solids	Total Non-Filterable Solids	Organic Carbon	Nitrate-Nitrogen	Nitrite-Nitrogen	Ammonia-Nitrogen	Total Nitrogen
1								
2 Surface	0.104	1554	5.39		6.19	0.210	4.66	13.63
2 Bottom	0.079	2010	8.36		3.78	0.018	2.00	7.87
3 Surface								
3 Mid-depth	0.051	2090	8.24		3.09	0.015	2.70	9.28
6								

	Inorganic Nitrogen	Organic Nitrogen	Orthophosphate Phosphorus	Total Phosphorus	Organic Phosphorus	Hg µg/ℓ	As µg/ℓ	Se µg/ℓ	Pb	Cd
1										
2 Surface	11.06	2.57	0.003	0.029	0.026	0.31	1.1	3.1	<0.1	<0.005
2 Bottom	5.80	2.07	0.005	0.037	0.032	0.28	1.3	2.6	<0.1	<0.005
3 Surface										
3 Mid-depth	5.81	3.48	0.004	0.027	0.023	0.14	1.3	2.2	<0.1	<0.005
6										

Table A-9. Water chemistry data for West Decker Mine settling pond, 6 April 1977

Site No.	Hg (μg/liter)	As (μg/liter)	Se (μg/liter)	Pb (mg/liter)	Cd (mg/liter)
1	0.58	1.6	<0.3	<0.1	<0.005
2 Surface	0.28	1.2	0.3	<0.1	<0.005
3 Surface	0.25	1.2	0.3	<0.1	<0.005
6	0.38	1.1	0.5	<0.1	<0.005

Table A-10. Productivity estimates at Site 3, West Decker Mine settling pond

A. Diurnal oxygen curves for community productivity

Date of measurement	Gross primary productivity (g $O_2/m^2/day$)	Respiration (g $O_2/m^2/day$)	Net primary productivity (g $O_2/m^2/day$)
27-28 July 1976	8.44	8.64	0
23-24 August 1976	7.63	5.52	2.11
19-20 September 1976	4.57	1.32	3.25
20-21 October 1976	0.23	0.58	0
17-18 November 1976	0.43	1.22	0
14-15 December 1976	0.16	0.47	0

B. Radioactive carbon uptake for planktonic productivity

Date of measurement	Productivity (g $C/m^2/day$)
28 July 1976	8.83×10^{-2}
25 August 1976	4.42×10^{-2}
21 September 1976	1.16×10^{-1}
22 October 1976	1.29×10^{-2}
18 November 1976	2.49×10^{-2}
15 December 1976	0

Table A-11. Pigment concentrations of samples taken at Site 3, West Decker Mine settling pond

Date of sample Collection	Pigment concentrations (mg/m^3)				
	Chlorophyll a monochromatic	Chlorophyll a trichromatic	Chlorophyll b	Chlorophyll c	Phaeopigments
29 June 1976	0	1.059	0.076	0.144	3.044
28 July 1976	1.255	3.269	0	0.553	3.277
24 August 1976	1.202	1.309	0	0	0.107
19 September 1976	4.606				3.571
21 October 1976	3.155	4.979	0.293	2.034	2.961
18 November 1976	4.806	3.699	0.077	1.402	0
14 December 1976	6.675	6.961	0	1.515	0.174
12 January 1977	521.2	356.9	0	60.3	0
9 February 1977	35.82	42.00	0	1.59	8.81

Table A-12. Pigment and bioaccumulation data for periphytic populations, West Decker Mine settling pond

Location of artificial substrates	Period of incubation	Pigment concentrations (mg/m^3)					
		Chlorophyll a					
		Mono-chromatic	Tri-chromatic	Chlorophyll b	Chlorophyll c	Phaeo-pigments	Bioaccumulation (mg $C/m^2/day$)[a]
Mine effluent below flume	12 May-27 June 1976 (46 days)	54.49	25.89	6.63	16.84	46.99	100.2
	27 June-28 July 1976 (31 days)	13.54	10.33	0	0.91	4.81	300.5
	28 July-25 August 1976 (28 days)	0.351	0	0.027	0.332	1.956	17.2
Within pond near stand-pipe	21 September-22 Oct 1976 (31 days)	24.66	23.78	0	8.42	0.45	439.0
	23 October-18 Nov 1976 (26 days)	data inconsistent					

[a] mg C calculated as 50% of the ash free dry weight of organic matter (Stockner and Shortreed 1976).

Table A-13. Summary of results of laboratory algal bioassays, West Decker Mine settling pond

Test water	Sampling date	Limiting nutrient(s)
Effluent (Site 6)	3 November 1975	nitrogen and phosphorus; primarily nitrogen
	15 January 1976	phosphorus
	19 March 1976	nitrogen and phosphorus; primarily phosphorus
	21 April 1976	phosphorus
	25 May 1976	nitrogen, phosphorus and trace elements; primarily phosphorus
	1 July 1976	nitrogen and phosphorus; primarily nitrogen
	29 July 1976	nitrogen and phosphorus
	26 August 1976	nitrogen and phosphorus
	22 September 1976	nitrogen and phosphorus; primarily phosphorus
	23 October 1976	phosphorus
	19 November 1976	phosphorus
Influent (Site 1)[a]	15 January 1976	nitrogen and phosphorus
	21 April 1976	phosphorus
	25 May 1976	nitrogen and phosphorus[b]
	1 July 1976	nitrogen
	22 September 1976	nitrogen and phosphorus
South Pond (Site 3)	16 December 1976	phosphorus[c]

[a] Prior to June 1976 all influent samples collected from sump pond.

[b] Poor replication.

[c] Productivity very low.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

Chemical and microbiological investigations of the settling pond system at the West Decker Coal Mine in southeastern Montana were undertaken during 1975-1977. Concentrations of total dissolved solids, bicarbonate, sodium, sulfate, and nitrogen species in pond water were elevated in comparison to those in other nearby surface waters; however, it was concluded that these would not significantly impact the nearby Tongue River or Tongue River Reservoir. Constituents of the mine drainage waters underwent chemical changes within the pond which were at least in part due to the activities of microorganisms. The increase in pH value and in concentration of dissolved oxygen, partially attributed to photosynthesis within the pond, oxidized reduced chemical species such as sulfide and ferrous iron. The high numbers and activity measurements of sulfate reducing bacteria as well as the significant amount of metal-bound sulfides in pond sediments made a strong case for the contributions of these organisms to metal precipitation in, and possible detoxification of, pond waters. Acidophilic iron and sulfur bacteria, responsible for the production of acid mine drainage, were consistently detected within the pond system and other locations at Decker. However, no acidic conditions were observed, most likely because relatively high concentrations of mineral carbonate in the overburden neutralized acid formed from pyritic materials present.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Settling ponds Microbial changes Acid bacteria Coal mine runoff Mine drainage	Western energy dev. Coal mining Water quality	68/D 06/F

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