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BACILLUS CEREUS RESULTS FROM THE MORRO DO OURO SURVEY AREA, MINAS GERAIS, BRAZIL

December 15, 1989

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INTRODUCTION

The *Bacillus cereus* exploration method was pioneered in 1982 by Watterson of the U.S. Geological Survey. Wattersons interest in the distribution of *Bacillus cereus* originated as a result of a study by Timoney and others (1978). They were the first to show that heavy metals in the environment can selectively enhance antibiotic resistance in soil bacterial populations associated with metalliferous sediments. Watterson (1986) has shown that penicillin resistance in total *Bacillus* species correlates significantly with soil metal content and that the most numerous of the *Bacillus* species displaying this resistance phenomenon is *Bacillus cereus*. It was concluded that either penicillin resistance in total *Bacillus* species or increases in *Bacillus cereus* are useful as an exploration method. Microbial populations of *Bacillus cereus* have been shown to increase in soils overlying subsurface copper, massive sulfide, and gold deposits (Parduhn and Watterson, 1984; Parduhn and others, 1985; Parduhn, 1988, Parduhn, in press; Watterson and others 1983a; Watterson and others, 1983b).

A total of thirty soils were collected from the Morro do Ouro survey area and assayed for *Bacillus cereus* populations. The purpose of the survey was to establish whether increases in *Bacillus cereus* populations coincide with the known location of disseminated gold mineralization.

Soil samples 1-8 and 20-26 were collected along a N85° W traverse with sample spacing at 20 meter intervals. After

crossing the Paracatu River, samples 9-19 were collected 33 meters S10°W offset from the original traverse because of the presence of cultivated land (Figure 1). Soils were collected 4-6 inches deep to avoid surface contamination. They were then air dried and sieved to -80 mesh. *Bacillus cereus* was assayed using standard methods (Watterson, 1985).

GEOLOGIC AND GEOMORPHIC SETTING

The Morro do Ouro deposit is hosted within carbonaceous shales and phyllites of the Paracatu Formation. Free gold occurs within boudin structures that have formed as a result of stretching of a competent bed during folding. The average ore grade is 0.64 gr/ton.

The survey area is within dry savanna consisting of intermittent grasslands and deciduous forests. Samples collected within the disseminated gold zone occur on a grassy hilltop and extend downslope to the Paracatu River. On the western side of the river the vegetation changes to dense shrubs and forest. The western-most sites show evidence of fire history (charcoal and burned stumps) and are vegetated by grasses. Soils along the grassy hilltop and west facing slope in the mineralized zone are red/brown in color, poorly-developed, and are rocky and shallow (immature laterite). "Background" areas on the west bank of the river are deep red in color, well-developed, and consist of clay and loam (podzols).

RESULTS

Bacillus cereus counts and plots are provided in Appendix A. Anomalous concentrations of soil/plant metals and *Bacillus cereus* populations were determined by inspection of histograms and are based on values one standard deviation above the mean.

Soils were found to contain a variety of *Bacillus cereus* that display a filamentous growth pattern, *Bacillus cereus* var. *Mycoides*. The most common colony growth pattern for *Bacillus cereus* in North America is small, pinpoint spindles. Because the filamentous growth pattern observed in soils cultivated from the Morro do Ouro survey obscures the enumeration of the more typical colonies, a 5 ppm solution of penicillin was added to suppress the growth of the atypical colonies. It was found, however, that a 5 ppm solution was too strong and resulted in the suppression of all *Bacillus cereus* colonies. Therefore, the un-amended data was plotted and used for interpretation. Further laboratory test work is required to establish the correct amount of penicillin amendment.

Bacillus cereus Populations

The majority of elevated *Bacillus cereus* populations occur within the mineralized zone (sites 2, 5, 7, and 8). Sporadic highs occur in the "background" area and may reflect the presence of local metal distributions (sites 12 and 13). A clustering of highs, however, is more indicative of potential areas of subsurface mineralization.

The need to integrate soil geochemistry with the microbial results is evident in areas where soils are very poorly developed. The shallow soils on the hilltop within the mineralized zone contain depressed values of *Bacillus cereus* and may relate to the poor development of the soils rather than the lack of metals within them. Poorly developed soils contain poorly developed microbial populations.

CONCLUSIONS

The correspondence of population highs within the zone of gold mineralization indicates that *Bacillus cereus* is a potential indicator of gold mineralization. Re-confirmation of the significance of the population highs with soil geochemistry will substantiate these results.

Further studies need to be performed to evaluate the effect of recent burning on the disturbance of soil microbial populations. The distribution of *Bacillus cereus* var. *Mycoides* needs to be investigated to determine their role in delineating metalliferous soil conditions, and to what extent they inhabit tropical climates.

The use of *Bacillus cereus* has been of particular success in areas where surface soil geochemical signatures are very subtle. The ability of *Bacillus cereus* to respond to very minute concentrations of metals, makes the method applicable in lateritic terrains where extensive leaching of metals has taken place.

REFERENCES

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APPENDIX A
Bacillus cereus Data and Plots

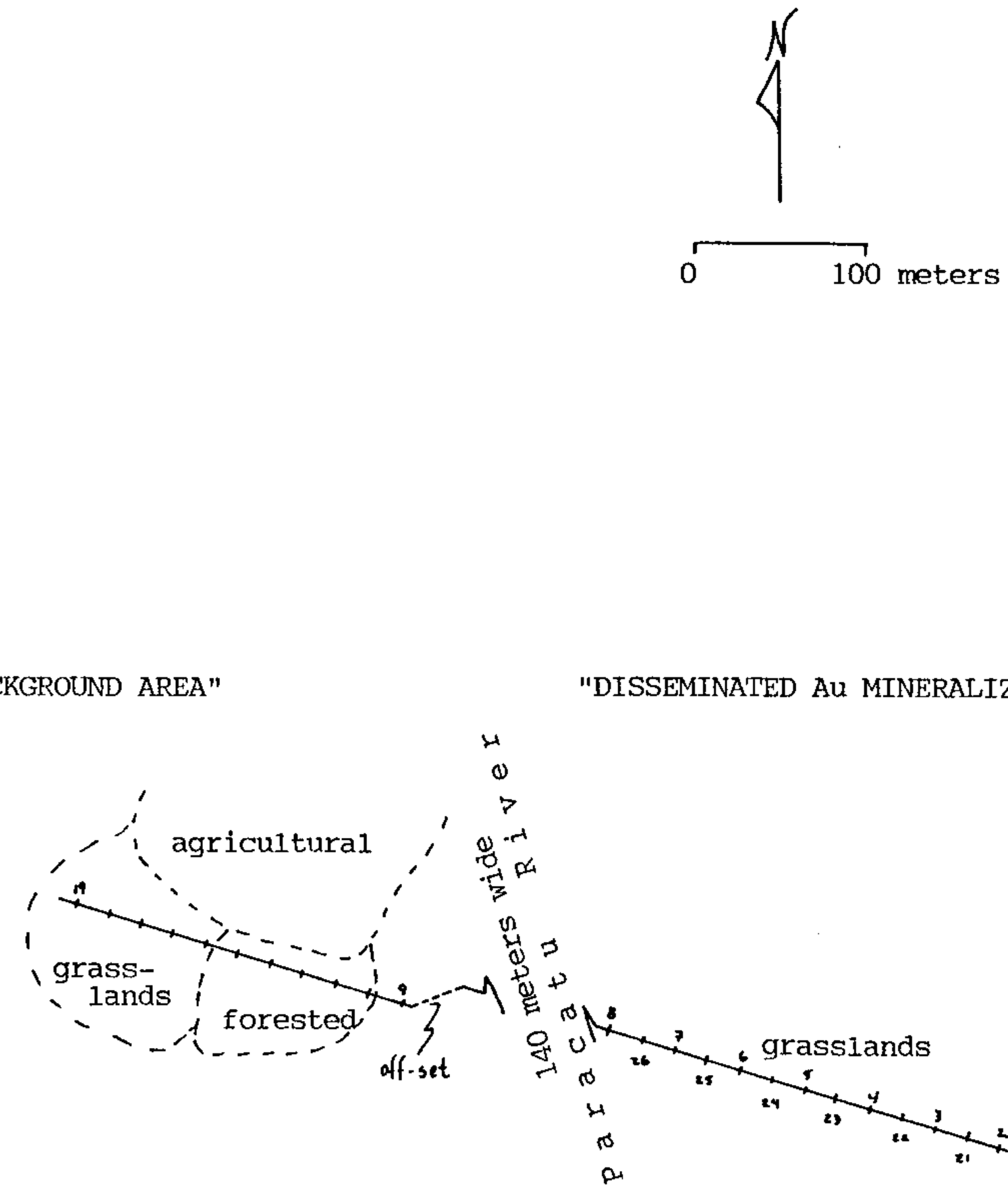
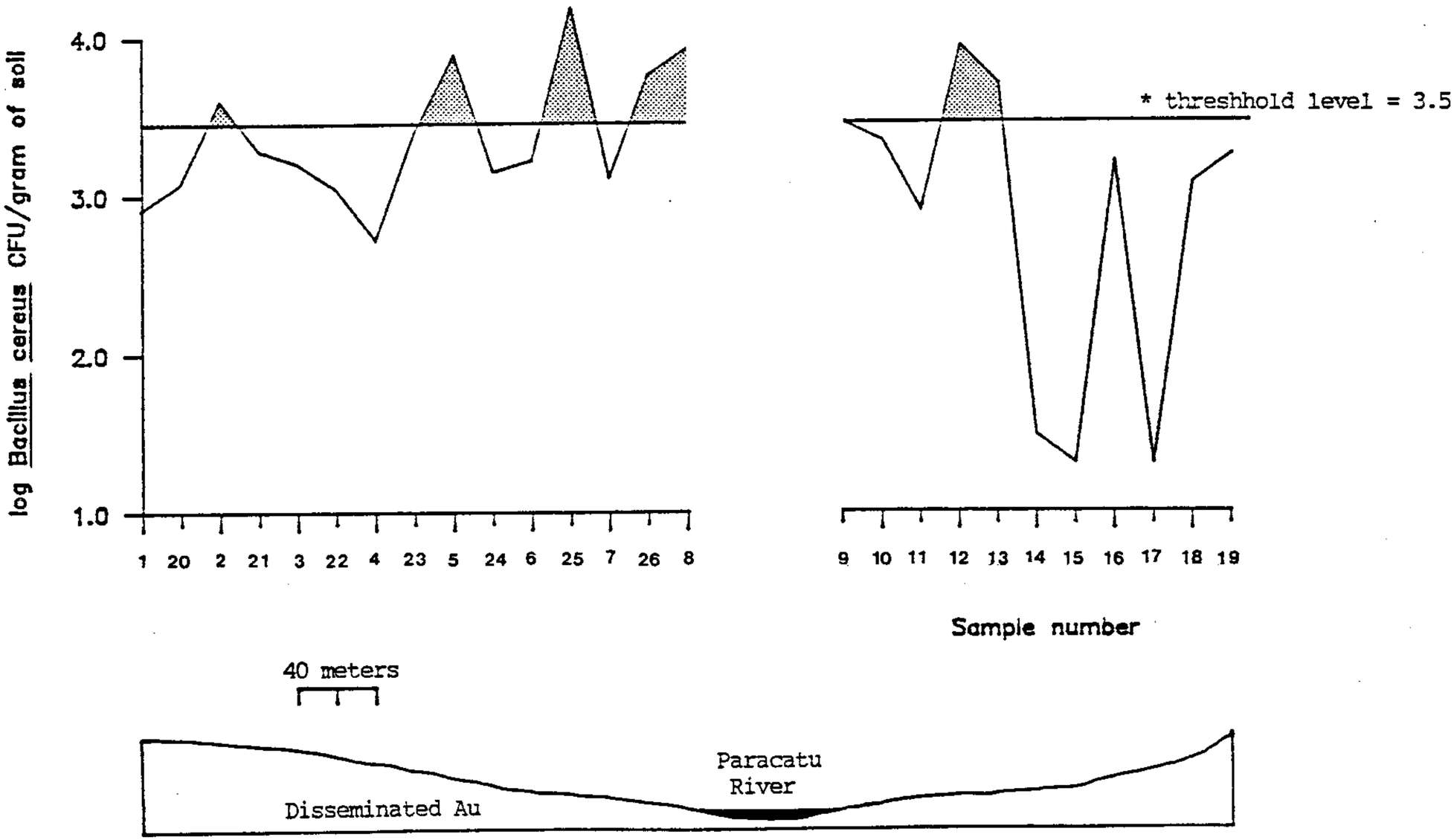


Figure 1. Sample location map.

MORRO DO OURO POPULATIONS

ROW	ID	B cer
1	1	810.0000
2	2	4000.0000
3	3	1600.0000
4	4	520.0000
5	5	2000.0000
6	5d	14000.0000
7	6	1700.0000
8	7	1300.0000
9	8	8800.0000
10	9	2900.0000
11	10	2200.0000
12	11	800.0000
13	12	9000.0000
14	13	5000.0000
15	14	30.0000
16	15	20.0000
17	16	2400.0000
18	16d	1100.0000
19	17	20.0000
20	18	1200.0000
21	19	1800.0000
22	20	1200.0000
23	21	1900.0000
24	22	1100.0000
25	23	2600.0000
26	24	1400.0000
27	25	16000.0000
28	26	6000.0000
29	XX-1	320.0000
30	XX-2	1200.0000

Morro do Ouro (Disseminated Gold Deposit) — Paracatu, Brazil



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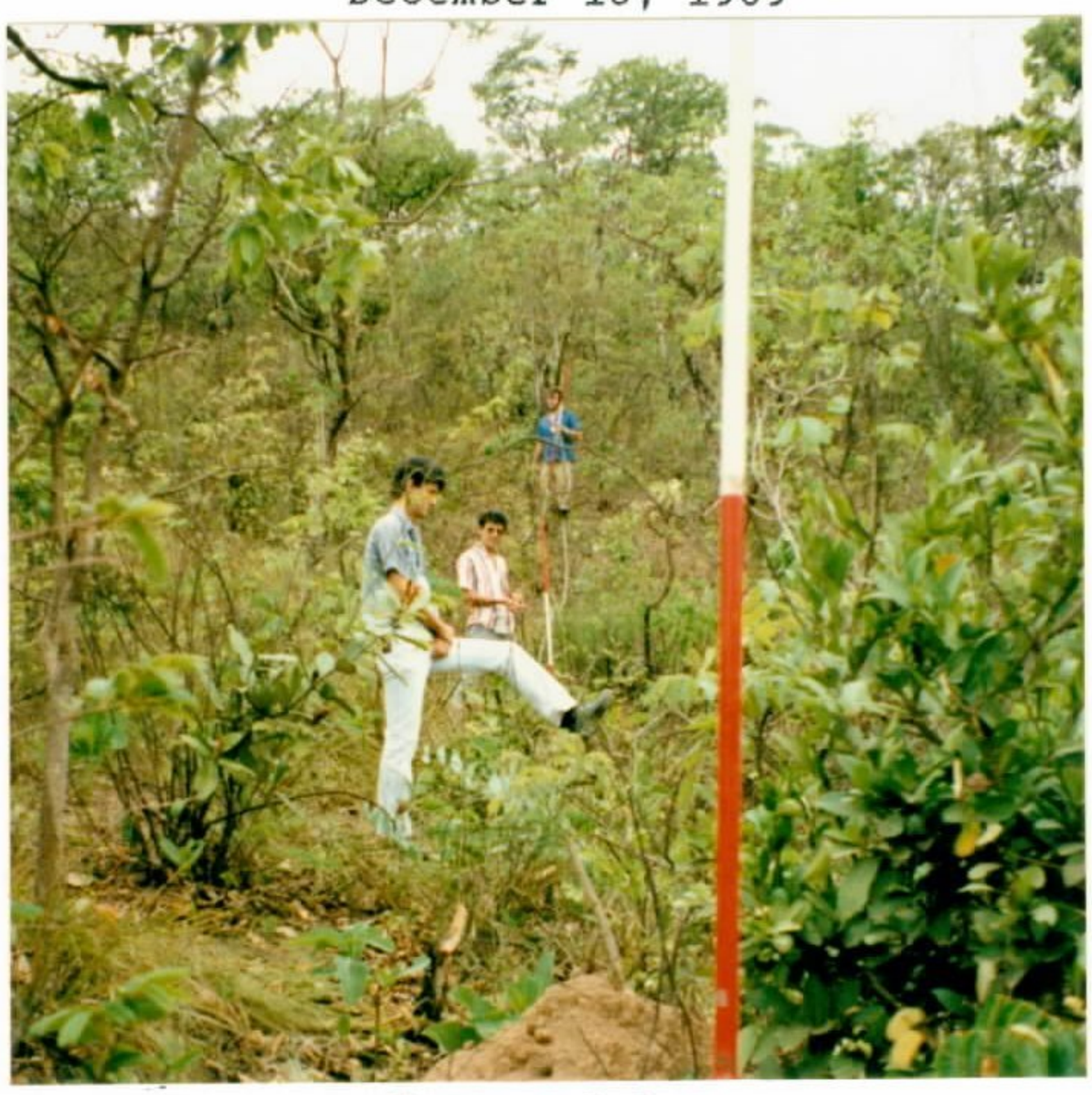

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Twenty-four soils from the Vazante survey area were assayed for *Bacillus cereus.* The purpose of the survey was to establish whether increases in *Bacillus cereus* populations coincide with the known location of zinc mineralization.

Soils were collected along two traverses at 10 meter intervals. The first line was sampled along strike, N35° E, of a mineralized outcrop containing zinc. The second traverse

intersects the center of the first line at 90° and extends S55° E upslope into "background" terrain (Figure 1). Soils were collected 4-6 inches deep to avoid surface contamination. They were then air dried and sieved to -80 mesh. *Bacillus cereus* was assayed by amending cultures with 2 ppm penicillin. Standard methods were used to isolate *Bacillus cereus* (Watterson, 1985).

GEOLOGIC AND GEOMORPHIC SETTING

Zinc and lead mineralization occurs in stromatolitic dolomite reefs of the Vazante Formation. It has been postulated that zinc mineralization was originally hosted within underlying shales. Zinc was then subsequently remobilized from the black shales into the overlying dolomite reef structures during a hydrothermal event. The primary zinc ore minerals are calomine ($Zn_4(Si_2O_7)(OH)_2 \cdot H_2O$), at the surface, and willemite (Zn_2SiO_4), at depth.

The survey area is within a dry savanna consisting of intermittent grasslands and deciduous forests. Soil textures range from poorly developed rocky/silty soils near the mineralized outcrop, to moderately developed silt/clay soils on the flanks and upslope from the outcrop. Alternating layers of shale and dolomite beds are present.

RESULTS

Bacillus cereus counts and plots are provided in Appendix A. Site 4 was not sampled because soils were not present.

Anomalous concentrations of *Bacillus cereus* populations were determined by inspection of histograms and are based on values one standard deviation above the mean.

Soils were found to contain a variety of *Bacillus cereus* that displays a filamentous growth pattern, *Bacillus cereus* var. *Mycoides*. The most common growth pattern in North America is very small, pinpoint, spindle shaped colonies. Because the filamentous growth pattern obscures the enumeration of the more typical colonies, a 2 ppm solution of penicillin was added to supress the growth of these atypical colonies. Very little has been published on the soil ecology of these filamentous varieties. Although not all filamentous varieties were supressed with the addition of penicillin, the number of colonies had decreased to the point where typical *Bacillus cereus* colonies could be counted.

Bacillus cereus Populations

Elevated *Bacillus cereus* populations occur on the north and south flanks of the mineralized outcrop (sites 5 and 8), along the second traverse on the eastern edge of the outcrop (sites 13-17), and at the eastern end of the second traverse (sites 23 and 25). The lack of elevated populations directly overlying the mineralized outcrop (sites 6 and 7) is attributed to the poorly developed nature of the soils. Poorly developed soils contain poorly developed microbial populations.

Elevated populations that extend from the eastern edge of

the outcrop area upslope, coincide with dense vegetation which contributes organic matter to the soil horizon. Elevated populations also occur in the eastern end of the traverse, and correspond to an area where organic-rich soils and dense shrub vegetation exists. Whether these population highs are related to the abundance of organic nutrients or whether they relate to the metalliferous condition of the soil, would be more clearly elucidated by analyzing the soils for base metals. The fact that populations have been subjected to dilute concentrations of penicillin, suggests that the populations are related to soil metals.

CONCLUSIONS

The apparent correspondence of population highs with the location of the mineralized outcrop (with the exception of the poorly developed soils directly overlying the exposure) indicate that *Bacillus cereus* can be used as a potential indicator of zinc mineralization. Soil analyses will further substantiate the significance of the populations increase. Additional penicillin resistance tests would also verify that population increases observed are clearly related to mineralization.

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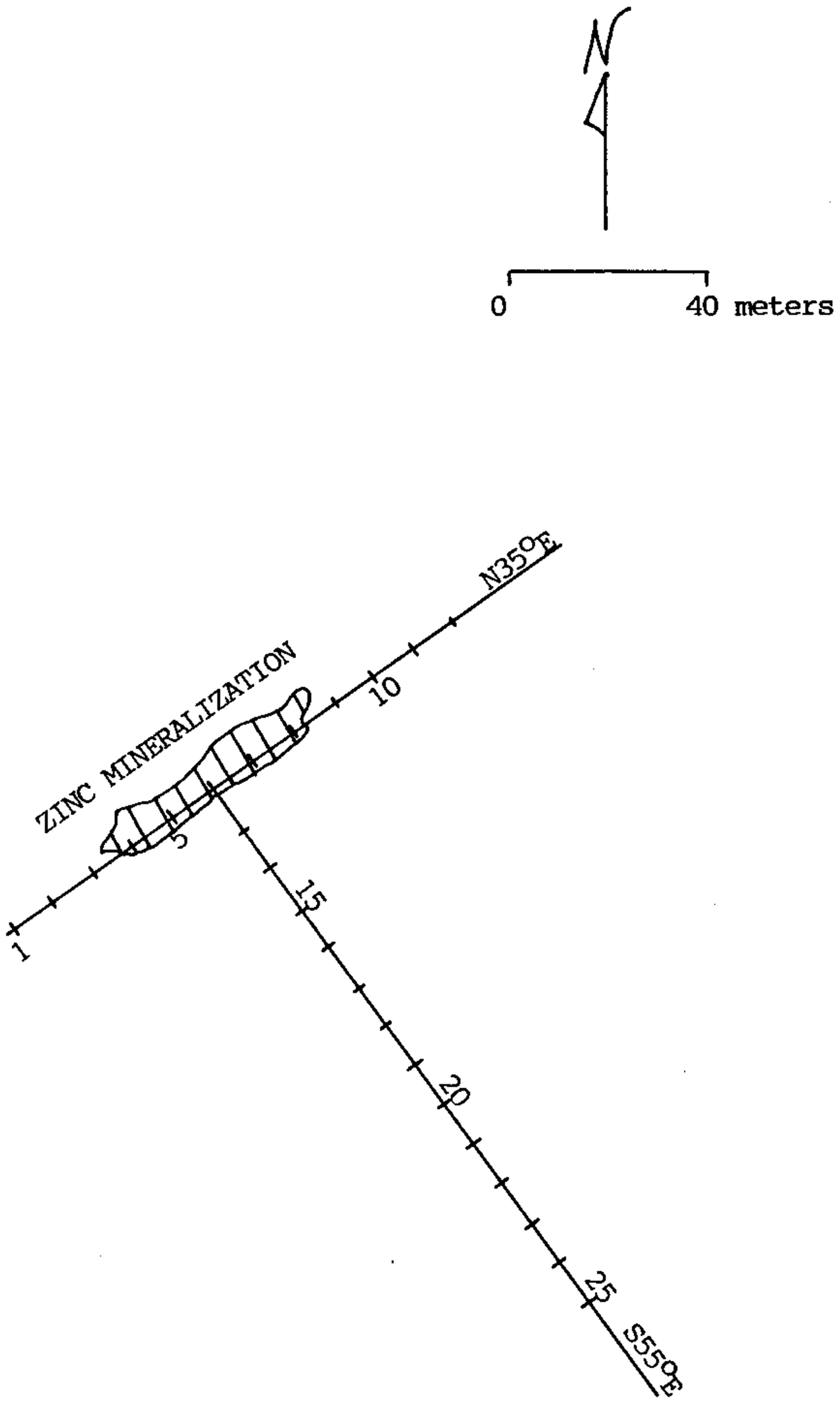


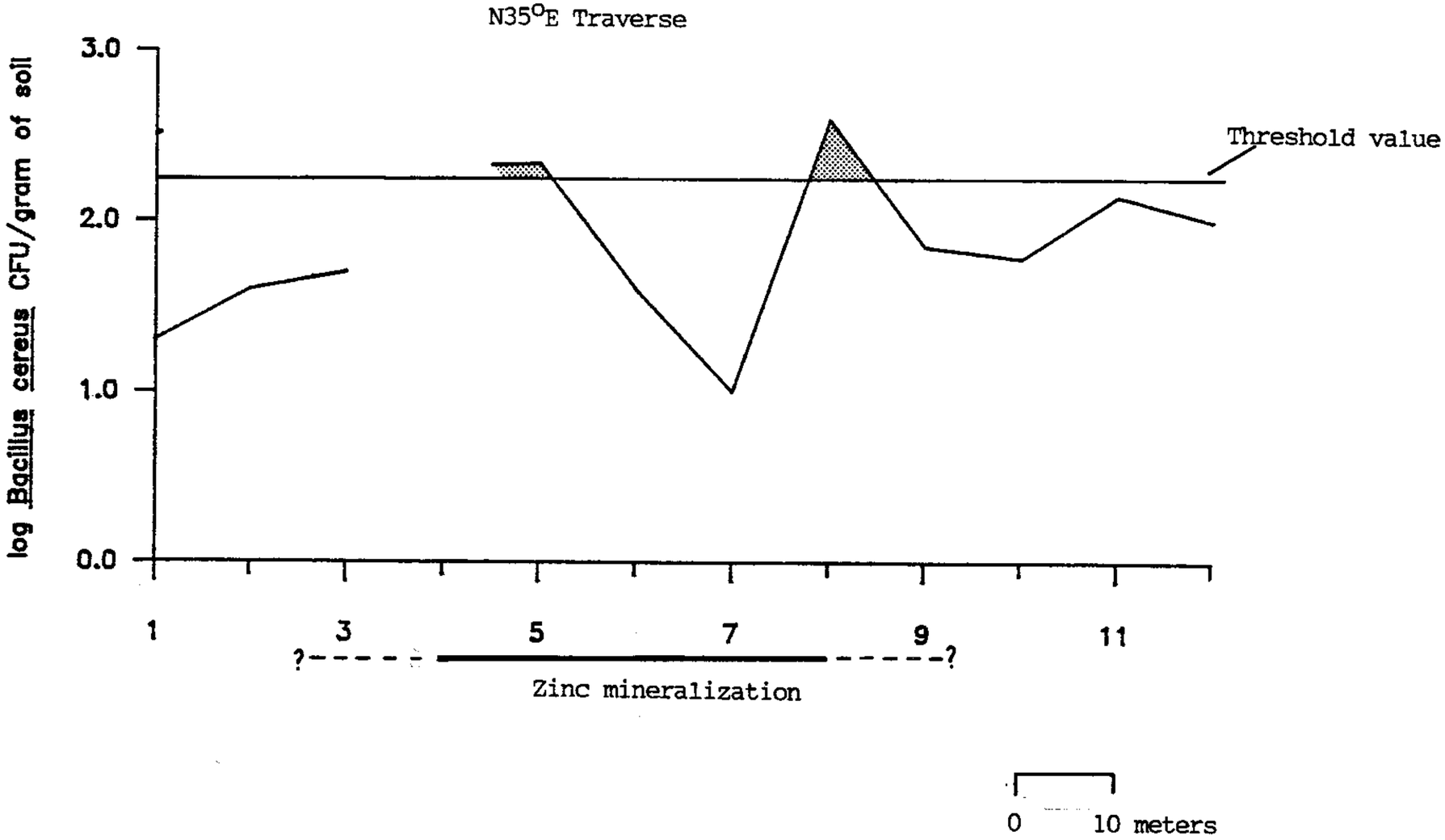
Figure 1. Soil sample locations relative to zinc mineralization.

APPENDIX A
Bacillus cereus Data and Plots

AMENDED VAZANTE *BACILLUS CEREUS* POPULATIONS

ROW	ID	BC/gram
1	1	20.0000
2	2	40.0000
3	3	50.0000
4	5	220.0000
5	6	40.0000
6	7	10.0000
7	8	410.0000
8	9	70.0000
9	10	60.0000
10	11	140.0000
11	12	100.0000
12	13	600.0000
13	14	220.0000
14	15	520.0000
15	16	170.0000
16	17	170.0000
17	18	80.0000
18	19	100.0000
19	20	10.0000
20	21	170.0000
21	22	150.0000
22	23	220.0000
23	24	110.0000
24	25	510.0000

Vazante Zinc Deposit — Central Brazil



Vazante Zinc Deposit — Central Brazil

S55°E Traverse