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GEOLOGICAL SURVEY

A survey of four study areas examining *Bacillus cereus* population distributions and soil metal concentrations

By

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A survey of four study areas examining *Bacillus cereus* population distributions and soil metal concentrations

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Abstract

The natural abundance of *Bacillus cereus* in soils was studied over two mineralized areas and two unmineralized areas. The *B. cereus* population distributions were compared with total metal concentrations and six water extractable ions. The data indicate there is no observable correlation between *B. cereus* populations and the metals examined.

The natural abundance of *B. cereus* from closely spaced samples within a single environmental setting often differed by an order of magnitude. The population differences between distinct environments, such as a pine forest and an open, grassy meadow, are as large as 3.5 orders of magnitude. These *B. cereus* population differences could not be attributed to metal concentrations nor water extractable ionic concentrations.

Introduction

The search for mineral deposits often incorporates new techniques or technologies that use a variety of subtle chemical or physical characteristics associated with the mineralization process. The distribution of metal-tolerant plant and fungal species has been used in mineral exploration with some success, although there are numerous environmental and physiological characteristics that are not well understood (Cannon, 1960; Brooks, 1972; and Kovalevskii, 1979).

The use of bacteria as a mineral exploration tool has been investigated near sulfur deposits (Miller, 1983) and precious- and base-metal mineralized areas (Parduhn, 1987; Parduhn and Watterson, 1984; Tucker, 1987; Tucker and others, 1989; Watterson, 1985; and Watterson and others, 1983, 1986).

Soil is a complex ecosystem dominated by numerous fungi and bacteria (Brock, 1974). Many kinds of fungi and bacteria compete for the same substrates (Brock, 1974; and Subba-rao and Alexander, 1985). Fungal-bacterial competition in a typical organic-rich soil may be minimal due to the large variety of substrate choices. However, in a stressed environment, competition for substrates will increase. Highly mineralized areas are examples of a stressed environment where biologically available metal concentrations in the soil may reach or exceed toxic limits for most organisms.

Elevated concentrations of most heavy metals within a soil greatly disrupts the natural ecology found in the surrounding unmineralized environment (Brooks, 1972; Kovalevskii, 1979; Tuovinen and others, 1971; and Watterson and others, 1986) and frequently causes environmental stresses (Brock, 1974; Ehrlich, 1978; Gottschalk, 1979; and Kuznetsov, 1963). Yet, many plants, fungi, and bacteria can, through adaptation, tolerate or even thrive in areas with elevated concentrations of heavy metals (Ballard and Grassle, 1979; Baross and Deming, 1983; Brierley, 1977, 1978; Cannon, 1960; Gottschalk, 1979; Lyalikova and Lebedeva, 1984; and Tuttle and others, 1968). In highly stressed environments, such as arctic tundra or deep sea fumaroles, only a few types of organisms tend to make the adaptations necessary for survival, but tend to occur in vast numbers (Ballard and Grassle, 1979; Baross and Deming, 1983; Kushner, 1978; and Nielson and Beck, 1972).

The successful use of *B. cereus* as a mineral deposit exploration tool depends greatly on their ability to adapt to, and thrive in, a highly mineralized environment. The complex physiological and intraspecies interactions are not fully understood but can be summarized as integrated, multifaceted dependencies: (1) *B. cereus* require organic substrates. The nutritional requirements are often met by grazing on a wide variety of fungi (Mitchell and Alexander, 1963).

(2) The fungi use many chemical and physical defensive strategies to ward off bacteria (Pollock, 1950; 1967; and Reading and Cole, 1977). One of the most important chemical defenses is the production of antibiotics. Many of these antibiotics contain the B-lactam bond.

(3) *B. cereus* can produce B-lactamase, an enzyme that breaks the B-lactam bond, which reduces the effectiveness of the antibiotic (Pollock, 1950; 1967; Ogawara, 1981; and Watterson and others, 1968). *B. cereus* produces at least two varieties of B-lactamase (which undoubtedly allows it to graze on a wide variety of fungi).

(4) Penicillin degrades into penicillamine, a strong metals-chelating agent (other B-lactam antibiotics undoubtedly form similar chelating agents).

(5) Bacteria, fungi, and other plants (which supply detrital and dissolved organic material to the soil) must be resistant to the effects of biologically available heavy metals. Studies with *B. cereus* have shown that heavy-metal resistance and B-lactamase production are transmitted on the same plasmid (Watterson and others, 1986). These characteristics confer survivability traits to *B. cereus* within a mineralized environment where the number of adaptable species is decreasing and intraspecies' competition for diminishing substrate resources is greatly increasing.

(6) For *B. cereus* to be effectively used as an exploration tool, the mineralized area must be an environment in which total number of organisms or other measurable physiological responses to this environment can be easily measured and compared to

these same responses or traits in an unmineralized environment. Two factors that facilitate the use of *B. cereus* as an exploration tool are: (a) the bacteria form spores that are long lived in the soil and geochemical samples, and (b) the culture assay is nearly species specific and easy to conduct and interpret.

The natural population distribution of *B. cereus* was examined over four distinctly different environments. The first study area consists of unmineralized pine forest and meadow environments. The second study area is in an unmineralized high desert environment near Carson City, Nevada. The third study area is located in an extensively hydrothermally altered area west of Virginia City, Nevada. The soil metal contents were similar in areas 2 and 3. The fourth study area is located on the Pacific Coast, north of Santa Cruz, California. This area contains a metal sulfide-rich zone, exposed along the cliffs, that is cross-cutting unmineralized mudstones and covered by stabilized dunes. These areas were chosen for study because of their differing geochemical histories and to compare the *B. cereus* population distributions within each environment.

Sample Collection

Four study areas were chosen for the examination of the natural abundance of *B. cereus*. In study area 1, 18 samples were collected, 28 samples were collected in study area 2, 21 samples were collected in study area 3, and 10 samples were collected in study area 4. At each site, the litter and the top 1 cm of soil were removed from the surface in an area some 20 cm in diameter. The soil was loosened to a depth of approximately 10 cm and hand mixed. Approximately 400 to 500 g of soil were placed in a cloth or paper bag and allowed to air dry. The dry soil wad disaggregated and sieved to minus-80 mesh. The minus-80-mesh fraction was used for all analytical procedures. The samples were collected during the summer of 1985.

Analytical Procedures

A. Geomicrobiology

The culture plate tests for *Bacillus cereus* were conducted using a nearly species-specific egg yolk agar culture medium (table 1, Watterson, 1985). The culture method is summarized in table 2. All water blanks, agar medium, and ceramic egg yolker were autoclaved at 121 °C for 15 min. The agar was kept in a 45 °C water bath and used within a few hours of preparation. The eggs were surface sterilized with 70 percent ethanol and aseptically added to the ceramic yolker in a sterile, laminar flow hood. The egg white was removed using the ceramic egg yolker, then 5 mL portions of the yolk were slowly added to sterile, 250-mL portions of agar medium.

Culture plates were inoculated using a 1-mL aliquot of the 10-fold serial dilutions (table 2). The test solution was added to the center of a petri dish, gently swirled, and

Table 1.--Selective egg yolk agar used for *Bacillus cereus* population studies

[L, liter, mL, milliliter, g, gram]

1.0 g K_2HPO_4 0.2 g $MgSO_4$ $7H_2O$ 0.01 g $FeSO_4$ $7H_2O$ 0.01 g $CaCl_2$ 1.0 g glucose 1.0 g NH_4Cl 0.1 g yeast extract 5.0 g trisodium citrate	All ingredients added to 1 L of deionized water, stirred well, then divided equally into 250-mL glass bottles containing agar.
Add 3-g agar to each of 4, 250-mL glass bottles.	
5-mL egg yolk per 250-mL agar, added at the time of plate pouring.	

Table 2.--Procedure for the preparation of samples for *Bacillus cereus* culture plate assay

[mL, milliliter; g, gram; °C, degree Celsius; CFU,colony-forming unit]

Step 1. Add 1 g of soil to 9 mL sterile DI water blank.
Step 2. Place tubes in a mechanical shaker for 10 min.
Step 3. Place tubes in a 90 °C water bath for 1 min.
Step 4. Remove tubes, quickly invert and place in cool water.
Step 5. Centrifuge at 1,200 rpm for 3 min.
Step 6. Make 10-fold serial dilutions in distilled water, usually 3.
Step 7. Beginning at the lowest dilution, add 1 mL of solution to a petri dish.
Step 8. Add approximately 8 mL of egg yolk agar to the petri dish directly on top of the 1 mL inoculum and swirl gently.
Step 9. Allow agar to solidify, invert, and allow to develop at 22-29 °C for about 18 h.
Step 10. Count colonies: plates with less than 30 CFU or greater than 300 CFU may not accurately reflect the population density of *B. cereus* in the soil sample.
Step 11. Average the number of CFU from the serial dilution plate counts.

about 8 mL of agar were poured directly on the solution and again swirled gently. The agar plates were allowed to cool, inverted, and incubated at room temperature for 18 h.

Population counts or colonies are a measure of one spore or one clump of spores that form a single colony, termed a colony-forming unit (CFU). The colonies form distinctive, diffuse white zones in the egg yolk agar due to extracellular enzymatic action. *B. cereus* var *mycoides* is identified by its branching growth.

Total *Bacillus spp.* populations were determined using a minimum salts medium (table 1) without the trisodium citrate or egg yolk. The fungalcide *cycloheximide* was added to the medium. The culture plates were incubated at 37 °C for approximately 48 h. The colonies appear as small, light tan or buff discus-shaped growths within the medium or circular colonies on the agar surface.

Bacterial assays should be viewed as a qualitative measure of the actual bacterial population within a sample. A factor of two variation between duplicate assays is generally acceptable. To maximize assay reproducibility, the lowest dilution factor that contains between 30 and 300 CFU/g should be used. Serial dilutions also help evaluate the assay techniques. Averaging results from several determinations may be useful. Many samples in the four study areas have bacterial counts less than 30 CFU/g in the 1:10 dilution. These populations are below the desired 30 CFU/g count, but are generally reproducible and are listed.

The results of duplicate analyses for two samples are given in table 3. The duplicate assays (A, B, C, etc.) were prepared separately from the original soil sample. The five duplicates listed for the 1:100 dilution represent a duplicate assay from that particular serial dilution. In sample AN5, the 1:10 and 1:100 dilutions give very similar population ranges (2,300 to over 3,000 CFU/g and 2,000 to 4,500 CFU/g, respectively) and show good agreement between the serial dilutions for each sample.

The results for sample TC2 are consistent within each serial dilution. The 1:1,000 dilution does not show the expected 10-fold reduction in bacterial colonies. This may reflect a separation of clumped spores, creating more colony-forming units in the lower dilution. Similar assay reproducibility problems are discussed in Parduhn (1987).

There are numerous factors that contribute to the population variations in this assay technique. Some of the physical factors contributing to population variations include the extremely small size of the bacterial spore, their ability to clump together, the often small population in some soils, and the presence of micro-environmental spheres within a soil. To minimize these effects, a large quantity (1 g) of well-dried, thoroughly disaggregated and well-mixed soil of at least minus-80 mesh should be used. Further studies into these factors are discussed in Parduhn (1987).

Table 3.--Results of replicate analyses for *Bacillus cereus* from two soil samples

Sample		dilution			
		1:10	1:100		1:1000
			A	B	
study area 4					
AN5	A	230	34	--	3
	B	280	39	39	2
	C	270	26	32	4
	D	260	29	45	2
	E	230	41	30	5
	F	>300	30	20	5
	G	280	34	--	3
	H	260	30	--	3
	I	290	36	--	6
	J	260	22	--	4
study area 1					
TC2	A	TNC*	165		41
	B	TNC*	172		50
	C	TNC*	166		39
	D	TNC*	130		43

*TNC denotes too numerous to count (>300 CFU/g).

Positive egg yolk culture tests have been noted for some strains of *B. anthracis* and *B. thuringiensis* (Watterson, 1985). These two bacteria may be variants of *B. cereus* (Gordon, 1973). *B. anthracis* and *B. thuringiensis* are rarely found in nonagricultural soils. In rare instances, *B. cereus* can cause ocular damage and is a causative agent of gastroenteritis (1). All cultured plates were considered a potential biohazard and were autoclaved before disposal.

B. Water Extraction of Soils

Water-leach extractions of the soil samples were used to examine the concentrations of some metals that may be readily available within the soil environment. One gram of soil was placed in a test tube with 10 mL of deionized water, thoroughly mixed (vigorous hand shaking) and placed on its side. The soil was resuspended every other day by vigorously hand shaking the test tube. Time-phased dissolution experiments indicated equilibrium was reached in 5 to 7 days (Tucker, 1987). At the end of 7 days, the samples were centrifuged and the supernatant was placed in a clean test tube. The concentration of Mg, Na, Ca, K, and Zn were determined by flame atomic-absorption spectrophotometry (Perkin-Elmer Corp., 1976). The concentration of Ag and Cu were determined by flameless atomic-absorption spectrophotometry (Perkin-Elmer Corp., 1977).

C. Emission Spectrography

The soils were analyzed for 31 elements by a 6-step d.c.-arc semiquantitative emission spectrographic method (Motooka and Grimes, 1976). This method measures elemental concentrations in a sample against standard concentrations. The standard concentrations are geometrically spaced (1, 2, 5, 10) over any given order of magnitude of concentration. Elemental concentrations that are estimated to fall between the above values are assigned values of 1.5, 3, 7 (of the appropriate magnitude). The precision of the analytical method has been determined to be within one reporting interval 83 percent of the time and within two reporting intervals 96 percent of the time (Motooka and Grimes, 1976).

Results and Discussion

A. Study Area 1

Study area 1 is located some 8 km north of Allenspark, Colorado, about 800 m east of CO 7 on Cabin Creek Road (gravel). This area was selected for the examination of the natural population variation of *B. cereus* because it is underlain by a uniform, unmineralized granite. Four distinct ecosystems or environments were identified within a 250-m by 140-m area (fig. 1). Eighteen samples were collected. Duplicate samples were collected at two locations within 5 m of each other. Samples 2 and 3, and

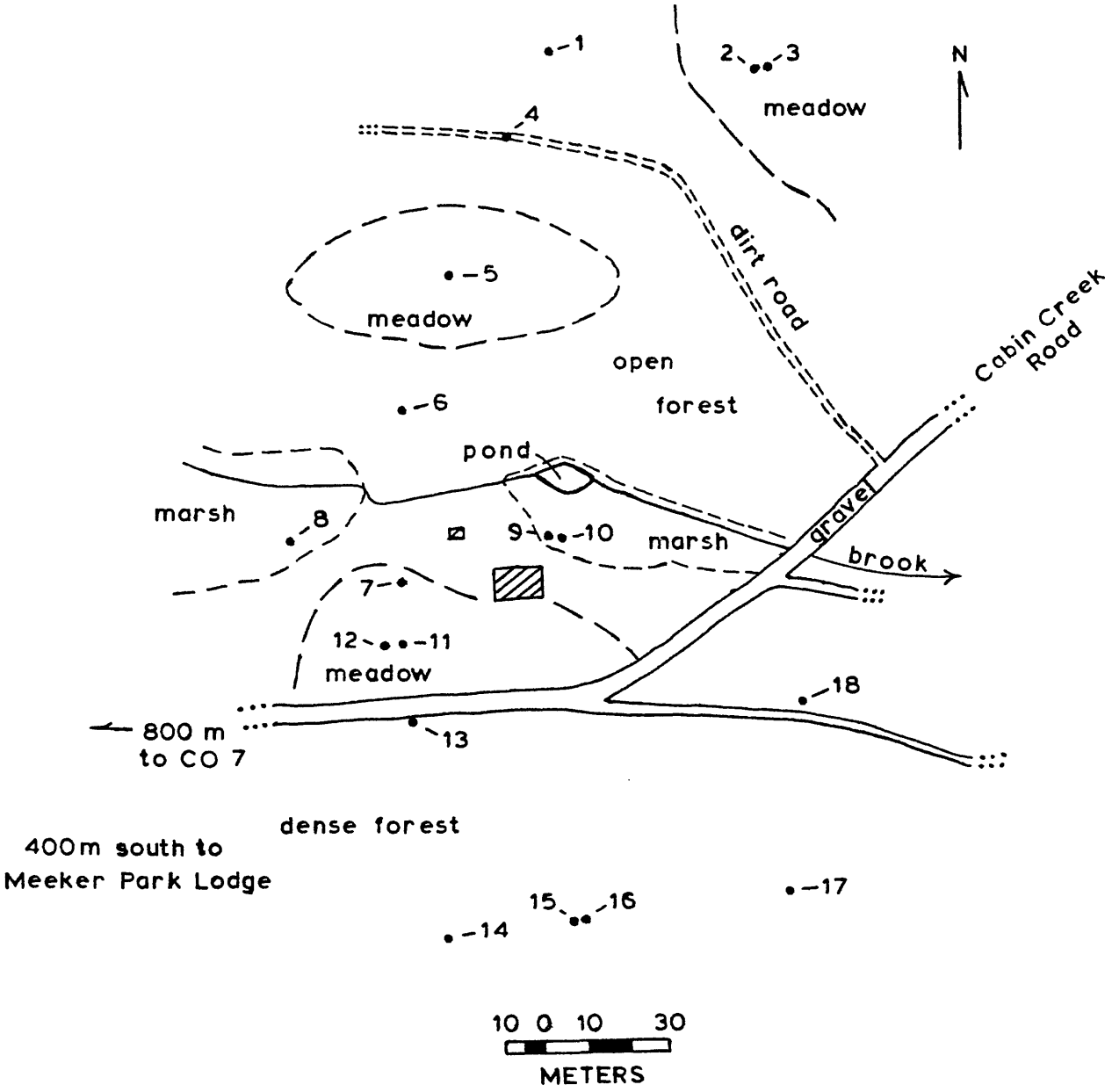

Figure 1. Site location map for study area 1, Allenspark, Colorado.

samples 15 and 16 are site duplicates. Samples 3 and 16 were split and analyzed as separate samples.

There are four rather distinct floral environments identified within the study area: (1) open meadows containing numerous grasses and wildflowers (sites 2, 3, 5, 7, 11, and 12); (2) forested area with lodge pole and ponderosa pines (sites 1, 6, 14, 15, 16, and 17); (3) marsh or wetland that contains tall grass and willows (sites 8, 9, and 10); and (4) disturbed areas on gravel roads or barren spots (sites 4, 13, and 18).

B. cereus and total *Bacillus* were determined for each sample. The population densities of *B. cereus* per gram of soil range from less than 10 to 64,000 CFU/g (table 4). The bacterial assays with less than 30 CFU/g are fairly reproducible and are listed for comparison. The duplicate location samples 2 and 3 have *B. cereus* populations of 17,000 and 2,100 CFU/g, respectively, and samples 15 and 16 have populations of less than 10 and 20 CFU/g, respectively. Duplicate determinations at site 3 are 2,100 and 6,100 CFU/g which is very high (table 4). Duplicate determinations at site 16 are 20 and 40 CFU/g (table 4). The population differences at sites 15 and 16 are within the normal fluctuations for plate cultures. The population variations at sites 2 and 3 are rather large considering the apparent uniformity of the meadow environment.

Several biogeochemical studies have indicated that soil metal concentrations can significantly alter the population densities of *B. cereus* (Parduhn, 1987; Parduhn and Watterson, 1984; and Watterson and others, 1986). The concentration of copper and lead was compared to *B. cereus* populations (fig. 2). The concentration range for Pb is 15-100 ppm with 13 samples containing 50-70 ppm Pb (table 5). The concentration range for Cu is 15-30 ppm (table 5). Given the geology, the very small range of the metal concentrations and the precision of the analytical method, it is very difficult to distinguish geochemically significant differences or anomalous metal concentrations that could have affected the observed *B. cereus* population ranges within the study area. There are also no apparent correlations with metals such as Be, Mn, La, Y, or Co with *B. cereus*.

The concentration of seven water-soluble metals within the soil were determined (table 6). The concentrations of Ca, Mg, Na, and K were summed and plotted against *B. cereus* (fig. 3). There is no definite correlation between the sum of major cations and *B. cereus* populations.

The ratios of total *Bacillus spp.* to *B. cereus* were calculated (see table 4). The samples with the lowest ratios are found in samples from meadows and marshy areas. There is a wide range in the concentration of soluble ions in these samples. Sites with the highest ratios occur in the forested environment and from disturbed areas. Sample 12 was collected under a lone ponderosa in a meadow 6 m from site 11. Sample 12 has a greater than two-fold decrease in *B. cereus* population densities (table 4).

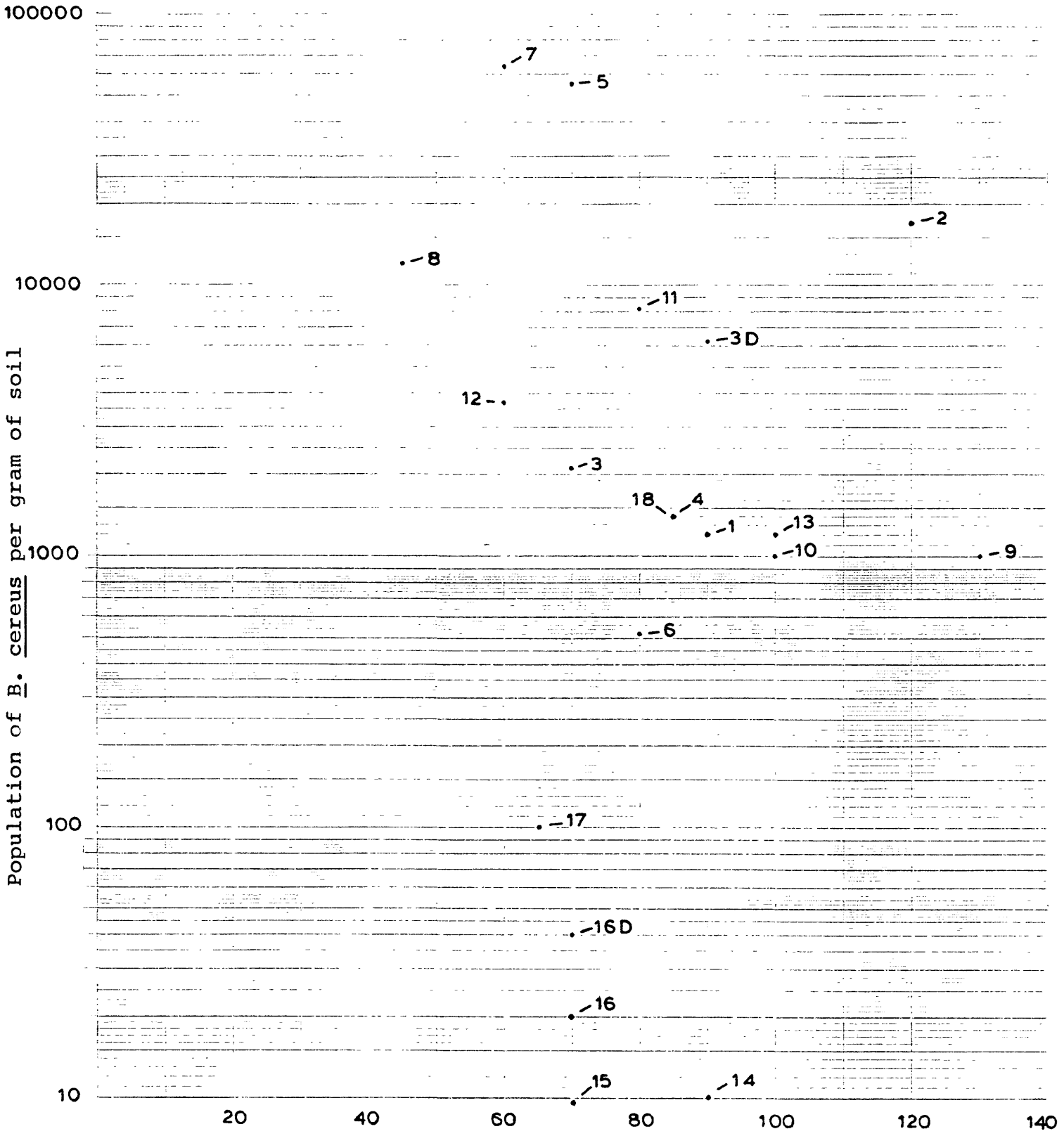

Figure 2. The sum of total soil Pb and Cu concentrations versus Bacillus cereus in study area 1, Allenspark, Colorado.

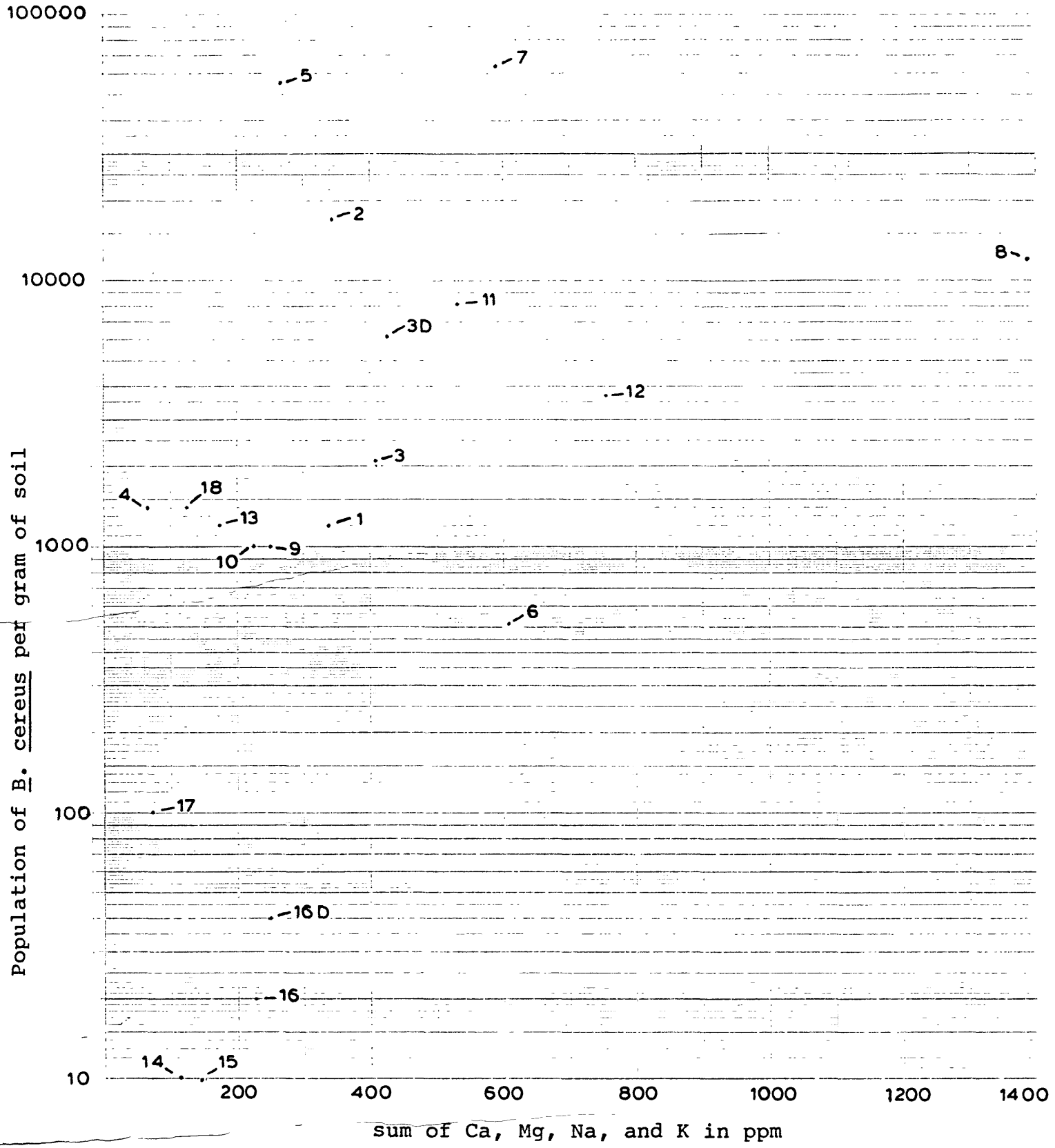

Figure 3. Distribution of Bacillus cereus populations versus the sum of water-extractable Ca, Mg, Na, and K in study area 1, Allenspark, Colorado.

Table 4. Results from culture tests for Bacillus cereus and total Bacillus in study area 1, Allenspark, Colorado

sample	dilution 1:10	1:100	1:1000	total CFU/g	sample	dilution 1:10	1:100	1:1000	total CFU/g
TC01	120	14	1	1200	TC10	100	5	0	1000
pines	TNC	300	50 (6)	30000	marsh	TNC	340 (12)	25	34000
TC02	TNC	171	40	17000	TC11	TNC	82	24	8200
meadow	TNC	TNC	89 (32)	89000	meadow	TNC	TNC	250 (14)	25000
TC03	210	34	7	2100	TC12	TNC	37	6	3700
meadow	TNC	TNC	92 (7)	92000	meadow	TNC	TNC	470 (5)	47000
TC03D	TNC	61	11	6100	TC13	116	16	1	1200
meadow	TNC	TNC	250 (16)	250000	dirt road	TNC	TNC	76 (1)	76000
TC04	142	16	3	1400	TC14	1	0	0	10
dirt road	TNC	320	29 (2)	32000	pines	TNC (8)	56	4	5600
TC05	TNC	TNC	55	55000	TC15	0	0	0	<10
meadow	TNC	TNC	>500(100)	>500000	pines	TNC	42	7	4200
TC06	51	11	3	510	TC16	2	0	0	20
pines	TNC	337	49 (2)	34000	pines	TNC	280	36	28000
TC07	TNC	TNC	64	64000	TC16D	4	0	0	40
meadow	TNC	TNC	480(130)	480000	pines	TNC	160 (3)	10	16000
TC08	TNC	120	38	12000	TC17	10	0	1	100
marsh	TNC	TNC(160)	160	160000	pines	TNC(12)	92	6	9200
TC09	105	9	1	1000	TC18	139	11	1	1400
marsh	TNC	200(9)	21	20000	dirt road	TNC	380 (6)	57	47000

top lines, B. cereus CFU/g; second line, total Bacillus CFU/g. TNC is too numerous to count. Number in parentheses represents B. cereus var. mycoides CFU/g.

Table 5. 6-step d.c.-arc semiquantitative emission spectrographic data results for study area 1, Allenspark Colorado. [Number in parentheses is detection limit; L is concentration lower than detection limit; N is concentration not detected.]

sample	Fe (.05)	Mg (.02)	Ca (.05)	Ti (.002)	Mn (10)	B (10)	Ba (20)	Be (1)	Co (5)	Cr (10)	Cu (5)	La (20)	Ni (5)	Pb (10)	Sr (100)	V (10)	Y (10)	Zr (10)
TC01	2	.3	.7	.3	700	30	300	2	10	50	20	300	15	70	150	70	100	150
02	1.5	.2	.5	.2	1000	30	300	3	N	30	20	20	7	100	100	50	20	150
03	2	.3	.5	.2	700	30	300	2	7	50	20	30	15	50	150	30	20	150
03D	2	.3	.7	.3	700	30	300	3	7	20	20	20	15	70	L	50	20	150
04	3	.5	.5	.3	300	30	500	2	10	50	15	70	15	70	150	70	50	200
05	2	.3	.7	.3	700	50	300	3	10	50	20	30	20	50	150	70	20	200
06	2	.2	.5	.2	700	50	200	5	5	30	30	50	10	50	L	50	30	150
07	2	.3	.3	.2	500	50	200	5	7	30	30	30	20	30	N	70	30	150
08	.7	.15	.3	.07	300	30	70	5	N	15	30	30	5	15	N	30	50	30
09	3	.5	.3	.3	300	30	300	2	10	100	30	50	50	100	N	50	20	200
10	2	.5	.3	.3	200	20	300	2	10	50	30	50	20	70	N	50	20	150
11	2	.3	.5	.3	700	70	300	3	7	30	30	30	15	50	L	50	30	150
12	2	.3	.5	.2	500	70	200	5	L	30	30	30	10	30	L	50	20	150
13	3	.5	.5	.3	500	30	300	2	10	70	30	30	30	70	L	70	30	200
14	2	.5	.5	.3	700	70	500	1.5	10	50	20	200	20	70	150	70	70	200
15	2	.5	.5	.3	500	30	500	2	10	70	20	30	20	50	100	50	15	200
16	2	.5	.5	.3	1000	30	300	1.5	5	50	20	30	15	50	100	70	30	200
16D	2	.5	.5	.3	700	70	500	2	10	70	20	20	15	50	100	70	15	200
17	1.5	.3	.5	.3	300	50	300	2	10	50	15	100	20	50	100	70	20	200
18	1.5	.3	.5	.2	1000	50	500	2	10	30	15	30	15	70	L	50	20	150

Fe, Mg, Ca, and Ti reported in percent. All other elements reported in ppm. The elements Ag, As, Au, Bi, Cd, Mo, Nb, Sb, and W were not detected.

Table 6.--Concentration of water-soluble cations and *Bacillus cereus* populations from study area 1, Allenspark, Colorado

[ppm, parts per million; ppb, parts per billion; CFU/g, colony-forming unit per gram]

Sample	Ca ppm	Mg ppm	Na ppm	K ppm	Zn ppm	Ag ppb	*B. cereus* CFU/g
TC01	200	47	8	84	0.2	<0.5	1,200
02	210	40	18	75	.6	<.5	17,000
03	285	45	11	68	1.2	.7	2,100
03D	295	45	10	76	.8	<.5	6,100
04	33	8	6	18	<.2	<.5	1,400
05	180	32	7	48	<.2	<.5	55,000
06	400	100	10	98	.2	.6	510
07	390	85	17	95	.2	.7	64,000
08	910	230	150	98	.2	.5	12,000
09	160	42	27	24	<.2	<.5	1,000
10	150	34	24	18	<.2	<.5	1,000
11	320	69	23	120	.2	<.5	8,200
12	435	105	17	200	.3	.6	3,700
13	91	24	14	45	<.2	<.5	1,200
14	72	12	9	23	<.2	<.5	10
15	95	19	8	22	.2	<.5	<10
16	135	32	7	52	<.2	<.5	20
16D	155	31	7	58	<.2	<.5	40
17	35	15	8	14	.2	<.5	100
18	85	18	6	16	<.2	<.5	1,400

Copper was not detected in any of the samples at a 0.5-ppm detection limit.

In the forested areas there was only a very thin A horizon, generally less than 2 cm, which directly overlaid the crumbled C horizon. In contrast, the meadow and marsh sites had very organic-rich soils with an A-horizon depth greater than the 10-cm sampling depth. The high population densities of *B. cereus* in organic-rich soils reflects a high nutrient content. The soils could contain numerous fungi on which the bacteria graze and (or) high concentrations of readily available dissolved nutrients.

The four floral environments in the study area show population densities of *B. cereus* ranging from less than 10 to 64,000 (table 4). The samples from the forest environment have the lowest populations of *B. cereus*. Very similar *B. cereus* populations occur in the three samples from the barren or sandy environment. The three samples from the marsh environment show a wide range in *B. cereus* populations. The highest *B. cereus* populations are found in the meadow environment; however, there is an order of magnitude variation in the *B. cereus* populations within the seemingly uniform meadow environment. The population variations may reflect subtle biochemical differences or the presence of distinct microenvironments within the soil.

The results from this limited study indicate a significant natural variation in *B. cereus* populations may occur within a small area underlain by a uniform, unmetallized rock type. The population of *B. cereus* within the study area does not appear to reflect or be greatly dependent on the metal concentrations or water-soluble ions found in the soils. The observed population variations appear to be influenced by the degree of soil development. The presence of distinct microenvironments within an apparently uniform area may explain the large variations of *B. cereus* between closely-spaced sites.

B. Study Area 2

Study area 2 is located some 16 km east of Carson City, Nevada. This area was selected because of the uniformity of the underlying welded ash flow tuff and vegetation types. Twenty-two samples were collected (fig. 4). A basalt flow unconformably overlies the tuff, to the west of the original traverse line. Six samples were collected over the basalt. The basalt is weathering very slowly, as indicated by the angularity of the rock fragments and fresh appearance. The soil is composed primarily of stabilized loess or wind-blown material.

The sites sampled over the tuff were collected predominantly from the base of a prominent outcrop. The soil is composed of fine sand-sized material that is predominantly weathered tuff. A few samples were collected under piñon and juniper trees, and sage and Mormon tea bushes. All other samples were collected in open areas. Sparse clumps of grass were found in the open areas but much denser growth occurs under the scattered bushes. Under the piñon pines, a thick mat of needles had collected to a depth of some 12-15 cm thick. Under the juniper trees, a layer of needles some 2-4 cm in thickness had collected. Sites 1 and 2 and sites 17 and 18 are location duplicates. Samples 2 and 18 were split and both splits analyzed.

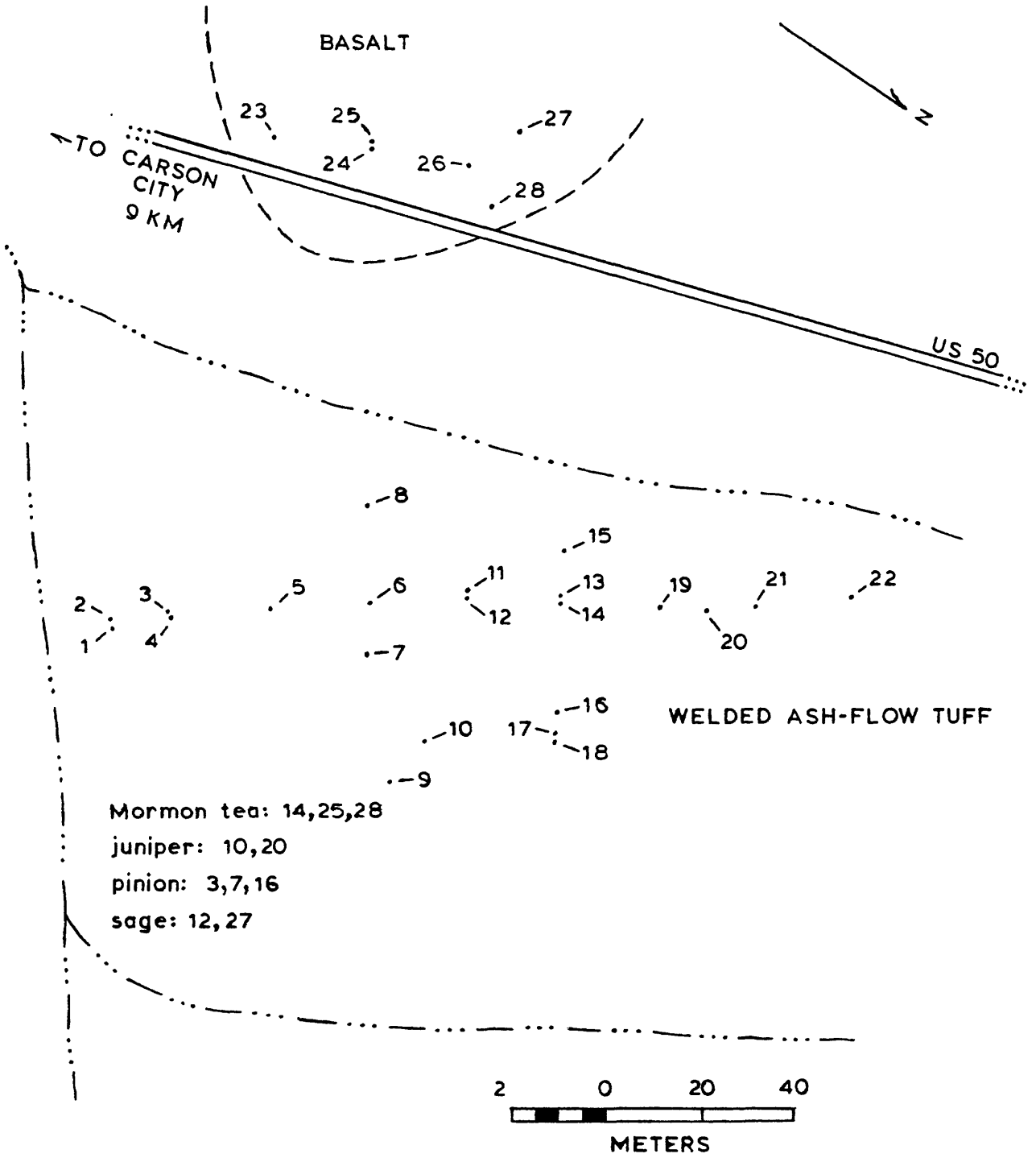

Figure 4. Site location map for study area 2, Carson City, Nevada.

The population densities of *B. cereus* are low, ranging from less than 10 to 820 CFU/g (table 7). Nineteen samples have population densities less than 30 CFU/g. Six of eight samples with *B. cereus* populations greater than 50 CFU/g of soil were collected under vegetation. Similar plant *B. cereus* correlations were noted by Parduhn (1987). Sample 23 was collected from between angular cobbles of basalt.

The metal concentrations are generally low and show small concentration ranges (table 8). The concentrations of Ag are anomalous for a tuff. The concentration of total Ag and water extractable Ag (table 9) were plotted versus the *B. cereus* populations (fig. 5). There are no apparent correlations between total-Ag concentrations and water-extractable Ag concentrations to *B. cereus* populations. There are no apparent correlations with any other metal examined.

B. cereus populations and the sum of the major cations from the water extraction were examined. The plot is similar to figure 4 showing no definite correlation between soluble ions and *B. cereus* populations.

There are only nine samples with *Bacillus spp.* to *B. cereus* ratios less than 10,000. Six of these samples were collected under vegetation, suggesting that *B. cereus* cannot effectively compete with other soil microorganisms, or survive in open, exposed soils.

The data plots suggest that vegetation (or presence of organic matter) has a greater influence on the population density of *B. cereus* than other factors. There may be other metals or ions that are more important to the ecology of *B. cereus* population dynamics but they have not been identified.

C. Study Area 3

Study area 3 is located approximately 20 km west of Virginia City, Nevada (fig. 6). This study area was chosen because of its botanical and climatic similarity to study area 2. However, this area has been intensely altered and is near the Comstock Silver district. The samples were collected along two traverse lines (fig. 6). The analyses indicated that the soils were not metal rich (table 10).

This study area is underlain by highly altered, welded ash flows that show abundant iron enrichment and argillation of the host rock. The vegetation has distinct variations that may be controlled by the geochemical characteristics of the underlying rocks. The most striking botanical feature is the presence of large barren areas that contain only widely spaced ponderosa pines. Surrounding the ponderosa islands or zones are forests of juniper and piñon with sage, Mormon tea, and a variety of grasses. The ponderosa zones contain virtually no soil and are composed of sand-sized to cobble-sized angular fragments of the weathered bedrock. A soil has developed over the rest of the area.

Table 7. Results from culture tests for Bacillus cereus and total Bacillus in study area 2, Carson City, Nevada

sample	dilution 1:10	1:100	1:1000	total CFU/g	sample	dilution 1:10	1:100	1:1000	total CFU/g
CC01	1	0	0	10	CC10	21	2	1	210
open	TNC	TNC	340	340000	juniper	TNC	TNC	>500	>500000
CC02	1	0	0	10	CC11	1	0	0	10
open	TNC	TNC	340	340000	open	TNC	TNC	320	320000
CC02D	0	0	<10	<10	CC12	1	0	0	10
open	TNC	TNC	450	450000	sage	TNC	TNC	420	420000
CC03	3	0	0	30	CC13	0	0	0	<10
pinion	TNC	TNC	50	50000	open	TNC	TNC	51	51000
CC04	7	0	0	70	CC14	25	1	0	200
open	TNC	TNC	260	260000	Mormon tea	TNC	TNC	450	450000
CC05	0	0	0	<10	CC15	0	0	0	<10
open	TNC	415	51	42000	open	TNC	TNC	340	340000
CC06	0	0	0	<10	CC16	1	1	0	<10
open	TNC	400	70	40000	pinion	TNC	TNC	350	350000
CC07	1	0	0	10	CC17	1	0	0	10
pinion	TNC	TNC	160	160000	open	TNC	TNC	230	230000
CC08	0	0	0	<10	CC18	0	0	0	<10
open	TNC	TNC	104	100000	open	TNC	TNC	150	150000
CC09	2	0	0	20	CC18D	0	0	0	<10
open	TNC	TNC	230	230000	open	TNC	TNC	310	310000

Top lines, B. cereus CFU/g; second line, total Bacillus CFU/g. TNC is too numerous to count. Number in parentheses represents B. cereus var. mycoides CFU/g.

Table 7. Results from culture tests for Bacillus cereus and total Bacillus in study area 2, Carson City, Nevada--Continued

sample	dilution factor -1	-2	-3	total	sample	dilution factor -1	-2	-3	total
CC19	1	0	0	10	CC24	1	0	0	10
	TNC	TNC	220	220000		TNC	TNC	410	410000
CC20	1	0	0	10	CC25	82	10	0	820
	TNC	TNC	>500	>500000		TNC	TNC	400	400000
CC21	6	6	0	60	CC26	2	0		20
	TNC	TNC	~800	>800000		TNC	TNC	86	86000
CC22	0	0	0	0	CC27	44	5		450
	TNC	TNC	300	300000		TNC	TNC	480	480000
CC23	78	10		780	CC28	8	1		80
	TNC	TNC	260	260000		TNC	TNC	90	90000

*Bacillus cereus populations per gram of soil; **Total Bacillus populations per gram of soil.

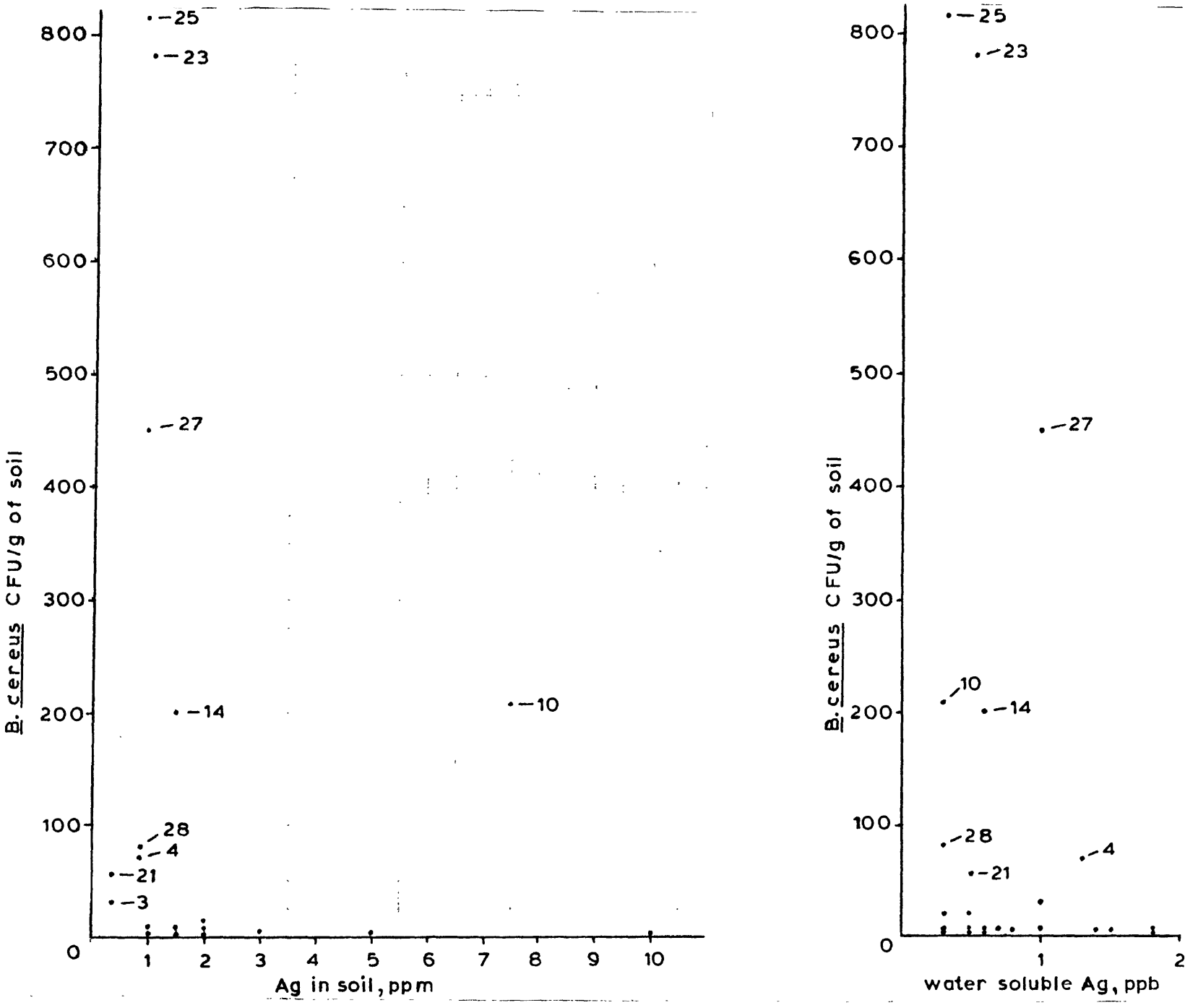

Figure 5 Distribution of total soil Ag and water extractable Ag versus Bacillus cereus populations, study area 2, Carson City, Nevada.

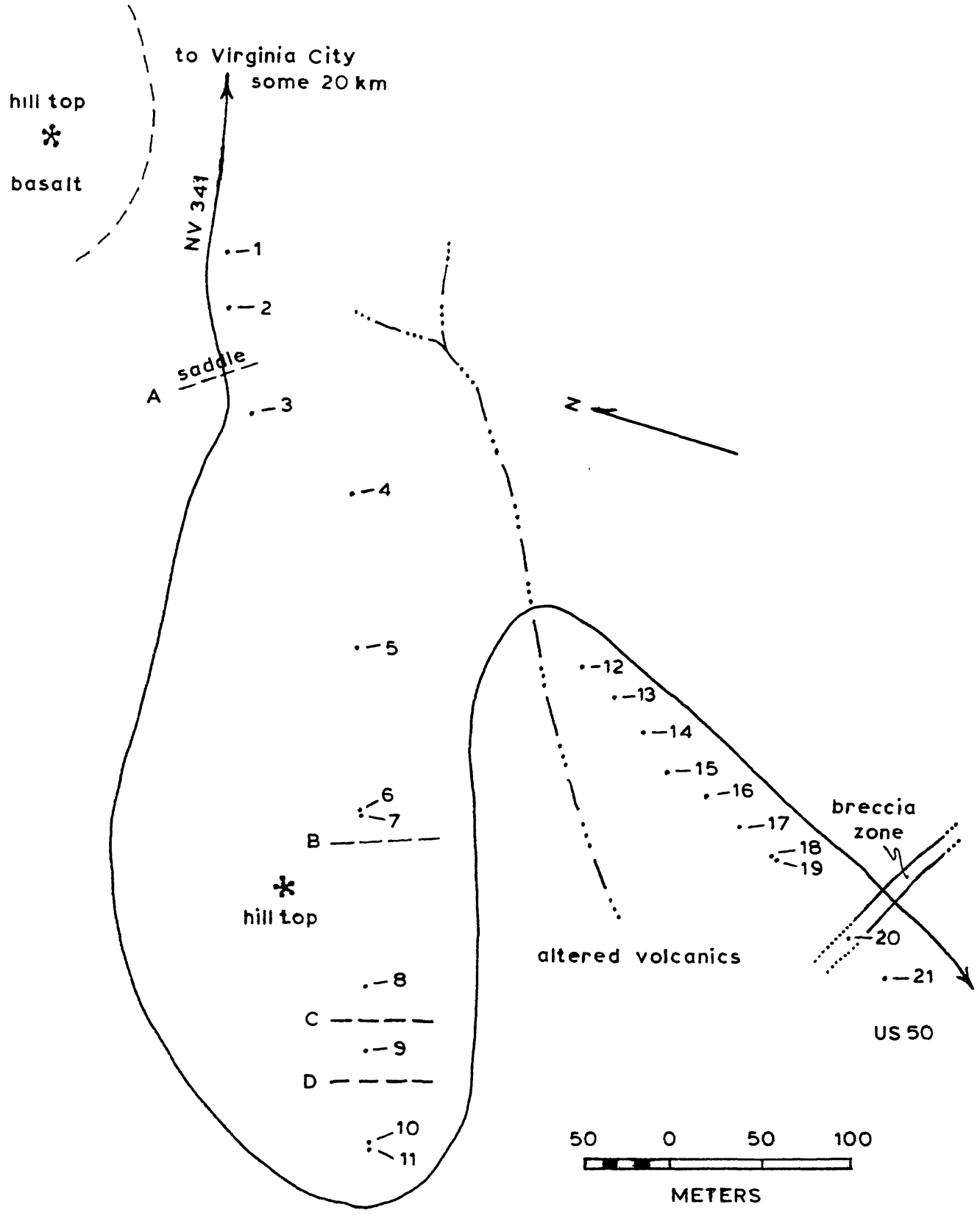

Figure 6. Site location map for study area 3, Virginia City, Nevada.

Table 8. 6-step d.c.-arc semiquantitative spectrographic analysis for samples from study area 2, Carson City, Nevada

sample	Fe (.05)	Mg (.02)	Ca (.05)	Ti (.002)	Mn (10)	B (10)	Ba (20)	Be (1)	Co (5)	Cr (10)	Cu (5)	La (20)	Ni (5)	Pb (10)	Sr (100)	V (10)	Y (10)	Zr (10)	Ag (.5)
CC01	3	.7	1.5	.3	700	50	700	1	10	30	20	20	15	70	500	100	15	200	1.5
02	3	.7	1.5	.3	700	70	700	1.5	10	50	20	20	15	70	500	100	10	150	2
02D	1.5	.7	1.5	.3	500	70	500	1.5	7	30	20	20	10	50	300	70	15	150	1.5
03	2	.7	1.5	.3	500	50	500	2	10	30	20	20	10	30	500	70	15	100	L
04	3	.5	1	.3	500	70	500	1.5	7	30	20	20	7	50	300	70	15	150	.7
05	3	.7	1.5	.3	500	50	500	1.5	10	20	20	20	10	50	500	70	10	100	1
06	3	.7	1.5	.3	700	70	500	1	10	50	30	20	15	50	500	100	15	150	2
07	2	.7	1.5	.3	500	70	500	1.5	10	30	20	20	10	50	500	70	15	150	2
08	2	.5	1.5	.2	300	70	500	1	7	30	15	20	10	30	300	70	15	50	1.5
09	3	.7	1.5	.3	500	50	500	1.5	10	30	30	20	15	50	500	150	15	200	2
10	2	.7	1.5	.3	500	50	500	1.5	7	20	20	20	10	70	500	70	15	150	7
11	2	.7	1.5	.3	700	70	500	1.5	10	50	20	20	15	50	500	100	15	200	3
12	2	.5	1	.2	500	50	300	1.5	7	20	15	20	7	70	300	70	10	150	1
13	2	.7	1.5	.3	700	70	500	1.5	10	50	20	20	15	70	500	70	15	150	5
14	2	.7	1.5	.3	500	70	500	1.5	10	20	20	20	10	70	500	70	15	150	1.5
15	2	.5	1	.3	500	70	500	1.5	10	30	20	20	10	50	300	70	15	200	1
16	2	.7	1.5	.3	700	30	300	1.5	10	30	20	20	15	30	300	70	10	70	1
17	3	.7	1.5	.3	700	70	500	1.5	10	30	20	20	10	50	500	100	15	150	1.5
18	2	.7	1.5	.3	500	70	500	1.5	10	70	20	20	10	50	500	70	20	200	2
18D	3	.7	1.5	.5	700	70	700	1.5	15	50	30	30	15	50	500	100	20	200	2
19	2	.7	1.5	.3	500	70	300	2	7	20	20	20	7	50	500	70	15	150	3
20	1.5	.5	1	.2	500	70	300	1	10	20	20	20	15	50	300	70	15	100	2

Table 8. continued

sample	Fe (.05)	Mg (.02)	Ca (.05)	Ti (.002)	Mn (10)	B (10)	Ba (20)	Be (1)	Co (5)	Cr (10)	Cu (5)	La (20)	Ni (5)	Pb (10)	Sr (100)	V (10)	Y (10)	Zr (10)	Ag (.5)
CC21	2	.7	1.5	.3	700	50	300	1.5	7	15	20	20	7	50	500	70	15	150	L
22	2	.5	1	.3	500	70	300	1.5	7	20	20	20	10	50	300	100	20	150	10
23	3	1	2	.3	700	70	700	1.5	15	50	30	20	15	70	500	100	15	200	1
24	3	.7	1.5	.5	700	50	700	1.5	15	70	30	20	20	50	500	150	20	150	2
25	3	.7	2	.5	700	70	500	L	15	50	30	20	15	70	700	150	20	150	.7
26	3	.7	1.5	.2	500	70	500	1	10	50	20	20	15	50	500	100	15	100	L
27	3	.7	1.5	.3	700	70	500	1.5	10	50	30	20	15	100	500	100	15	150	1
28	3	.7	1.5	.5	700	70	500	1	15	50	30	20	20	100	500	100	15	150	.7

Fe, Mg, Ca, and Ti reported in percent; all other elements reported in ppm.
Detection limit listed in parentheses; N=not detected; L=concentration just below the first standard.
The elements As, Au, Bi, Cd, Mo, Nb, Sb, Sn, and W were not detected.

Table 9.--Concentration of water-soluble cations and *Bacillus cereus* populations from 28 soil samples collected from study area 2, Carson City, Nevada

[ppm, parts per million; ppb, parts per billion; CFU/g, colony-forming units per gram]

Sample	Ca ppm	Mg ppm	Na ppm	K ppm	Zn ppm	Ag ppb	*B. cereus* CFU/g
CC01	145	24	11	45	<0.2	0.7	10
02	83	15	11	50	.2	<.5	10
02D	70	12	9	45	<.2	.5	<10
03	140	32	22	250	<.2	1.0	30
04	52	16	21	28	<.2	1.3	70
05	100	17	17	67	<.2	.6	<10
06	68	15	11	30	<.2	<.5	<10
07	105	26	10	100	.3	1.4	10
08	53	15	9	38	<.2	<.6	<10
09	46	10	11	15	<.2	.5	20
10	530	42	22	120	<.2	<.5	210
11	55	13	10	34	<.2	<.5	10
12	335	58	15	195	<.2	.6	10
13	76	14	10	48	<.2	.5	<10
14	350	56	13	310	.2	.6	200
15	67	16	10	36	<.2	<.5	<10
16	220	58	17	270	<.2	.8	10
17	91	19	21	38	<.2	1.0	10
18	71	18	11	20	<.2	.5	<10
18D	66	16	20	24	<.2	1.8	<10
19	48	12	20	30	<.2	1.8	10
20	300	43	13	86	<.2	.5	10
21	135	26	13	110	.3	.5	60
22	75	16	12	38	.3	.5	<10
23	105	21	20	32	<.2	.5	780
24	155	29	15	70	<.2	<.5	10
25	380	36	26	145	<.2	<.5	820
26	90	19	24	27	<.2	<.5	20
27	205	37	17	140	<.2	1.0	450
28	315	38	22	135	<.2	<.5	80

Copper was not detected in any of the samples at a detection limit of 0.5 ppm.

Table 10. 6-step d.c.-arc semiquantitative emission spectrographic data results for study area 3, Virginia City, Nevada

sample	Fe (.05)	Mg (.02)	Ca (.05)	Ti (.002)	Mn (10)	B (10)	Ba (20)	Be (1)	Co (5)	Cr (10)	Cu (5)	La (20)	Ni (5)	Pb (10)	Sr (100)	V (10)	Y (10)	Zr (10)
VC01	3	.3	1	.5	300	20	1000	1	7	20	30	20	7	30	500	100	15	500
02	3	.7	.7	.3	700	50	500	1.5	10	20	50	30	20	30	700	150	10	70
03	3	.5	.3	.3	150	30	300	L	L	30	30	20	7	30	500	150	15	50
04	3	.5	.15	.2	150	50	500	L	N	30	20	20	L	20	150	150	L	50
05	2	.2	.05	.2	50	100	200	L	N	50	20	20	L	30	300	150	10	100
06	2	.5	.2	.3	150	70	300	1	L	20	20	20	L	30	300	100	15	150
07	2	.7	.2	.3	150	70	300	1	L	30	20	20	5	20	300	100	10	70
07D	3	.7	.3	.3	150	70	200	L	L	20	30	20	5	20	300	150	15	50
08	2	.15	.7	.3	500	70	300	L	N	30	20	20	7	30	30	150	15	50
09	2	.3	.7	.3	700	30	500	1.5	10	100	30	20	15	30	300	100	10	70
10	2	.5	1.5	.3	700	20	700	1	70	50	30	20	30	30	500	100	15	70
11	3	.7	1.5	.3	700	30	700	1.5	70	100	30	20	30	50	700	100	10	50
12	3	.7	1	.3	300	50	500	1	7	20	20	L	7	30	500	150	15	200
13	2	.5	.7	.2	300	70	500	L	L	15	30	20	7	30	300	70	N	150
14	2	.3	.7	.3	700	50	500	1	10	10	20	20	L	30	300	70	L	150
15	3	.5	1	.5	300	20	500	L	7	10	30	20	5	30	500	100	L	500
16	2	.5	1.5	.3	500	30	500	1.5	10	10	20	20	5	30	500	100	15	100
17	3	.7	1.5	.3	700	15	500	1	15	20	30	20	10	30	500	100	15	150
18	2	.5	1.5	.3	300	30	500	1	7	30	15	20	5	20	300	70	15	150
19	2	.5	1.5	.5	300	30	500	1	10	20	15	20	7	30	500	100	10	150
19D	2	.5	1.5	.3	300	30	500	L	7	20	20	20	7	30	500	100	15	70
20	3	.5	.15	.2	200	70	1000	L	L	L	30	20	L	20	300	150	N	100
21	3	.5	.5	.3	200	50	700	1	L	30	30	20	7	30	500	100	L	100

Fe, Mg, Ca, and Ti reported in percent; all other elements reported in ppm.
Detection limit listed in parentheses; N=not detected; L=concentration just below the first standard concentration.
The elements Ag, As, Au, Bi, Cd, Mo, Sb, Sn, and W were not detected.

The first sampling traverse line crossed into one of the ponderosa zones, then crossed back into the piñon-juniper forest. The samples along the second traverse were collected at 25-m intervals in a level area south of the first traverse line. At the end of the second traverse line is a highly iron-enriched breccia zone. Line A on figure 6 is the boundary between the sage-grass flora and the barren ponderosa zone. Virtually no other plants are found within the ponderosa zone. The rocks are yellow to whitish in color. There is no soil development on the slopes. Samples 3-7 were collected from essentially mechanically weathered rock that contained a wide range of particle sizes. At line B the ponderosa zone ended rather abruptly and piñon pine, sage, and scattered grass clumps appeared. A low growing, blue-grey ground shrub was also present. There was a marked increase in soil to the west of line B. At line C there was a distinct increase in the number of sage bushes and grass clumps. At line D the blue-grey shrub disappeared and Mormon tea appeared to the west. The rocks were not as altered near site 8, as in the ponderosa zone. Near site 10, it was possible to see the texture of the host rock, indicating the intensity of alteration had decreased. Near site 20, the blue-grey bush and ponderosa pine appeared and sage and grasses greatly decreased in number.

The ponderosa pine zones are an environmental curiosity. This type of geobotanical environment occurs in several places in Utah. Geochemical studies on the Utah sites suggest that the alteration was produced by thermal springs cogenetic with the emplacement of intrusives. There is geochemical evidence of intrusive emplacement and extensive hydrothermal activity associated with the Comstock lode located only a few kilometers to the east of the study area. Geochemical studies at one Utah site similar to this study area found the ground waters to have pH values less than 2. A low soil pH in these highly altered zones may account for the sparse vegetation and low *B. cereus* populations.

Duplicate site samples were collected at three locations within the study area. Samples 6 and 7, 10 and 11, and 18 and 19 are site duplicates. The duplicate samples (numbers 7, 11, and 19) were collected about 2 m from the original sample split and analyzed as separate samples.

The elemental concentrations from the soils in this study area are very similar to those in study area 2, except for the absence of Ag in this study area (table 10). The mean Cu concentrations are 26 and 22 in study areas 3 and 2, respectively. The mean Pb concentrations are 29 and 57 in study areas 3 and 2, respectively. The metal concentration differences between the two study areas are not analytically or geochemically significant. The data results indicate that, although this study area has been significantly altered, the hydrothermal fluids responsible for the alteration were not charged with metals. There may be localized metal concentrations in small veins, but the sample density was too sparse to detect them.

The population densities of *B. cereus* and *Bacillus spp.* vary widely within the study area (table 11). The culture tests indicate that the ponderosa zone is virtually devoid of any *Bacillus* species (table 11, samples 3-7). A similar reduction in *Bacillus spp.* population occurs near the breccia zone (table 11, samples 20 and 21).

The results of the water extraction are given in table 12. The plot of *B. cereus* versus major soluble cations shows no definite correlation (fig. 7). Examination of water soluble Zn and Ag concentrations versus *B. cereus* populations show no observable correlation (no plot). There is no observable correlation between the ratio of *Bacillus spp.* to *B. cereus* versus major soluble cations (no plot).

With the exception of the ponderosa zone, samples in this study area appear to have a high amount of organic material and soil formation is common. The distribution of *B. cereus* closely follows these qualitative observations. The range of *B. cereus* populations along the second traverse (samples 12-19) cannot be adequately explained on the basis of metal content or major environmental factors. The population ranges may be due to physiological, chemical, or physical stresses that are not fully understood.

A comparison of study areas 2 and 3 indicates that the metal concentrations are analytically and geochemically similar, with the exception of Ag. The *B. cereus* populations in both areas show wide ranges. In study area 2, the *B. cereus* populations vary between less than 10 to 820 CFU/g. Seventeen samples (61 percent) have *B. cereus* populations of 10 CFU/g or less. In study area 3, the *B. cereus* populations vary between less than 10 and 1,700 CFU/g of soil. Nine samples have *B. cereus* populations of 10 CFU/g or less. There are slightly higher *B. cereus* populations in area 3, but this does not appear to be related to metal content or extractable major or minor cations examined. In study areas 1 and 3, the amount of soil development seemed to have a significant influence on *B. cereus* populations. The degree of soil development was qualitatively more in most of the samples in study area 3 compared to study area 2, which may account for the higher *B. cereus* populations in study area 3.

The populations of *Bacillus spp.* range from 40,000 to greater than 500,000 CFU/g in study area 2. The mean population is approximately 280,000 CFU/g. In study area 3, *Bacillus spp.* populations range from less than 10 to over one million CFU/g with a mean population of approximately 250,000 CFU/g. It should be noted that the *Bacillus spp.* are greatly reduced over the ponderosa zones in study area 3 (table 11).

The results from the *B. cereus* plate cultures studied and the geochemical analyses from study areas 2 and 3 suggest that three factors need to be examined when comparing population densities of *B. cereus* found within a study area. (1) The degree of soil development appears to have a significant impact on the populations of *B. cereus*. This finding is in agreement with Parduhn (1987). Samples collected from open areas in a sandy soil may have much lower *B. cereus* populations than more developed soils.

Table 11. Results from culture tests for Bacillus cereus and total Bacillus in study area 3, Virginia City, Nevada

sample	dilution 1:10	1:100	1:1000	total CFU/g	sample	dilution 1:10	1:100	1:1000	total CFU/g
VC01	6	0	0	60	VC11D	27	1	0	270
	TNC	345 (1)	38	35000		TNC	TNC	140 (1)	140000
VC02	0	1	0	<10	VC12	45	3	1	450
	TNC	TNC	>1000	>1000000		TNC	60 (4)	5	6000
VC03	0	0	0	<10	VC13	20	3	0	200
	123	12 (13)	0	1200		TNC	TNC	200 (10)	200000
VC04	0	0	0	<10	VC14	172	17	1	1700
	13 (3)	0	0	130		TNC	TNC	287 (4)	290000
VC05	0	0	0	<10	VC15	13	2	0	130
	0	0	0	<10		TNC	300 (8)	42	30000
VC06	0	0	0	<10	VC16	150	20	3	1500
	7	0	0	70		TNC	TNC	250 (8)	250000
VC07 & VC07D	0	0	0	<10	VC17	14	0	0	140
	19 (1)	0	0	190		TNC	TNC	280 (5)	280000
VC08	0	0	0	<10	VC18	13	2	0	130
	TNC	TNC	93 (2)	93000		TNC	TNC	97 (1)	97000
VC09	2	1	0	20	VC19	25	2	0	250
	TNC	TNC	>1000	>1000000		TNC	TNC	>1000	>1000000
VC10	25	1	0	250	VC19D	40	2	0	400
	TNC	290 (5)	48	30000		TNC	TNC	>1000	>1000000
VC11	39	0	0	390	VC20	0	0	0	<10
	TNC	TNC	178 (1)	180000		113 (1)	11	2	1100
					VC21	0	0	0	<10
						57 (7)	4	0	570

Top lines, B. cereus CFU/g; second line, total Bacillus CPU/g. TNC is too numerous to count. Number in parentheses represents B. cereus var. mycoides CFU/g.

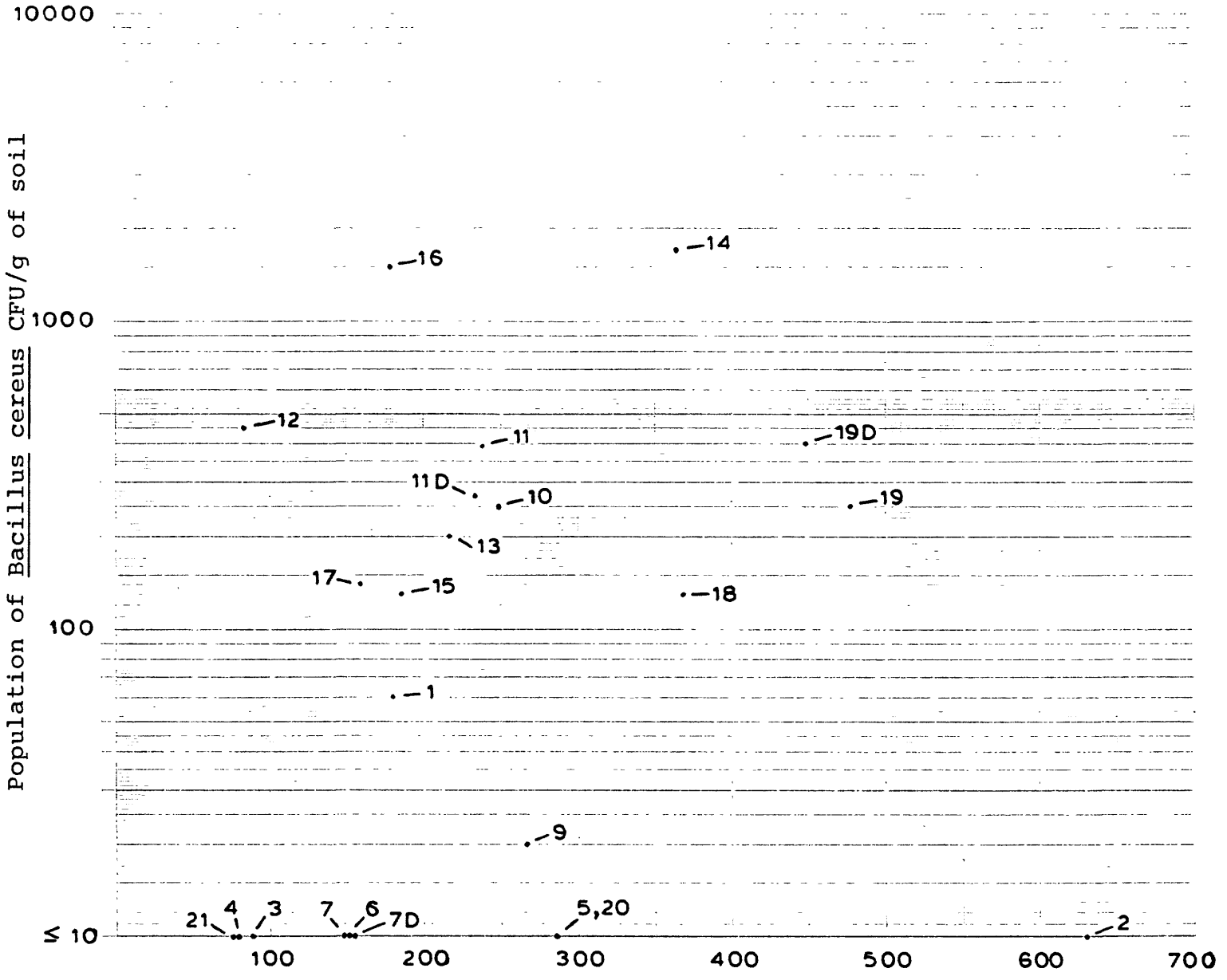

Figure 7. Distribution plot of Bacillus cereus populations versus the sum of water soluble Ca, Mg, Na, and K in study area 3, Virginia City, Nevada. (Site 8 has 1400 ppm total cations and less than 10 CFU/g B. cereus but is not shown.)

Table 12.--Concentrations of water-soluble cations and *Bacillus cereus* populations from 21 soil samples from study area 3, Virginia City, Nevada

[ppm, parts per million; ppb, parts per billion; CFU/g, colony-forming units per gram]

Sample	Ca ppm	Mg ppm	Na ppm	K ppm	Zn ppm	Ag ppb	*B. cereus* CFU/g
VC01	40	33	75	31	<0.2	<0.5	60
02	400	90	14	125	<.2	<.5	<10
03	12	22	10	43	<.2	<.5	<10
04	6	16	12	43	<.2	<.5	<10
05	50	17	48	170	3.3	<.5	<10
06	12	30	25	84	.4	<.5	<10
07	20	30	31	68	<.2	<.5	<10
07D	20	29	35	70	.2	<.5	<10
08	795	150	73	360	1.0	.5	<10
09	135	40	20	72	.3	<.5	20
10	145	58	21	24	<.2	<.5	250
11	135	47	16	40	<.2	<.5	390
11D	135	42	15	41	<.2	<.5	270
12	25	18	13	27	<.2	<.5	450
13	62	26	24	105	<.2	<.5	200
14	200	52	27	85	.4	<.5	1,700
15	6	28	115	36	<.2	.5	130
16	29	34	73	42	<.2	<.5	1,500
17	92	26	12	29	<.2	<.5	140
18	15	27	310	16	<.2	.9	130
19	23	24	400	30	.2	1.2	250
19D	24	22	380	22	.2	1.0	400
20	35	46	35	170	.4	.6	<10
21	5	23	14	33	<.2	<.5	<10

Copper was not detected in any sample at a detection limit of 0.5 ppm.

Samples collected under vegetation such as Mormon tea, sage, or juniper may have higher populations of *B. cereus* than adjacent samples collected in the open. (2) The population densities of *B. cereus* are not greatly influenced by the soluble metals examined in this study. (3) The natural variation of *B. cereus* population densities often fluctuates by an order of magnitude, even from closely spaced samples, in soils that are seemingly within the same geochemical and botanical environment.

D. Study Area 4

Study area 4 is located at Aña Nuevo State Reserve, some 35 km north of Santa Cruz, California, approximately 2.5 km from the Reserve parking lot. The study area affords the first access to the beach from the established trail. The area was selected for study because it contains a metallized fault zone bordered by sedimentary rocks that are unmetallized. The metallized zone is exposed for about 50 m along the cliff. The zone contains iron sulfide minerals (predominantly pyrrhotite) occurring as crisscrossing veinlets up to some 3 cm in width. The surrounding rocks are thick sequences of limy siltstones and mudstones that dip to the west. The metallized zone vertically cuts the sedimentary rocks and is unconformably overlain by mudstone. There are stabilized and moving dunes overlying the horizontal mudstone. Only ten samples were collected in this study area due to Reserve regulations (fig. 8).

Sites 1, 3, and 4 are located in the gossan, above the unweathered rock containing sulfides. There are only a few widely spaced plants near these sites. The sample material is composed primarily of loose and weathered gossan and is not a true soil. Site 2 is located in the dense gray clay that overlies the gossan. There appeared to be no alteration within the clay and its horizontal aspect indicates that the clay layer was deposited after the tilting and alteration of the sedimentary rocks. Sample 5 was collected from stabilized dune sands that had ice plant cover. Sample 6 was collected under willows that are growing on stabilized dunes. Samples 7 and 8 were collected along a small runoff depression cut into the clay. Some grasses are present at site 8; site 7 was barren. Sample 9 was collected from clay on top of the cliffs to the east of the metallized zone. Some tuffs of grass and a large pampus grass clump are present at the site. Sample 10 was collected in a wind-swept area of gray-black clay that may be occasionally reached by high waves.

B. cereus populations range from less than 10 to 5,100 CFU/g (table 13). The greatest populations of *B. cereus* occur in samples 5 and 6, collected from stabilized dune sands. Samples from the gossan and clay soils have *B. cereus* populations of 10 CFU/g or fewer (table 13).

The metallized zone has slightly higher concentrations of Fe, Be, Co, Cu, Ni, and Zn compared to the surrounding rocks (table 14). It is probable that unweathered samples of the metallized zone would have much higher metal concentrations. The elevated concentrations of Fe, Ti, and Cr in samples 5 and 6 are probably related to the

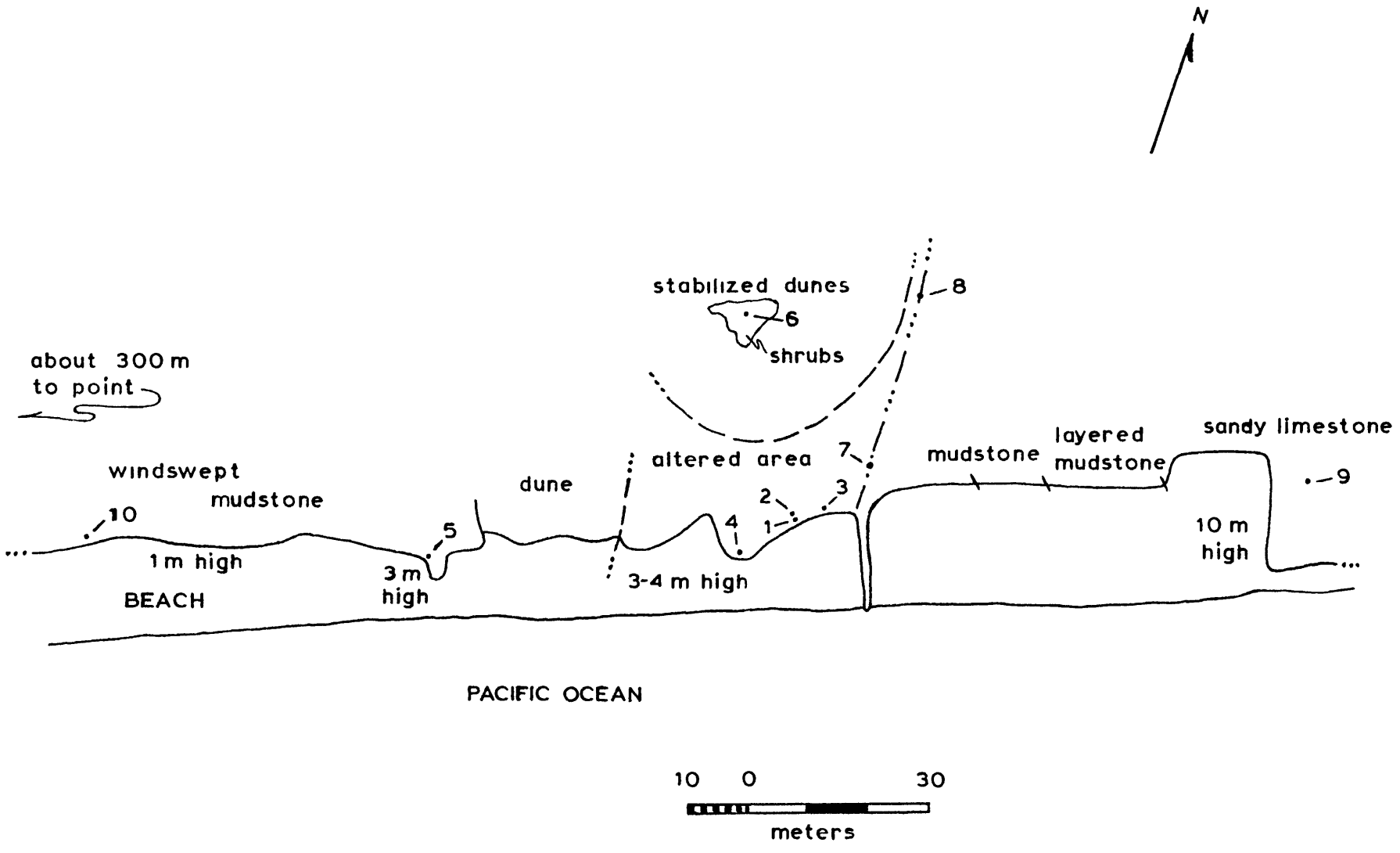

Figure 8. Sample location map for study area 4, Aña Nuevo State Reserve, California.

Table 13. Results from culture tests for *Bacillus cereus* and total *Bacillus* at Aña Nuevo State Reserve, California

Sample	Dilution 1:10	1:100	1:1000	Total
AN01	1	0	0	<10
	3	0	0	30
02	1	0	0	10
	52	6	0	520
03	0	0	0	<10
	220	2	1	2,200
03D	0	0	0	<10
	88 (15)	2	0	880
04	1	0	0	10
	2	0	0	20
05	264	22	4	2,600
	TNC	TNC	210 (10)	210,000
06	TNC	51	3	5,100
	TNC	TNC	300 (11)	300,000
07	0	0	0	<10
	108 (6)	3	0	1,100
08	1	0	0	10
	TNC	120	12	12,000
09	0	0	0	<10
	TNC	160	9	16,000
10	0	0	0	<10
	44 (3)	0	0	440

Top lines, *B. cereus* CFU/g; second line, total *Bacillus* CFU/g. TNC is too numerous to count. Number in parentheses represents *B. cereus* var *mycoides* CFU/g.

Table 14. 6-step d.c.-arc semiquantitative spectrographic analysis for samples from study area 4, Ana Nuevo State Reserve, California

	Fe (.05)	Mg (.02)	Ca (.05)	Ti (.002)	Mn (10)	B (10)	Ba (20)	Be (1)	Co (5)	Cr (10)	Cu (5)	La (20)	Ni (5)	Pb (10)	Sr (100)	V (10)	Y (10)	Zn (200)	Zr (10)
AN01	5	.7	1.5	.5	300	15	150	L	20	300	30	N	100	L	300	150	20	N	70
02	3	.5	.7	.3	500	50	300	1	10	100	10	L	30	15	150	100	10	N	100
03	10	.7	1.5	.3	700	20	1000	1	50	150	50	L	150	L	300	100	20	L	50
04	5	.7	.5	.2	700	50	300	5	50	100	30	30	200	15	150	100	50	L	70
05	3	1	2	.5	700	50	200	L	10	1000	L	20	30	10	200	150	30	N	150
06	5	1.5	2	1	1000	70	200	L	20	3000	10	200	70	20	300	150	30	N	200
07	2	.5	1	.15	200	30	300	L	20	150	15	L	70	10	200	70	15	N	150
08	1.5	.3	.7	.3	1000	30	500	1.5	10	150	7	20	30	20	200	70	15	N	150
09	1.5	.5	1	.3	500	30	300	L	7	300	L	20	15	15	150	70	15	N	200
10	3	.5	1	.3	300	30	300	1.5	10	150	10	20	30	15	200	150	20	N	100

Fe, Mg, Ca, and Ti are reported in percent, all other elements are reported in ppm.
The elements Ag, As, Au, Bi, Cd, Mo, Nb, Sb, Sn, and W were not detected.
(The number in parentheses is detection limit; L is concentration lower than detection limit; N is concentration not detected).

presence of heavy minerals such as magnetite or illmenite. These minerals are found in the black sand streaks that are abundant within the dunes. The small concentration range for most of the elements are geochemically and analytically insignificant. There appears to be no correlation between heavy-metal concentrations and *B. cereus* populations.

The water extraction was performed to determine what effects the proximity of a highly saline body of water may have on the concentration of major cations within the soil (table 15). The results indicate that sites in clay or sand have Ca, Mg, and K concentrations similar to the soils from the other study areas. The concentration of Na in some samples is elevated compared to the soils from the other study areas. The soils from the gossan have a significant increase in the concentrations of all four major cations compared to the surrounding soils. The anomalous concentrations of Ca, Mg, Na, and K in the gossan zone are probably a function of the chemical and physical characteristics of the zone. The gossan zone is more permeable than the surrounding clay layers. Sea water spray or aerosols would tend to soak into the gossan but sit on the surface of the clay. Any of the salts deposited on the clay surface would be easily removed by the wind.

The dune areas are composed of rounded mineral grains, predominantly quartz and some heavy minerals. These minerals are relatively resistant to chemical attack. Any salts that collected in the dunes would be easily washed out during rains due to high permeability of the sands.

Previous studies of the gossan zone (Tucker, unpub. data) identified *Thiobacillus* and *Desulfovibrio* in the soils. The presence of *Thiobacillus* indicates the oxidation of sulfide minerals releasing sulfuric acid to the soil. The soil pH in the gossan zone was near 2. The low pH may explain the low populations of *B. cereus* and *Bacillus spp.* in the gossan samples. The effects of low pH on *Bacillus spp.* in this study area coincides with the results observed in the ponderosa zone in study area 3. It is apparent that many unresolved complex geochemical and biogenic processes are contributing significantly to the observed complexity in the data.

The range of soluble cations is very large and the range of *B. cereus* populations is over four orders of magnitude. However, there is no apparent correlation between *B. cereus* populations and the sum of the major soluble cations (fig. 9). The ratio of *Bacillus spp.* to *B. cereus* versus the major soluble cations also shows no correlations (no plot).

Interpretations of the *B. cereus* population data is tentative due to the complexity observed in the geochemical characteristics of the various sample groups, i.e., gossan, sand dunes, and clay sediments. Samples from the gossan zone and the clay soils have population densities of *B. cereus* of 10 CFU/g or fewer. The population densities of *Bacillus spp.* are greater in the clay soils than the gossan, suggesting the clay environment is more hospitable than the gossan zone. The high-metal content of the

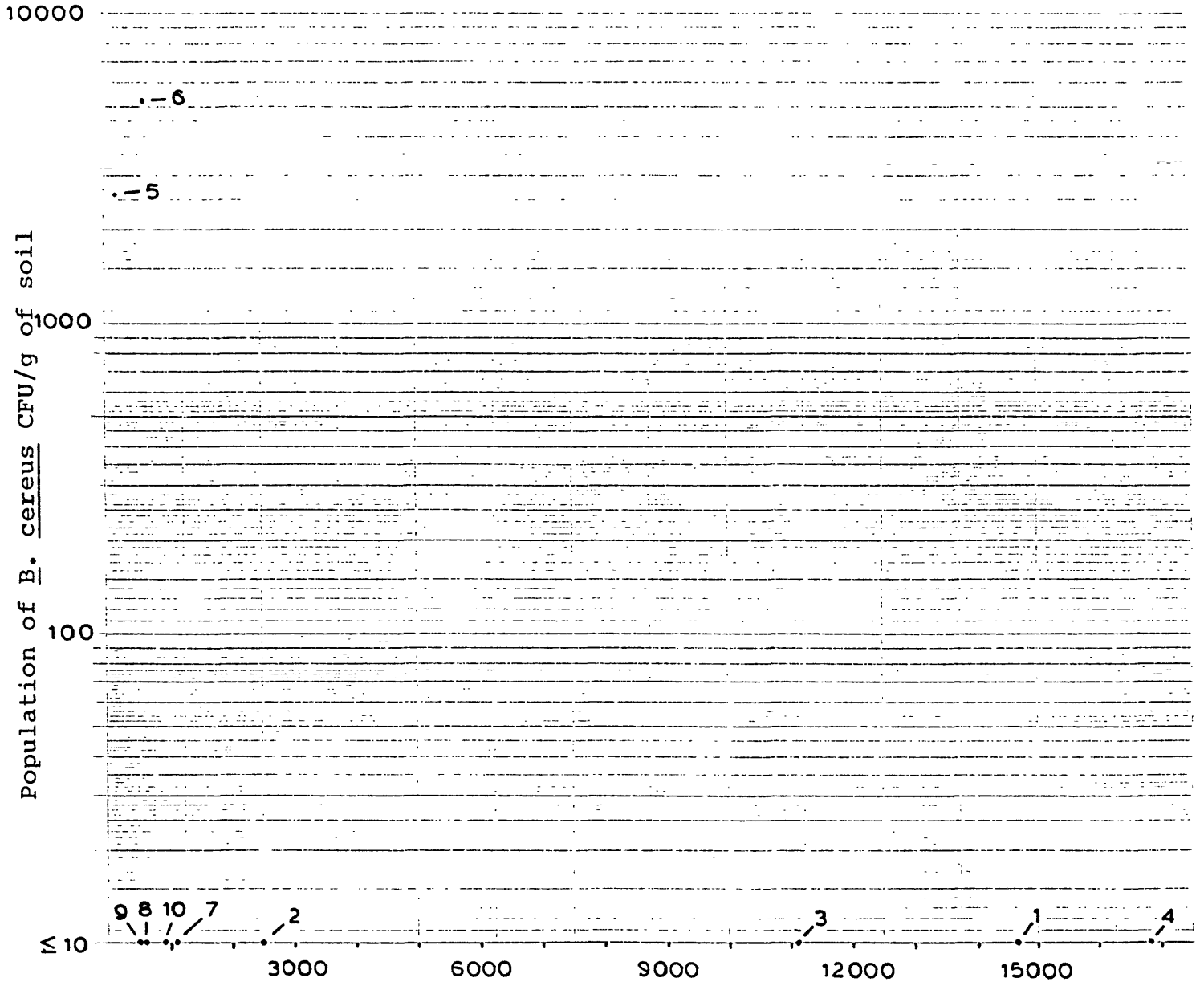

Figure 9. Distribution plot of *Bacillus cereus* versus the sum of water soluble Ca, Mg, Na, and K in study area 4, Aña Nuevo State Reserve, California.

Table 15.--Concentration of water-soluble cations and *Bacillus cereus* poulations from 10 soil samples collected in study area 4, Aña Nuevo State Reserve, California

[ppm, parts per million; ppb, parts per billion; CFU/g, colony-forming units per gram]

Sample	Ca ppm	Mg ppm	Na ppm	K ppm	Zn ppm	Cu ppm	Ag ppb	*B. cereus* CFU/g
AN01	3,000	1,500	10,000	180	<0.2	<0.5	1.0*	<10
02	210	62	2,200	17	<.2	<.5	1.0	10
03	320	590	10,000	220	<.2	<.5	1.3*	<10
04	1,100	630	15,000	73	<.2	<.5	1.4*	10
05	49	23	130	17	<.2	<.5	<.5	2,600
06	430	57	52	80	<.2	<.5	<.5	5,100
07	130	45	840	93	4.0	.8	2.2	<10
08	210	65	370	16	<.2	<.5	<.5	10
09	180	74	200	52	<.2	<.5	.8	<10
10	240	47	670	12	<.2	<.5	<.5	<10

* Sodium concentrations greater than 10,000 ppm may cause a silver interference equal to 1.3 ppb.

gossan zone should be a favorable environment for *B. cereus*. The very high concentrations of soluble ions, lack of organic substrate, low pH, or other factors appear to create a very hostile environment that precludes *B. cereus* survival.

Low populations of *B. cereus* were found in the clay soils. Chemical analysis of the clay soils does not suggest they contain a toxic chemical environment, and qualitatively, the soils appear to have high organic content. It appears that some set of physical and chemical characteristics, such as oxygen content, permeability, amount of water, organic compounds, chemical species, etc., adversely affect *B. cereus*.

The samples with the highest *B. cereus* and *Bacillus spp.* populations are from stabilized dunes. The dune sample is composed of highly sorted, refractory mineral grains. There is some organic detritus present under the willows. These soils are highly aerated and somewhat damp. The environmental conditions would enhance the growth of many fungi, aerobic bacteria, and *B. cereus*.

The number of samples in this study area is very small, which precludes definite conclusions. However, the *B. cereus* assay results seem to be ineffective at delineating the metallized zone. More study is required to better understand the interactions between soil characteristics, such as pH and organic matter content, and *B. cereus* population dynamics.

Conclusions

Four study areas were selected to examine the natural population density of *B. cereus*. Geochemical characteristics of the soil samples were examined with respect to possible effects metal concentrations would have on *B. cereus* populations. The concentrations of 31 elements were determined using 6-step d.c.-arc semiquantitative emission spectrographic analysis. The concentrations of water extractable Ca, Mg, Na, K, Zn, Cu, and Ag were determined by atomic absorption analyses.

In the four study areas examined, the data results indicate there is no observable correlation between *B. cereus* populations and the concentration of total soil metals examined in this study. There is no apparent correlation between the concentration of soluble metal ions and the populations of *B. cereus*. There may be some chemical parameter of the soil not measured that significantly affects the distribution of *B. cereus* populations and would explain the population fluctuations. There is a strong qualitative correlation between the degree of soil development and *B. cereus* populations in all study areas. Low soil pH, from organic sources such as pine forests and inorganic sources such as weathering sulfides, appears to have an adverse effect on *B. cereus* populations.

The population variations within a sample may fluctuate by a factor of two. Samples from what appears to be a single environmental setting exhibit *B. cereus*

population variations in the range of an order of magnitude. This indicates that subtle population differences will be very difficult to detect. *B. cereus* population ranges of about four orders of magnitude between distinct environments, such as forest and meadow, were deserved. These population differences could not be attributed to total metals or water-extractable ion concentrations in the soil. The very high, naturally occurring, population variations suggest the problematical nature of using *B. cereus* to detect zones of mineralization. These results indicate that the relationships between many geochemical and physical soil characteristics and the population dynamics of *B. cereus* are only poorly understood.

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