 0030-0950/83/0004-0191 $2.00/0

OHIO STRIP MINE SPOILS: PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION AND CHANGES DUE TO LIMING AND ORGANIC ADDITIONS[1]

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ABSTRACT. Southeastern Ohio strip mine spoils and garden soil were characterized physico-chemically and microbiologically. Spoils, limed spoils and garden soil that had been sterilized were amended with cellulose or sucrose and some minerals, including ammonium-nitrogen. The samples were inoculated with microbes from a garden compost infusion and incubated for 3 wk at 25 C. Soil water content was held at 80% field capacity. Changes in microbial populations, nitrate concentration and soil aggregation were followed for 3 wk. Microbial populations and soil aggregation increased mostly during the first week in all samples. Molds predominated in acid spoils while bacteria and actinomycetes declined. In limed spoils and garden soil bacteria and actinomycetes outgrew the molds. In spite of the inoculation with nitrifying bacteria, nitrification did not occur in acid spoils but continued for 3 wk in the other samples. It was shown that liming and proper amendments did improve the characteristics of acid spoils.

OHIO J. SCI. 83 (4): 191–197, 1983

INTRODUCTION

Microorganisms are involved in litter decomposition and mineralization processes as well as in the modification of the physical structure of soils (Stroo and Jencks 1982). Indeed, microbial activity and soil fertility are often closely related (Fresquez and Lindemann 1982, Tisdall et al. 1978). Acid mine spoils show a characteristic deficiency of nutrients and vegetation, high levels of acidity, toxic concentrations of some ions (e.g. Al and Mn) and low numbers of microbes (Fresquez and Lindemann 1982, Lawrey 1977). It was of interest to us to see if additions of lime, organic carbon, minerals and microorganisms could improve the properties of acid mine spoils. Garden soil was included as an example of properties of a complete and productive soil. The differences between the samples in densities and varieties of microorganisms were overcome by sterilization and uniform inoculation with the rich microflora from garden compost. We report here on the resultant changes with time in microbial populations, in nitrification and in soil aggregate stabilization.

METHODS AND MATERIALS

SOIL SAMPLES. Strip mine spoils were obtained from spoil banks located in Green Township of Hocking County in southeastern Ohio. Garden soil was taken from a garden plot in Athens County. Each sample consisted of 36 randomly chosen subsamples which were taken to a depth of 10–15 cm and transported to the laboratory in polyethylene bags.

PHYSICAL AND CHEMICAL ANALYSES. Respective air-dried and sieved (2 mm) samples were thoroughly mixed. Subsamples were used for the following physical determinations: Mechanical analysis by the hydrometer method (Bouyoucos 1951), field capacity by the method of Bouyoucos (1935) and soil aggregate stability by the dispersion ratio method (Griffiths and Burns 1972).

The chemical analyses were pH (soil : water, 1 : 1), exchangeable bases (Ca, Mg, K), extracted according to Jackson (1958), by atomic absorption spectrophotometry, total N by the macro-Kjeldahl method (Jackson 1958), KCl-extractable Al according to Jones and Thurman (1957) and Mn according to Cornfield and Pollard (1950). Nitrate-N was ana-

[1]Manuscript received 20 October 1982 and in revised form 14 March 1983 (#82–27).

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lyzed by the phenoldisulfonic acid method (Bremner 1965), available P by the method of Bray and Kurtz (1945) and lime requirement by the method of Dunn (1943).

MICROBIOLOGICAL ANALYSES. For microbiological analyses samples, consisting of 4 subsamples, were collected aseptically and returned immediately to the laboratory. A sterile buffer (0.5% KH_2PO_4, 0.5% K_2HPO_4; pH 7.0) was used for blending and serial dilutions. Aerobic heterotrophic bacteria and actinomycetes were enumerated on Casein-Peptone-Starch (CPS) agar plates (Casein 0.5 g, K_2HPO_4 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, $FeCl_3$ 0.001 g, Actidione 0.1 g (Cycloheximide, Sigma Chem. Co.), agar 20 g, distilled H_2O 1 L, pH 7.5) and molds on Glucose-Peptone-Rosebengal-Streptomycin (GPRS) agar plates (Glucose 10 g, peptone 5 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, agar 20 g, distilled H_2O 1 L, rosebengal 1:30,000, streptomycin 30 μg/ml). The most probable number (MPN) of ammonium and nitrite oxidizers was estimated by the 5-tube method using the media of Meiklejohn (1968). Microbial isolations were made from representative colonies developing on plates during the enumeration of microorganisms from acid spoils and identified to the genus level. Bacteria were identified according to Bergey's Manual (Buchanan and Gibbons 1974), actinomycetes according to Waksman (1967) and molds according to Gilman (1957). *Thiobacillus* was enriched and isolated on the autotrophic media for aciduric species of Skerman (1959).

SOIL TREATMENTS. Three samples were used: acid spoils, limed (0.8%) spoils and garden soil. One hundred g of sample was placed into a 250-ml beaker, wrapped with aluminum foil, and autoclaved at 121 C for 30 min. For each sample replicate beakers were prepared. Sterile basal fertilizer solution was added to supply final concentrations in soil of 1% sucrose and (parts per million) K as K_2SO_4 40.0, B as H_3BO_3 1.0, P as $Ca(H_2PO_4)_2 \cdot H_2O$ 80.0, N as $(NH_4)_2SO_4$ 170.0. Enough sterile distilled H_2O was added to bring samples to 80% field capacity. Cellulose (1% w/w, microcrystalline cellulose powder, 20 μm, Sigma Chem. Co.) was added to samples before autoclaving. Samples amended with cellulose received sterile basal fertilizer solution without sucrose and supplying 340 ppm nitrogen. All samples, except controls, were inoculated with 1 ml of a compost infusion (10 g compost shaken for 20 min in 90 ml distilled H_2O). Samples were equilibriated at 4 C for 18 hr and mixed before incubation at 25 C for 3 wk. Water loss was replaced regularly with sterile distilled H_2O. All treatments were repeated twice, and the experiment was repeated twice. Replicate samples were sacrificed weekly for the analysis of microbial populations, NO_3^--N and soil aggregate stability. Before each analysis, sterile controls were checked for sterility by subculturing on CPS and GPRS agar plates.

The differences and changes in soil properties with time due to the 2 substrate treatments were compared using the Student's t-tests. A probability ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

The physical and chemical properties of samples are shown in table 1. Acid spoils contained a higher content of clay which probably resulted from the mixing of shale with the original parental materials during the mining operations. Acid spoils aggregates showed a much lower stability compared to garden soil aggregates. Total nitrogen, available phosphorus and exchangeable bases were lower in acid spoils than in garden soil. This is consistent with the deficiency of nutrient elements observed in overburden (Lawrey 1977). The concentrations of exchangeable Al and Mn were highest in acid spoils, much lower in limed spoils and absent in garden soil. All 3 samples differed significantly in pH.

Garden soil contained much higher populations of bacteria and actinomycetes than did acid spoils (table 2). About twice as many molds were counted in acid spoils than in the compost. In compost and garden soil large populations of nitrifiers were present. Their total absence from acid spoils makes nitrification impossible, a serious implication which adversely affects the fertility of acid spoils.

Identification of representative colonies on plate count plates revealed the presence in acid spoils of 5 genera of molds (*Acrostalagmus, Aspergillus, Botrytis, Penicillium, Trichoderma*), 2 genera of actinomycetes (*Micromonospora, Streptomyces*) and 3 genera of bacteria (*Alcaligenes, Bacillus, Enterobacter*). In addition *Thiobacillus* was isolated after enrichment.

Since each of the 3 samples contained different densities of microorganisms and because acid spoils were devoid of all nitrifiers (table 2), we chose to eliminate this uncontrolled variable by sterilization and uniform inoculation with the rich microflora of compost (table 2). The patterns of microbial growth in amended soils are shown in fig. 1A–C. With a few excep-

TABLE 1

Physical and chemical properties of acid mine spoils and garden soil.

		PHYSICAL					
		Particle Size Distribution					
Sample	Color	Sand (0.05–2 mm) (%)	Silt (2–50 μm) (%)	Clay (<2 μm) (%)	Water Content at Field Capacity (%)	Dispersion Ratio	Textural Class
Acid spoils	Black/gray	38.2	13.6	48.2	28.0	74.4	clay
Garden soil	Brown	19.6	70.3	10.1	24.0	32.5	silt loam

		CHEMICAL							
		Exchangeable bases (meq/100 g)					Exchangeable		
Sample	pH	Ca	Mg	K	Total N (%)	Avail. P (ppm)	Al (ppm)	Mn (ppm)	Lime requirement (tons/acre)
Acid spoils	3.6	3.4	0.4	0.7	0.04	14.4	580.0	12.0	8.0
Limed (0.8%) spoils	6.3	ND*	ND	ND	ND	ND	14.0	2.5	—
Garden soil	7.1	10.1	3.4	0.8	0.2	1925.0	0.0	0.0	0.0

*Not determined

tions, growth occurred only during the first week and then remained stationary. In acid spoils (fig. 1A), molds predominated and bacteria and actinomycetes declined. In limed spoils (fig. 1B) and garden soil (fig. 1C), bacteria predominated and molds were suppressed. The difference between the 2 substrates was insignificant except that in the acid spoils the actinomycetes showed a slight stimulation with sucrose only.

Nutrient availability and pH are among the numerous factors which control the survival and growth of microorganisms in soils. Under acid conditions it appeared that the actinomycete population was incapable of cellulose hydrolysis. The dominance of molds in acid spoils could be attributed to their acid tolerance and minimal nutritional competition from bacteria and actinomycetes. Traaen (1974) noted that dominance shifted from bacteria to fungi at pH 3.5 in laboratory respirometry studies with glucose and glutamic acid substrates and the effective antagonism to *Aspergillus niger* by *Serratia marcescens* in soil clay mixtures increased with an increase in soil pH (Rosenzweig and Stotzky 1979).

TABLE 2

Initial numbers of microorganisms per gram of acid spoils, compost and garden soil.

Sample	Molds	Bacteria	Actinomycetes	NH_4^+-oxidizers	NO_2^--oxidizers
Acid spoils	4.1×10^3	3.7×10^3	6.1×10^2 Est.*	<1	<1
Compost	2.3×10^3	2.6×10^8	3.5×10^3	1.8×10^7	2.3×10^7
Garden soil	3.4×10^3	4.9×10^7	5.9×10^6	3.8×10^4	3.3×10^4

*Est. = estimated count from invalid plates (Amer. Publ. Health Assoc. 1972)

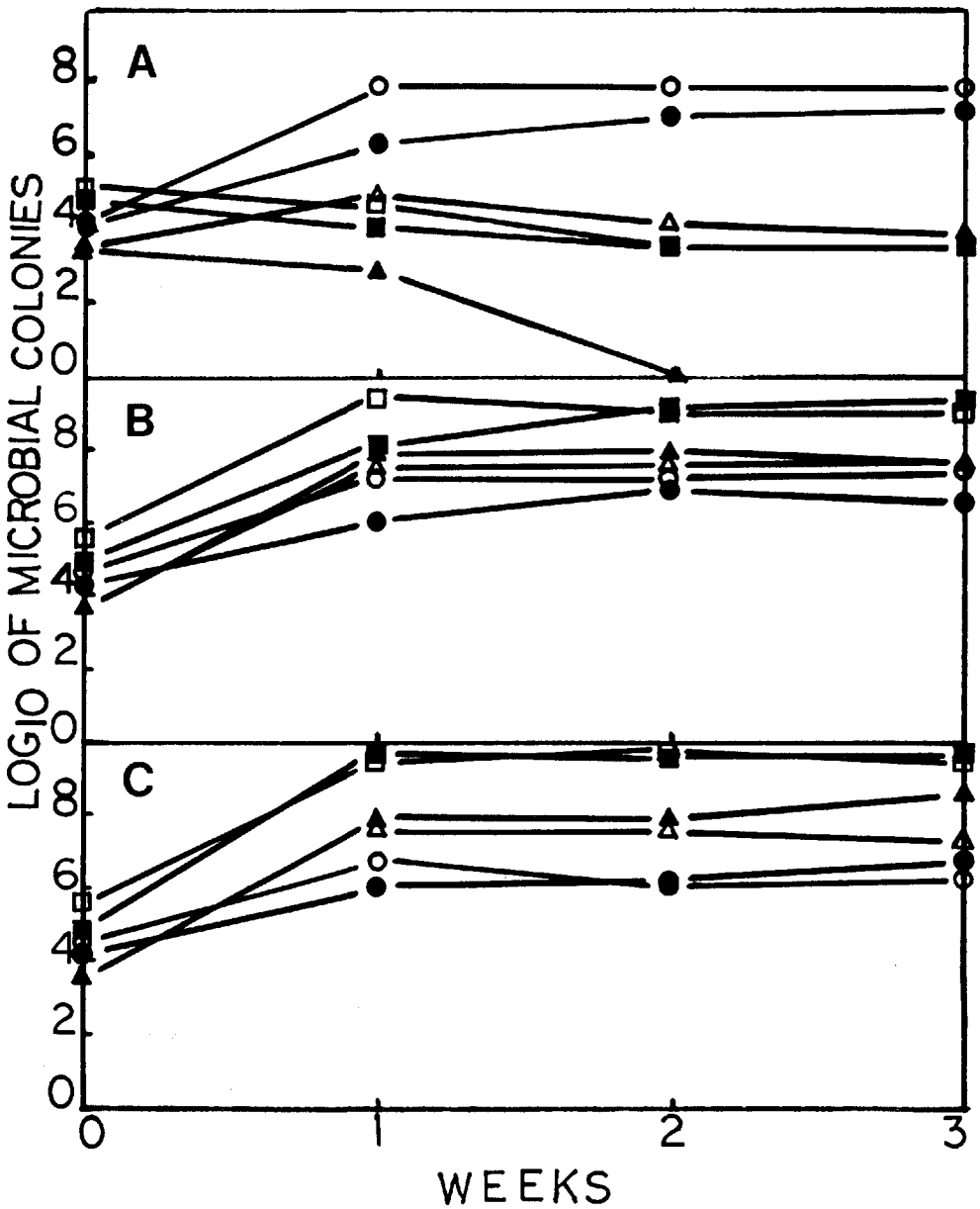

FIGURE 1. Population changes of molds (○), bacteria (□), and actinomycetes (△) derived from garden compost in acid spoils (A), limed spoils (B), and garden soil (C) amended with sucrose (open symbols) and cellulose (closed symbols) and incubated at 25 C (based on plate counts/g).

The suppressed growth of molds in limed spoils and garden soils may result from increased utilization of nutrients by the larger populations of bacteria and actinomycetes which are favored by an abundance of lime and neutral pH conditions of soils (Alexander 1977). While the molds seemed to be the most active cellulose de-

composers in acid spoil samples, the bacterial populations were the most active in limed and garden soils.

Nitrification activity in samples is shown in table 3. In acid spoils nitrification of the added NH_4^+-nitrogen did not occur. The nitrifiers in the inoculum from compost (table 2) were inhibited or killed by the acid conditions. Nitrate levels increased in limed spoils and in garden soil. Apparently the initial NH_4^+-nitrogen levels were high enough that the metabolism of either organic substrate and the resulting demand for nitrogen by the growing microbial population (fig. 1) did not prevent nitrification.

Our results agree with previous observations that soil acidity decreased the survival (Sarathchandra 1978) and activity (Hons and Hossner 1980) of nitrifying bacteria. However, phosphate deficiency may also have an effect. Purchase (1974) noted delay and suppression of nitrification in savannah soils under phosphate deficiency. Since phosphate also complexes with Al and Fe through adsorption to clay surfaces (Ayodele and Agboola 1981), we analyzed the available phosphorus in our acid and limed spoils after 3 wk of incubation. Only 18.0% of the added phosphorus was available in acid spoils, but 87.5% in limed spoils. Whether the high levels of Al and Mn in acid spoils had additional adverse effects on nitrification is not clear at this time. However, liming of acid spoils makes effective nitrification possible, although at a lower efficiency than in garden soil.

Soil aggregate stability, expressed as dispersion ratio, is shown in table 4. Lower values indicate greater stability. At the first week of incubation, qualitative differences in microbial growth patterns could be detected in soils. Acid spoils were covered with a dense growth of hyphal filaments with abundant spores. A rapid increase in stability of acid spoils correlated with increased growth of molds in the samples, however, stability did not increase significantly beyond the second week of incubation. In limed spoils, the presence of mold filaments became only visible at week 3 and fungal spores were few. In garden soils scant filamentous growth was detectable. Sucrose induced higher stability than cellulose in garden soil and limed spoils. This had been reported previously for arable and garden soils (Griffiths and Jones 1965). But in acid spoils cellulose supported higher stability than sucrose. The predominantly filamentous growth in acid spoils was responsible for the greatest improvement in stability among the 3 samples.

TABLE 3

The progress of nitrification during incubation (25C) of acid spoils, limed spoils and garden soil amended with cellulose or sucrose and with ammonium nitrogen.*

Sample	Amendment	Weeks Incubated			
		0	1	2	3
Acid spoils	Cellulose	11.3a**	13.3a	9.0a	12.7a
	Sucrose	14.7a	14.0a	11.7a	13.7a
Limed spoils	Cellulose	14.7b	146.7c	333.0d	533.4f
	Sucrose	11.3b	136.7c	260.5d	406.4e
Garden soil	Cellulose	326.7g	660.0j	946.0k	1553.0m
	Sucrose	336.0g	430.4h	987.6k	1486.5m

*Nitrification is expressed as μg nitrate produced per gram of oven-dried soil.

**Dissimilar subscripts within each sample indicate a significant difference at 5% probability. Data were analyzed as paired comparisons (t-test).

TABLE 4

Changes in aggregate stability in soil samples amended with cellulose or sucrose and with ammonium nitrogen.

		Weeks Incubated			
		Dispersion Ratio			
Sample	Amendment	0	1	2	3
Acid spoils	Cellulose	47.9	14.4	11.3	10.4
	Sucrose	47.9	21.2	16.9	16.8
Limed spoils	Cellulose	44.3	36.9	31.1	29.9
	Sucrose	44.3	31.8	27.5	24.9
Garden soil	Cellulose	45.9	34.5	31.2	24.2
	Sucrose	45.9	21.6	22.4	20.4

It is obvious that liming of acid spoils resulted in definite improvements in pH and decreased availability of Al and Mn. Amendment with organic carbon and minerals was followed by growth of microorganisms and improved aggregate stability. Nitrification became possible in spoils only after liming and addition of nitrifiers. How these changes influenced plant growth will be shown in a later publication.

LITERATURE CITED

Amer. Public Health Assoc. 1972 Standard methods for the examination of dairy products. 13th ed. Amer. Public Health Assoc., Inc. New York, NY.

Alexander, M. 1977 Introduction to soil microbiology. John Wiley & Sons, NY.

Ayodele, O. and A. A. Agboola 1981 Evaluation of phosphorus fixation capacity of tropical savannah soils of western Nigeria. Soil. Sci. Soc. Amer. J. 45: 462–464.

Bremner, J. M. 1965 Inorganic forms of Nitrogen. *In*: C. A. Black (ed.), Methods of soil analysis, part 2, p. 1179-1232. Amer. Soc. Agron. Madison, WI.

Bray, R. H. and L. T. Kurtz 1945 Determination of total organic and available forms of phosphorus in soils. Soil Sci. 59: 39–45.

Bouyoucos, G. J. 1935 A comparison between the suction method and the centrifuge method for determining moisture equivalent of soils. Soil Sci. 40: 165–170.

——— 1951 A recalibration of the hydrometer method for making mechanical analysis of soils. Agron. J. 43: 434–438.

Buchanan, R. E. and N. E. Gibbons (eds.) 1974 Bergey's manual of determinative bacteriology. The Williams & Wilkins Co., Baltimore, MD.

Cornfield, A. H. and A. G. Pollard 1950 The use of Tetramethyldiamino-di-phenylmethane for the determination of small amounts of manganese in plant and soil extracts. J. Food Sci. Agric. 1: 107–109.

Dunn, L. E. 1943 Lime requirement determination of soils by means of titration curves. Soil Sci. 56: 341–351.

Fresquez, P. R. and W.C. Lindemann 1982 Soil and rhizosphere microorganisms in amended coal mine spoils. Soil Sci. Soc. Amer. J. 46: 751–755.

Gilman, J. C. 1957 A manual of soil fungi. Iowa State Univ. Press. Ames.

Griffiths, E. and D. Jones 1965 Microbial aspects of soil structure. Plant and Soil. 23: 17–32.

——— and R. G. Burns 1972 Interactions between phenolic substances and microbial polysaccharides in soil aggregation. Plant and Soil. 36: 599–612.

Hons, F. M. and L. R. Hossner 1980 Soil nitrogen relationships in spoil material generated by surface mining of lignite coal. Soil Sci. 129: 222–228.

Jackson, M. L. 1958 Soil chemical analysis. Prentice-Hall, Englewood Cliffs, NJ.

Jones, L. H. and D. A. Thurman 1957 The determination of aluminum in soil, ash and plant materials using Eriochrome Cyanine R. A. Plant and Soil. 9: 131–142.

Lawrey, J. D. 1977 The relative decomposition potential of habitats variously affected by surface coal mining. Can. J. Bot. 55: 1544–1552.

Meiklejohn, J. 1968 Numbers of nitrifying bacteria in South Rhodesian soils under natural grass and improved pastures. J. Applied Ecol. 5: 291–300.

Purchase, B. S. 1974 The influence of phosphate deficiency on nitrification. Plant and Soil. 41: 541–547.

Rosenzweig, W. D. and G. Stotzky 1979 The influence of environmental factors on antagonism of fungi by bacteria in soil: clay minerals

and pH. Appl. Environ. Microbiol. 38: 1120–1126.
Sarathchandra, S. U. 1978 Nitrification activities and the changes in the populations of nitrifying bacteria in soil perfused at two different H-ion concentrations. Plant and Soil. 50: 99–111.
Skerman, V. B. D. 1959 A guide to the identification of the genera of bacteria. Williams & Wilkins Co. Baltimore, MD.
Stroo, H. F. and E. M. Jencks 1982 Enzyme activity and respiration in mine soils. Soil Sci. Soc. Amer. J. 46: 548–553.
Tisdall, J. M., B. Cockroft and N. C. Uren 1978 The stability of soil aggregates as affected by organic materials, microbial activity and physical disruption. Aust. J. Soil Res. 16: 9–17.
Traaen, T. 1974 Effect of pH on microbiological decomposition of organic matter, p. 15–22. *In*: F. H. Braekke (ed.), Hydrokjemiske og hydrobiologiske rapporter fra NIVA. Sur Nedbors Virkning Pa Skog og Fisk, Oslo, Norway.
Waksman, S. A. 1967 Actinomycetes. A summary of current knowledge. Ronald Press Co., NY.