An investigation of microbiological parameters in the revegetation of coal mine spoils
by Rosemary Celia Stewart

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Microbiology
Montana State University

Abstract:
Soil samples were collected from six major plots at Colstrip, Montana during April, August, October and December, 1974. Three of these plots were native soil plots and the other three plots were spoils which resulted from the disturbance caused by coal strip mining.

At each plot, samples were collected from within the root region of several different plant species. Sampling experiments were conducted to determine the amount of variability which could be expected within the soil system itself and that which was due to different plant species.

Duplicate samples were collected in April 1974 and soil chemical and physical analyses were run on the duplicate samples.

The soil samples were air-dried, sieved and blended in the laboratory before performing analyses. Following this treatment, measurements were made of respiratory activity (by measuring O2 uptake on a Gilson respirometer), phosphatase activity, pectinolyase activity and the number of pectinolytic bacteria for each soil sample.

Attempts were then made to correlate the measurements of each microbiological parameter with the physical and chemical properties of the soil, seasonal variation and every other microbiological parameter. The phosphatase activity of native soil was the only microbiological parameter which correlated with several of the physical and chemical properties of the soil in April, 1974. Those correlations included positive correlations with percent clay and magnesium and negative correlations with potassium, calcium, sodium and percent silt. These correlations appear to be related to the clay, which may be adsorbing the enzyme onto its surface. Respiratory activity in native soil correlated with only one soil characteristic, water-holding capacity, and that was only for native soil. This relationship is related to the pore space of the soil. Spoils did not exhibit this correlation, lack of which could be attributed to the more heterogeneous textural types.

All four microbiological parameters were observed to exhibit significant seasonal variations for both native soil and spoils, although these seasonal variations did not coincide for all four parameters. Respiratory activity and the number of pectinolytic bacteria both tended to peak in October whereas phosphatase activity tended to peak in August and pectinolyase activity was high in August and peaked in December. The possibility of seasonal variation in physical and chemical characteristics of the soil is also suggested.

The variation in the four microbiological parameters were found to be not significantly due to different plant species.

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140

AN INVESTIGATION OF MICROBIOLOGICAL PARAMETERS IN THE REVEGATION OF COAL MINE SPOILS

by

ROSEMARY CELIA STEWART

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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in

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Approved:

____
Head, Major Department

____
Chairman, Examining Committee

____
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

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TABLE OF CONTENTS

	Page
VITA	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vii
LIST OF FIGURES	x
ABSTRACT	xi
Chapter	
1. INTRODUCTION	1
Statement of Purpose	3
2. MATERIALS AND METHODS	5
Study Area	5
Sampling	7
Soil Analyses	18
Respiration	19
Phosphatase	20
Pectinolyase	21
Pectinolytic plate counts	22
Isolation and Identification of Soil Organisms	25
Isolation of DNA and Determination of T_m	25
Statistics	27

Chapter Page

3. RESULTS 28

Variation in a Plot at large. 28

Variation Under a Particular Plant Species. 28

Variation in Sampling Techniques. 30

Soil Horizon Comparison 35

Soil Analyses 35

Water-holding Capacity for Optimal Respiratory Rate . . 43

Variability in Respiratory Rate Due to Quantity of Soil 43

Interrelationship of Respiratory Activity and Soil Chemical and Physical Properties 46

Respiratory Activity for Three Native and Three Spoils Plots. 46

Seasonal Variation of Respiratory Activity. 50

Effects of Air-drying Soil on Phosphatase Activity. . . 50

Interrelationship of Phosphatase Activity and Soil Chemical and Physical Properties. 54

Phosphatase Activity for Three Native and Three Spoils Plots. 54

Seasonal Variation of Phosphatase Activity. 57

Effects of Air-drying on Pectinolyase Activity 62

Interrelationship of Pectinolyase Activity and Soil Chemical and Physical Properties. 62

Pectinolyase Activity for Three Native and Three Spoil Plots 64

Seasonal Variation of Pectinolyase Activity 64

Chapter Page

Effects of Purifying Pectin on Selective Plating of Pectinolytic Bacteria 69

Effects of Air-drying Soil on Pectinolytic Bacteria Enumeration 72

Relative Proportion of Total Soil Bacterial Population Estimated as Pectinolytic Bacteria 72

Interrelationship of Pectinolytic Bacterial Population and Soil Chemical and Physical Properties 72

Pectinolytic Bacterial Population for Three Native and Three Spoils Plots 75

Seasonal Variation of the Number of Pectinolytic Bacteria 79

Interrelationship of Microbiological Parameters 82

Overall Analysis of Variance for the Microbiological Parameters 82

Isolation and Identification of Soil Organisms 87

Isolation of DNA and Determination of T_m to Identify a Soil Organism 87

4. DISCUSSION 89

5. SUMMARY 131

APPENDIX 135

LITERATURE CITED 156

LIST OF TABLES

Table		Page
1.	Sampling sites, description, dates sampled and plant species	8
2.	Six major plots and their descriptions	15
3.	Variation in a plot at large - Demo plot, August, 1974	29
4.	Variation under a particular plant species, Demo plot, August, 1974	31
5.	Variation under a plant species at different plots, April, 1974	32
6.	Variation of plants with sampling time - Demo and Native Range Unfertilized plots	33
7.	Analysis of variance for Quartered and Staked sampling treatments	39
8.	Soil horizon comparison - Demo plot, August 1974	40
9.	Significant correlations within soil analyses for native soils and spoils, April, 1974	42
10.	Water-holding capacity for optimal respiratory rate	44
11.	Variability in respiratory rate due to quantity of soil	45
12.	Analysis of variance due to plant and time for respiratory activity of six major plots	48
13.	Analysis of variance due to plant species for respiratory activity of six major plots	49
14.	Seasonal variation of respiratory activity of six major plots	51
15.	Significant correlations of seasonal variation for respiratory activity of six major plots	52
16.	Phosphatase activity for air-dried and field moisture treatments of soils	53

Table		Page
17.	Significant correlations between phosphatase activity and the soil analyses for native soils in April, 1974	55
18.	Analysis of variance due to plant species for phosphatase activity of six major plots	58
19.	Analysis of variance due to plant and time for phosphatase activity of six major plots	59
20.	Seasonal variation of phosphatase activity of six major plots	60
21.	Significant correlations of seasonal variation for phosphatase activity of six major plots	61
22.	Pectinolyase activity for air-dried and field moisture treatments of soils	63
23.	Analysis of variance due to plant species for pectinolyase activity of six major plots	66
24.	Analysis of variance due to plant and time for pectinolyase activity of six major plots	67
25.	Seasonal variation of pectinolyase activity for six major plots	68
26.	Significant correlations of seasonal variation for pectinolyase activity of six major plots	70
27.	Comparison of pectinolytic bacteria selective media with commercial pectin and purified pectin	71
28.	Number of pectinolytic bacteria for air-dried and field moisture treatments of soils - Manhattan silt loam	73
29.	Relative proportion of soil bacteria as pectinolytic bacteria	74
30.	Analysis of variance due to plant species for number of pectinolytic bacteria of six major plots	77

Table Page

31. Analysis of variance due to plant and time for number of pectinolytic bacteria of six major plots 78

32. Seasonal variation of the number of pectinolytic bacteria for six major plots. 80

33. Significant correlations of seasonal variation for numbers of pectinolytic bacteria of six major plots 81

34. Correlations of four microbiological parameters as measured at three native soil and three spoils plots during April, August, October and December, 1974. 83

35. Chi-square for the four microbiological parameters. . . . 85

36. Pooled variances of microbiological parameters. 86

37. Conversion of the number of pectinolytic bacteria to number of bacteria/gram of organic matter for April, 1974 123

38. Mean values of all four microbiological parameters for all sites and plants sampled. 136

39. Physiochemical properties of soils as determined April, 1974. 151

39b. Additional physiochemical properties of soils as determined April, 1974. 153

LIST OF FIGURES

Figure	Page

1. Photograph of Demo spoils plot 10
2. Photograph of Gouge spoils plot 11
3. Photograph of Topsoil Gradient spoils plot. 12
4. Photograph of BN native soil plot 13
5. Photograph of Native Range Unfertilized and Native Range Fertilized native soil plots 14
6. Respiration rate for sampling variation 34
7. Phosphatase enzymatic activity for sampling variation 36
8. Pectinolyase enzymatic activity for sampling variation 37
9. Pectinolytic plate count for sampling variation 38
10. Respiratory activity for six major plots. 47
11. Phosphatase activity for six major plots. 56
12. Pectinolyase activity for six major plots 65
13. Number of pectinolytic bacteria for six major plots 76
14. Linear regression of pectinolyase activity against pectinolytic plate count. 84
15. Normal probability plot of T_m for <u>Arthrobacter</u>. 88
16. Soil microbiology field sampling data sheet 155

ABSTRACT

Soil samples were collected from six major plots at Colstrip, Montana during April, August, October and December, 1974. Three of these plots were native soil plots and the other three plots were spoils which resulted from the disturbance caused by coal strip mining. At each plot, samples were collected from within the root region of several different plant species. Sampling experiments were conducted to determine the amount of variability which could be expected within the soil system itself and that which was due to different plant species.

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Attempts were then made to correlate the measurements of each microbiological parameter with the physical and chemical properties of the soil, seasonal variation and every other microbiological parameter. The phosphatase activity of native soil was the only microbiological parameter which correlated with several of the physical and chemical properties of the soil in April, 1974. Those correlations included positive correlations with percent clay and magnesium and negative correlations with potassium, calcium, sodium and percent silt. These correlations appear to be related to the clay, which may be adsorbing the enzyme onto its surface. Respiratory activity in native soil correlated with only one soil characteristic, water-holding capacity, and that was only for native soil. This relationship is related to the pore space of the soil. Spoils did not exhibit this correlation, lack of which could be attributed to the more heterogeneous textural types.

All four microbiological parameters were observed to exhibit significant seasonal variations for both native soil and spoils, although these seasonal variations did not coincide for all four parameters. Respiratory activity and the number of pectinolytic bacteria both tended to peak in October whereas phosphatase activity tended to peak in August and pectinolyase activity was high in August and peaked in December. The possibility of seasonal variation in physical and chemical characteristics of the soil is also suggested.

The variation in the four microbiological parameters were found to be not significantly due to different plant species.

The statistical analyses of the microbiological parameters shows that differences exist between each plot for each of the four parameters, but the differences are not significant for spoils plots as compared to native soil plots.

The only correlation found between the four parameters was that of pectinolyase activity with the numbers of pectinolytic bacteria. This correlation is higher for native soils than for spoils, which might suggest that by following the correlation of these two measurements, an ecological trend could be observed.

It was concluded that the four microbiological parameters as applied to the ecological state of the soil were effective in showing differences. The importance of these differences is a question which requires further research.

Chapter 1

INTRODUCTION

The surfaces of land altered by coal strip mining are a diverse mixture of rocks and soils originating from the overburden lying over the coal. This overburden is commonly termed "spoils" following replacement after mining and is essentially a new medium for plant growth.

There are numerous publications dealing with the revegetation of spoil areas which report both successes and failures in revegetation of such areas. In the past, reclamation research has been carried out intensively on spoils from strip mining in the eastern United States. Recently, such research has been initiated to study the reclamation potential of western coal lands. These western coal lands differ most markedly from those in the eastern portions of the United States by generally low levels of pyritic materials in the coal and by occurring in a much more arid climatic zone. Therefore, the well-known problem of acidic spoils associated with eastern coal is not a problem involved in reclamation of western coal lands.

Reclamation research conducted up to this time has been focused on such factors as: surface manipulation, topsoiling, degree of slope, native and introduced plant species trials, fertilizer treatments and time of seeding -- all of which are designed to enhance successful reclamation. However, the long-range success of these treatments is difficult to predict, for once vegetation is established on spoil its continued success depends upon the physical, chemical and biological factors that are so complexly interrelated in the soil condition known as fertility. Therefore, the reclamation research activities cited above can no longer be considered as an all inclusive approach to restoration of a desirable and productive environment in the shortest time possible, since it is necessary to observe the trends of the spoils' potential for productivity.

It has long been known that microorganisms are the active agents in the soil-forming processes and in the cycling of nutrients between soil and plant. Microbial activity can be said to prepare the environment for the plant, and it then depends on the direction and intensity of biological processes in soil whether the requirements of plants for nutritional elements will be satisfied. Therefore, the degree and type of microbial activity is probably important in determining the speed and permanency of reclamation of spoils from strip mining.

In newly manipulated spoils, it would be expected to find relatively low levels of microbial activity initially due to a few rather widely-adapted physiological groups of microorganisms. As the spoils progress to the state which supports plant life and is self-maintaining (i.e. approaching natural conditions) the level of microbial activity can be expected to increase, and populations of specifically-adapted physiological groups of microorganisms, especially those aiding in the turnover of materials in the soil, would likewise increase. This developing ecosystem in the spoils is probably quite different from that of the pre-existing native rangeland, which would lend even more importance to the use of microbial activity as a measure of the progress of reclamation.

Traditionally, studies of soil bacteria have been concerned with taxonomic identification and populations. However, in recent years increasing attention has been given to the detection of specific enzymes and metabolic pathways in the soil in addition to the usual enumeration techniques. These parameters have been used to obtain information on the intensity of biological processes taking place in the soil. The sum of these metabolic approaches has been termed "biological activity". The main drawback of these procedures is not the use of conventional methods, but rather, the absence of standardized procedures.

Statement of Purpose

The long-range success of surface reclamation of strip mined spoils is related to the microbial activity of the spoils in proceeding from pioneer to climax ecosystems. The purpose of the present research is to combine the tools of biochemistry and physiology to evolve parameters of biological activity of the soil. Specifically, parameters that measure that biological activity which is mediated by or directly related to microorganisms in the soil. These parameters would be useful in determining the ecological state of the disturbed soil as related to its natural state and in determining its potential for maintaining a steady-state population.

The specific aspects of this study involve: (1) soil sampling field experimentation in an attempt to determine the inherent variation of the system; (2) an <u>in vitro</u> examination of the oxygen uptake or respiratory activity of the soils, microbial extracellular phosphatase enzymatic activity, the extracellular pectinolytic enzymatic activity and a plating technique for the isolation and enumeration of pectinolytic organisms as the microbiological parameters; (3) an examination of the physical and chemical properties of soils as they interrelate to the microbiological parameters; and (4) a study of a specific biochemical taxonomic tool for the identification of soil organisms.

Chapter 2

MATERIALS AND METHODS

Study Area

The study area, as described by Sindelar, et. al. (1975), is located in southeastern Montana at Colstrip, where coal strip mining has occurred for over forty years. Western Energy Company and Peabody Coal Company have active coal mines in the immediate area. They disturb approximately 80 hectares (198 acres) of rangeland annually while producing about 8 - 10 million tons of sub-bituminous coal, and these figures are expected to increase in future years due to current energy demands.

Southeastern Montana is part of the Missouri Plateau, an unglaciated region of the Northern Great Plains in the Midland physiographic area of Montana. Geologic materials are primarily Cenozoic sedimentary rocks with some colluvium laid down in the Quaternary period. Distinctive sandstone ridges, mesas and escarpments dominate much of the landscape with broad valley lined with alluvial fans, foot slopes and stream terraces. The dendritic drainage pattern generally flows northward to the Yellowstone River.

The elevation of Colstrip is 981 meters (3,200 feet). The continental climate is cold in winter, warm in summer and has large variations in seasonal precipitation. Average annual precipitation is

38.4 centimeters (15 inches) and maximum precipitation occurs in April through July, with about 30 percent of annual precipitation occurring as snow. The growing season extends from April through July with a frost-free period of 120-124 days. July is generally the warmest monthe and summers are hot, dry and windy with extreme evaporation rates.

Most soils in the study area are formed on weakly consolidated sandstone, and siltstone, stratified sands, residuum and colluvium from sandstone, clay and silt shales and siltstone. Soil development is often limited to a weak accumulation of organic matter to form the A horizons. Residuum from shale or other salty materials often is affected by the dry climate and patterns of soil drainage to produce occassionally saline and alkaline soils. These soils and the area climate restrict vegetation to adapted drought and saline-alkali tolerant species.

Vegetation of the Colstrip area is primarily mixed-prairie grassland interspersed with ponderosa pine occurring on scattered sandstone outcroppings. Livestock carrying capacity is about 1.2 - 2.4 hectare (3.0 - 6.0 acres) per animal unit month depending on range conditions.

Sampling

Samples were collected from a total of fifteen sample plots consisting of 7 spoils sites and 8 native soil sites (Table 1) during the period of September 1973 through December 1974 on five different dates. The three dominant plants at each site were selected and samples were taken from an arbitrary location under these dominant plants at each plot. Stakes were placed at each of these sites during the April sampling and subsequent sampling in August, October and December was conducted at these stakes. Following the April sampling, three representative spoils plots (Figures 1 - 3) and three representative native soil plots (Figures 4 and 5) were selected for further study (Table 2). A data sheet was completed for each sample in the field (Appendix).

Samples were taken as close to the particular plant as possible to include the finer plant roots. The litter was scraped aside and soil was sampled to the depth of 6 inches with a spade. The samples, consisting of approximately one quart of soil, were placed in plastic lined brown-paper sampling bags supplied by the M.S.U. Soils Lab. The collected samples were immediately placed on ice and subsequently placed in a 4C refrigerator upon arrival back in the laboratory, after approximately 6 hours had elapsed.

Each sample was stored at 4C until it was air-dried for eight hours, sieved through a 1 mm mesh brass sieve, blended in a Twin shell

Table 1. Sampling sites, descriptions, dates sampled and plant species.

Site	Description, Dates Sampled and Plant Species
BN	Native soil; sampled September 1973, April, October and December 1974; *Agropyron smithii*, *Artemisia frigida* and *Stipa comata*.
Cape Oliver	Spoils; sampled September 1973 and April 1974; *Agropyron smithii*, *Chrysothamnus nauseosus* and *Gutierrezia sarothrae*.
Demo	Spoils; sampled September 1973, April, August, October and December 1974; *Agropyron cristatum*, *Bromus inermis* and *Melilotus officinalis*.
Dryland Pasture	Spoils; sampled September 1973 and April 1974; *Agropyron cristatum*, *Agropyron elongatum* and *Dactylis glomerata*.
Gouge	Spoils; sampled September 1973, April, August, October and December 1974; *Agropyron cristatum*, *Agropyron elongatum*, *Artemisia cana* and *Bromus inermis*.
McDonald	Native soil; sampled September 1973 and April 1974; *Koeleria cristata* and *Stipa comata*.
MP Lower	Native soil; sampled September 1973 and April 1974; *Gutierrezia sarothrae* and *Koeleria cristata*.

Table 1. (continued)

Site	Description, Dates Sampled and Plant Species
MP Upper	Native soil; sampled September 1973 and April 1974; *Bouteloua curtipendula*, *Rhus trilobata* and *Schizachyrium scoparium*.
Native Range Fertilized	Native soil; sampled September 1973, April, August, October and December 1974; *Carex* spp., *Gutierrezia sarothrae* and *Stipa comata*.
Native Range Unfertilized	Native soil; sampled September 1973, April, August, October and December 1974; *Carex* spp., *Gutierrezia sarothrae* and *Stipa comata*.
Raw Spoils - 1 month old	Spoils; sampled April 1974; No vegetation.
Raw Spoils - 1 year old	Spoils; sampled September 1973 and April 1974; No vegetation.
Streeter	Native soil; sampled September 1973 and April 1974; *Agropyron spicatum*, *Pinus ponderosa* and *Stipa comata*.
Old Spoils	Spoils; sampled April 1974; *Chrysothamnus nauseosus* and *Oryzopsis hymenoides*.
Topsoil Gradient	Spoils; sampled September 1973, August, October and December 1974; *Bromus inermis*.