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Environmental Factors Affecting the Occurrence of Mycobacteria in Brook Waters

E. K. IIVANAINEN,[1]* P. J. MARTIKAINEN,[1] P. K. VÄÄNÄNEN,[2] AND M.-L. KATILA[3]

Department of Environmental Microbiology, National Public Health Institute, P.O. Box 95,[1] and Geochemical Department, Geological Survey of Finland,[2] 70701 Kuopio, and Department of Clinical Microbiology, Kuopio University Hospital, 70211 Kuopio,[3] Finland

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To evaluate the impact of environmental factors on the occurrence of environmental mycobacteria, viable counts of mycobacteria were measured in samples of brook water collected from 53 drainage areas located in a linear belt crossing Finland at 63° north latitude. The numbers of mycobacteria were correlated with characteristics of the drainage area, climatic parameters, chemical and physical characteristics of the water, and counts of other heterotrophic bacteria in the water. The numbers of mycobacteria in the water ranged from 10 to 2,200 CFU/liter. The counts correlated positively ($P < 0.001$) with the presence of peatlands, precipitation data, chemical oxygen demand, water color, and concentrations of Fe, Al, Cu, Co, and Cr. The mycobacterial counts correlated negatively ($P < 0.001$) with water pH, whereas other heterotrophic bacterial counts lacked any correlation with pH. A linear regression model with four independent variables (i.e., peatlands in the drainage area, chemical oxygen demand, concentration of potassium, and pH) explained 83% of the variation in mycobacterial counts in brook waters. Our results suggest that acidification may enhance the growth of environmental mycobacteria.

Environmental mycobacteria, which are also called atypical mycobacteria or mycobacteria other than *Mycobacterium tuberculosis*, are acid-fast bacteria that are related to tuberculous bacilli (*M. tuberculosis*). They are common saprophytes in all natural ecosystems (14). However, some species are also pathogenic to humans or animals, causing a disease similar to tuberculosis (5, 16, 32, 42). Infections caused by mycobacteria other than *M. tuberculosis* are considered to be environmentally derived.

The numbers of isolations of environmental mycobacteria from clinical samples are increasing in industrialized countries (6, 17, 41), and new potentially pathogenic species have been detected recently (40). The ecology of mycobacteria is poorly understood, and the reasons for their increased numbers in clinical samples and the appearance of new pathogenic species are unknown. Low pH values of culture media are known to enhance the growth (19, 34) and detection (18) of environmental mycobacteria. In the environment, mycobacteria have also been found more frequently under acidic conditions (4, 21, 26). More information about the effects of pH and other environmental factors on the occurrence of mycobacteria is needed to determine whether acidification of the environment could explain the observed increases in numbers of nontuberculous mycobacterial infections. In this study, the relationship between occurrence of environmental mycobacteria and several environmental factors was examined in Finnish brook waters.

MATERIALS AND METHODS

Study site. The study site was a 550-km-long linear belt crossing Finland at 63° north latitude (Fig. 1); in this study site we examined 53 drainage areas that are situated in rural regions (2 to 20 inhabitants per km^2) (31) where the main source of livelihood is agriculture. Each drainage area examined covered a region that is 10 to 50 km^2. The brooks which we studied flow through woodlands (20 to 90% of the soil area) (37) or peatlands (3 to 80% of the drainage area) (30). Of the surrounding land, a maximum of 25% is cultivated. The most common soil type is moraine. In the westernmost coastal area, there are also clay and silt soils containing sulfides and acid sulfates. The bedrock of the belt contains mainly granitoids and mica schists, as well as some local mafic volcanic and plutonic rocks.

Sampling. Water samples were collected at the outlet of each drainage area, where the brook was 2 to 5 m wide. A total of 53 water samples (500 ml for microbiological analyses and 600 ml for geochemical analyses) were collected directly into polyethylene flasks from the surfaces of the brooks. The samples were collected in June and July 1990 and were stored at 4°C until they were processed (mean, 36 days; standard deviation, 6.8 days).

Climatic data. The local precipitation data were the cumulative precipitation data for the 3 days and 2 and 3 weeks preceding each sampling. The mean air temperature during the 7 days before each sampling was also recorded. These data were obtained from the Finnish Meteorological Institute.

Physical and chemical analyses. Water temperature, pH, and electrical conductivity were measured at the time of sampling with model WTW pH91 and LF91 meters (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Color was determined by comparison with the platinum standard (Hellige GmbH, Freiburg, Germany). Chemical oxygen demand (COD) was measured titrimetrically by using $KMnO_4$ and the Finnish standard (11), and HCO_3^- alkalinity, which reflects water buffer capacity, was also determined titrimetrically by using the Finnish standard (10). SO_4^{2-}, NO_3^-, and Cl^- contents were analyzed by using a model 2000i ion chromatography apparatus (Dionex Corp., Sunnydale, Calif.) (1) equipped with a type 37042 Ion Pac AS4A precolumn and a type 37041 Ion Pac AS4A analytical column. The eluent contained 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$, and the flow rate was 1.5 ml/min.

* Corresponding author.

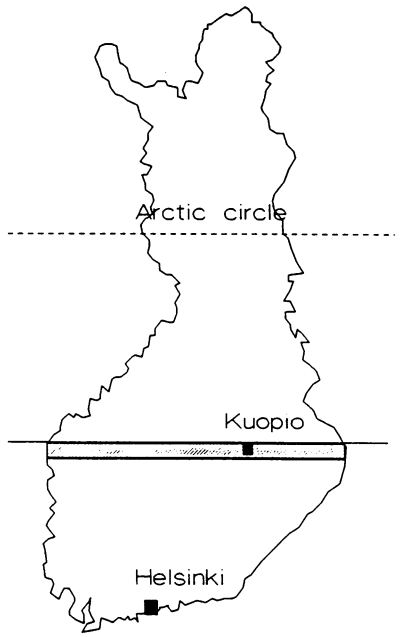

FIG. 1. Location of the study belt consisting of 53 drainage areas in Finland.

Fluoride was measured with an ion selective electrode (type 555A; Findip, Espoo, Finland), and SiO_2 was determined spectrophotometrically (Shimadzu, Tokyo, Japan) by using a molybdate method (1). For cation analyses, 100 ml of each water sample was filtered through a 0.45-μm-pore-size filter and acidified with 0.5 ml of highly purified HNO_3 (Suprapur; Merck, Darmstadt, Germany) immediately after sampling. Ca, Mg, Na, K, Fe, Mn, and Zn were analyzed by using an atom absorption spectrometry flame method (model 5000 apparatus; Perkin-Elmer Corp., Norwalk, Conn.). Al, Cu, Ni, Co, Cr, Pb, Mo, and Cd were analyzed by graphite oven atom absorption spectrometry (model SpectrAA 400P apparatus; Varian Techtron, Mulgrave, Victoria, Australia).

Cultivation for heterotrophic bacteria. The numbers of heterotrophic bacteria were determined by using R2A medium (Difco Laboratories, Detroit, Mich.) (35). The plates were incubated in the dark at room temperature for 4 weeks.

Cultivation for mycobacteria. Mycobacteria were concentrated by filtering 125 ml of a water sample through a nylon membrane filter (Pall N_{66} Posidyne Nylon 66 membrane filter; pore size, 0.2 μm; diameter, 47 mm; Pall Trinity Micro Corp., Cortland, N.Y.). The membrane filter was cut into small pieces, and the pieces were placed into a sterile centrifuge tube containing 5 ml of sample water and glass beads. The microbes were eluted from the membrane filter by shaking the tube in a rotatary mixer (Vortex Genie 2 mixer; Scientific Industries, Inc., Bohemia, N.Y.) for 5 min. We prepared two concentrates of each sample, one for each of the two decontamination methods used (methods A and B).

Method A was a modification of the decontamination technique described by Beerwerth (2). A sample concentrate (5 ml) was first treated with 10 ml of 1 M NaOH for 20 min. After centrifugation at 8,600 × *g* and 4°C for 15 min in a Sorvall model RC-5B centrifuge (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.), the resulting sediment was treated with 5 ml of 5% (wt/vol) oxalic acid for 20 min. The centrifuged sediment was neutralized with 30 ml of sterile distilled water, centrifuged again, and resuspended in 0.7 ml of sterile distilled water. In method B, instead of being treated with NaOH and oxalic acid, 5 ml of a sample concentrate was treated with 5 ml of 2 M H_2SO_4 and 0.7 ml of cycloheximide (8 mg/ml) for 30 min. Otherwise, the steps of this procedure were the same as the steps in method A.

The following four types of egg media were used: (i) egg medium supplemented with glycerol (pH 6.5); (ii) egg medium supplemented with glycerol and adjusted to pH 5.5; (iii) egg medium supplemented with pyruvate (pH 6.5); and (iv) egg medium supplemented with pyruvate and adjusted to pH 5.5 (18). All media contained 0.5 mg of cycloheximide per ml. Two parallel slopes of each medium in sealed 30-ml universal containers (Sterilin GSUC/Z/C containers) were inoculated with 50-μl portions of the resuspended sediment, and the resulting preparations were incubated for 6 months at 30°C. Additional tubes of the media at pH 5.5 were also incubated at 42°C.

At each reading (readings were performed at 4- to 5-week intervals), newly detected colonies were checked for acid fastness by Ziehl-Neelsen staining. Acid-fast colonies were subcultured for identification studies to be performed later. The concentration of mycobacteria in a sample was calculated as the average number of acid-fast colonies obtained with the two decontamination methods.

Statistical analyses. The results were analyzed by Spearman rank correlation analyses and stepwise linear multiple-regression analyses (SPSS/PC+ for the PC/XT/AT; SPSS Inc., Chicago, Ill.). Before the multiple-regression analyses, the variables were transformed logarithmically, if necessary, to obtain normal distributions.

RESULTS

Environmental and climatic data and physical and chemical characteristics of water samples. As shown in Table 1, the mean water and air temperatures during sampling were 15 and 14°C, respectively. Before sampling, the precipitation varied greatly. There was also great variation in the chemical characteristics of the water samples from different brooks. The pH varied from almost neutral to acidic (pH 4.4). Variations of 10- to 30-fold were detected in alkalinity values, electrical conductivity values, color values, and COD values in different brooks. The concentrations of anions (NO_3^-, Cl^-, SO_4^{2-}) and cations (Fe, Mn, Na, K, Mg, Ca) also varied greatly; the same was true for concentrations of trace metals and Al. The concentrations of Zn, Cd, Pb, and Mo exceeded the limits of detection in only a few samples, and these variables were omitted from the statistical analyses. Both the presence of peatlands and precipitation affected the chemical characteristics of the water (Table 2).

Bacteriological characteristics of the brook waters. Mycobacteria were isolated from all 53 samples. Among the strains isolated there were both rapidly and slowly growing species. In cultures grown at 30°C, the concentrations of mycobacteria varied from 10 to 2,200 CFU/liter (mean, 618 CFU/liter; standard deviation, 603 CFU/liter). More than 10^3 CFU/liter was detected in 12 (23%) of the samples (Fig. 2). In five (9%) of the samples mycobacteria were also isolated by incubating the preparations at 42°C. The counts obtained from incubations at 30°C were used for statistical analyses. There was distinct geographical variation in the occurrence of mycobacteria (Fig. 3C). Large numbers were found in a limited area at the eastern end of the belt, as well as in a

TABLE 1. Environmental and climatic data and chemical and physical characteristics of the 53 water samples

Variable	Minimum	Maximum	Mean	SD
Peatlands (%)	3	80	39	21
Water temp (°C)	11	19	15	2
Air temp (°C)	9	18	14	2
Precipitation (3 days) (mm)[a]	0	26	12	7.1
Precipitation (2 weeks) (mm)[a]	3.4	60	29	14
Precipitation (3 weeks) (mm)[a]	16	75	43	16
pH	4.4	6.7	5.7	0.6
Alkalinity (mmol of HCO_3^- per liter)	0.1	1.8	0.3	0.3
Electrical conductivity (mS/m)	2.3	30	6.5	5.8
Color (mg of Pt per liter)	20	600	210	170
COD (mg of $KMnO_4$ per liter)	16	180	75	38
Concn of:				
NO_3^- (mg/liter)	0	11	1.1	2.0
Cl^- (mg/liter)	0	32	4.0	5.4
F^- (mg/liter)	0	0.4	0.1	0.1
SO_4^{2-} (mg/liter)	1.4	52	7.9	9.2
SiO_2 (mg/liter)	1.2	15	6.1	3.3
Fe (mg/liter)	0.1	3.7	1.3	1.0
Mn (mg/liter)	0.01	0.21	0.06	0.04
Zn (mg/liter)	0	0.03	0.01	0.01
Na (mg/liter)	1.1	30	4.4	6.1
K (mg/liter)	0.4	11	1.5	1.7
Mg (mg/liter)	0.5	8.6	1.9	1.6
Ca (mg/liter)	1.8	18	4.5	3.0
Al (μg/liter)	21	960	270	220
Cd (μg/liter)	0	0.3	0.1	0.1
Pb (μg/liter)	0	5.9	0.2	0.8
Cu (μg/liter)	0.6	11	2.5	2.1
Co (μg/liter)	0	3.8	0.8	0.8
Ni (μg/liter)	0	24	3.0	5.2
Cr (μg/liter)	0	2.3	0.8	0.5
Mo (μg/liter)	0	1.3	0.2	0.2

[a] Cumulative precipitation for the 3 days and 2 and 3 weeks before each sampling.

larger area in the westernmost areas. The lowest mycobacterial counts were obtained in the central parts of the belt. The numbers of other heterotrophic bacteria varied from 2.3 × 10^6 to 7.0 × 10^8 CFU/liter (mean, 1.5 × 10^8 CFU/liter; standard deviation, 1.3 × 10^8 CFU/liter) (Fig. 3D).

Correlations between bacteria and environmental, climatic, and water characteristics. The occurrence of mycobacteria correlated positively ($P < 0.001$) with the presence of peatlands (Fig. 3 and Table 3), 3-week precipitation data, water color, COD, and the presence of some metals (Fe, Al, Cu, Co, and Cr) (Table 3) and negatively ($P < 0.001$) with water pH (Fig. 3 and Table 3). A linear multiple-regression model in which the presence of peatlands, COD, the concentration K, and pH were used as independent variables explained 83% of the variation in the numbers of mycobacteria in the brook waters tested (Table 4).

There was a positive correlation between the numbers of mycobacteria and the numbers of other heterotrophic bacteria (Table 3). Both of these values also correlated similarly with values of several chemical variables, including water color, COD, and concentrations of metals (Table 3). However, there was a distinct difference in the correlation of these values with water pH. In contrast to mycobacteria, which exhibited a negative, highly significant correlation with water pH, the counts of the other heterotrophic bacteria did not correlate with pH. The mycobacteria and the other heterotrophic bacteria also differed in their correlations with precipitation data (Table 3).

DISCUSSION

The frequency of isolation of mycobacteria (100% of the samples) in our study was higher than the frequency of isolation from water environments reported previously (9, 13, 15). The laboratory techniques used may partially explain our high yield. Our membrane filtration technique enabled us to enumerate mycobacteria even in samples containing low counts of these bacteria. On the other hand, the two procedures used for decontamination and acidified medium modifications may have allowed growth of more mycobacterial species, some of which may be sensitive to alkaline treatment or may grow only in media supplemented with pyruvate or glycerol (18, 19).

Previous observations have shown that the occurrence of mycobacteria in the environment varies geographically (4, 9). Our results suggest that variation in surface waters is due to the association of mycobacteria with hydrogeochemical characteristics of the drainage areas.

Peatlands may enhance the occurrence of mycobacteria in brook waters in several ways. First, peatlands can be a reservoir from which runoff disperses mycobacteria to the environment (23). Kazda (22) and Kazda et al. (24) isolated mycobacteria from sphagnum vegetation and showed that this vegetation provides the basic nutrients and the temperature needed for mycobacteria. Second, acidic runoff from peatlands may enhance the survival and perhaps the multiplication of mycobacteria in brook waters. Correlations of the occurrence of peatlands with COD and pH values in our study showed that the runoff from peatlands both increased the content of organic matter and decreased the pH values in the waters. Our results also indicate that rainy periods further increased the color, COD, acidity, and counts of mycobacteria in the waters. Our results are consistent with those of Kirschner et al. (26), who recently found a positive correlation between the numbers of members of the *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* complex and the content of humic and fulvic acids, two important constituents of organic matter. The negative correlation between the presence of mycobacteria and water pH values supports previous results obtained with natural waters (21, 26). Previous studies have also indicated that mycobacteria benefit from acidity in growth media (18, 34).

About 60% of the peatlands in Finland (6.3 × 10^6 of the 10.4 × 10^6 ha) have been ditched and drained, mainly for forestry (27). Ditching changes the runoff conditions and may influence the quality of brook waters. Judging by the numbers of mycobacterial isolations from respiratory specimens, colonization of the respiratory tract by mycobacteria has increased at least fivefold in Finland from 1975 to 1990 (20) (i.e., during a period of intensive draining of peatlands) (33). The possibility that such widespread environmental manipulation has influenced the increase in clinical isolations of mycobacteria to some extent cannot be excluded. In our material, the highest mycobacterial count (2,200 CFU/liter) was obtained in a brown-colored brook flowing from a peat-harvesting area where ditches were being dug at the time of sampling.

High organic matter contents in waters also favored the overall occurrence of heterotrophic bacterial flora, which explains the positive correlation between the presence of mycobacteria and the presence of other heterotrophic bac-

TABLE 2. Correlation coefficients of environmental and climatic data and chemical characteristics of waters with the presence of peatlands, precipitation data, pH values, color, and COD values

Variable	Peatlands		Precipitation (3 weeks)[a]		pH		Color		COD	
	r[b]	P	r	P	r	P	r	P	r	P
Peatlands					−0.53	<0.001	0.50	<0.001	0.53	<0.001
Water temp			−0.37	<0.05						
Air temp			0.20							
Precipitation (3 weeks)					−0.47	<0.001	0.46	<0.001	0.32	<0.05
pH	−0.53	<0.001	−0.47	<0.001			−0.37	<0.01	−0.32	<0.05
Alkalinity	−0.34	<0.01	−0.15		0.61	<0.001	−0.03		−0.27	<0.05
Electrical conductivity	−0.18		0.11		0.44	<0.01	0.41	<0.01	0.24	<0.05
Color	0.50	<0.001	0.46	<0.001	−0.37	<0.01			0.82	<0.001
COD	0.53	<0.001	0.32	<0.05	−0.32	<0.01	0.82	<0.001		
Concn of:										
NO_3^-	−0.04		0.38	<0.01	−0.06		0.49	<0.001	0.28	<0.05
Cl^-	−0.19		0.25	<0.05	0.27	<0.05	0.48	<0.001	0.28	<0.05
F^-	0.09		0.31	<0.05	0.06		0.67	<0.001	0.34	<0.01
SO_4^-	−0.33	<0.01	0.07		0.40	<0.01	−0.02		0.09	
SiO_2	0.24	<0.05	0.14		0.10		0.50	<0.001	0.39	<0.01
Fe	0.45	<0.001	0.42	<0.01	−0.21		0.94	<0.001	0.69	<0.001
Mn	0.11		−0.06		0.0002		0.49	<0.001	0.31	<0.05
Na	−0.005		0.32	<0.05	0.18		0.63	<0.001	0.39	<0.01
K	−0.29	<0.05	0.06		0.47	<0.001	0.27	<0.05	0.07	
Mg	−0.11		0.08		0.43	<0.01	0.48	<0.001	0.33	<0.01
Ca	−0.10		0.10		0.42	<0.01	0.40	<0.01	0.23	<0.05
Al	0.40	<0.01	0.51	<0.001	−0.41	<0.001	0.79	<0.001	0.76	<0.001
Cu	0.19		0.28	<0.05	−0.22		0.62	<0.001	0.48	<0.001
Co	0.32	<0.01	0.34	<0.01	−0.19		0.65	<0.001	0.55	<0.001
Ni	0.07		0.15		0.12		0.43	<0.01	0.40	<0.01
Cr	0.38	<0.01	0.43	<0.01	−0.28	<0.05	0.71	<0.001	0.63	<0.001

[a] Cumulative precipitation for the 3 weeks before each sampling.
[b] r, Spearman rank correlation coefficient.

teria. This positive correlation is in contrast to the results of a moorland water study (21). In our study, the presence of other heterotrophic bacteria did not correlate negatively with water pH values, as the presence of mycobacteria did, indicating a basic difference between mycobacteria and other heterotrophic bacteria.

Our results revealed several correlations between mycobacterial counts and ion concentrations, in contrast to the results of some previous surface water studies (26) and groundwater studies (28). However, the complex effects of pH and organic matter on bacterial counts and ion contents make evaluation of the effects of individual variables problematic. Low pH values are known to elevate concentrations of metals in waters (3) by mobilizing the metals from the soil (36). Also, in our material Al and Cr concentrations correlated negatively with pH values. Thus, the positive correlations between the concentrations of these metals and mycobacterial numbers may merely reflect the favorable effects of low pH values on mycobacteria. Several of the cation concentrations that correlated positively with the counts of mycobacteria and total heterotrophic bacteria also correlated positively with COD and color, indicating that there was binding to humus. The cations may have been carried from the soil to the brook waters as humus-bound ions. Therefore, the positive correlation between concentrations of cations and bacterial numbers may reflect the positive effect of organic matter on bacteria, rather than the effect of cations on microbes. This applies also to heavy metals. In previous studies workers have shown that some mycobacterial strains are tolerant of Cu, Cd, and Hg (7, 8, 29). We have no evidence that mycobacteria in brook waters are more tolerant of heavy metals than the other heterotrophic bacteria; the total counts of mycobacteria and the total counts of heterotrophic bacteria correlated similarly with the heavy metal concentrations.

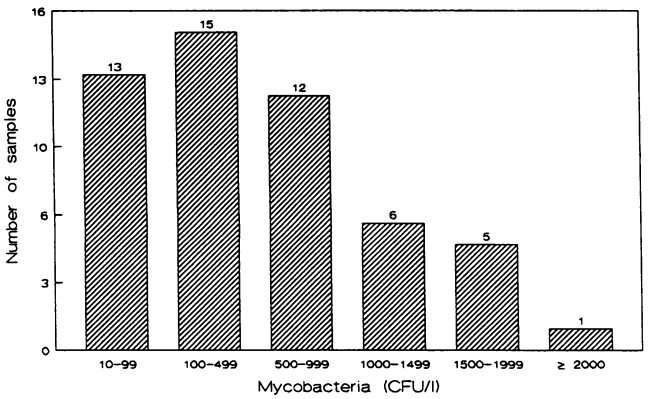

FIG. 2. Frequency distribution of numbers of mycobacteria in brook waters.

We found no correlation between the numbers of mycobacteria and water or air temperature, in contrast to the results of Kirschner et al. (26). The difference between the findings may have been due to the wide temperature range

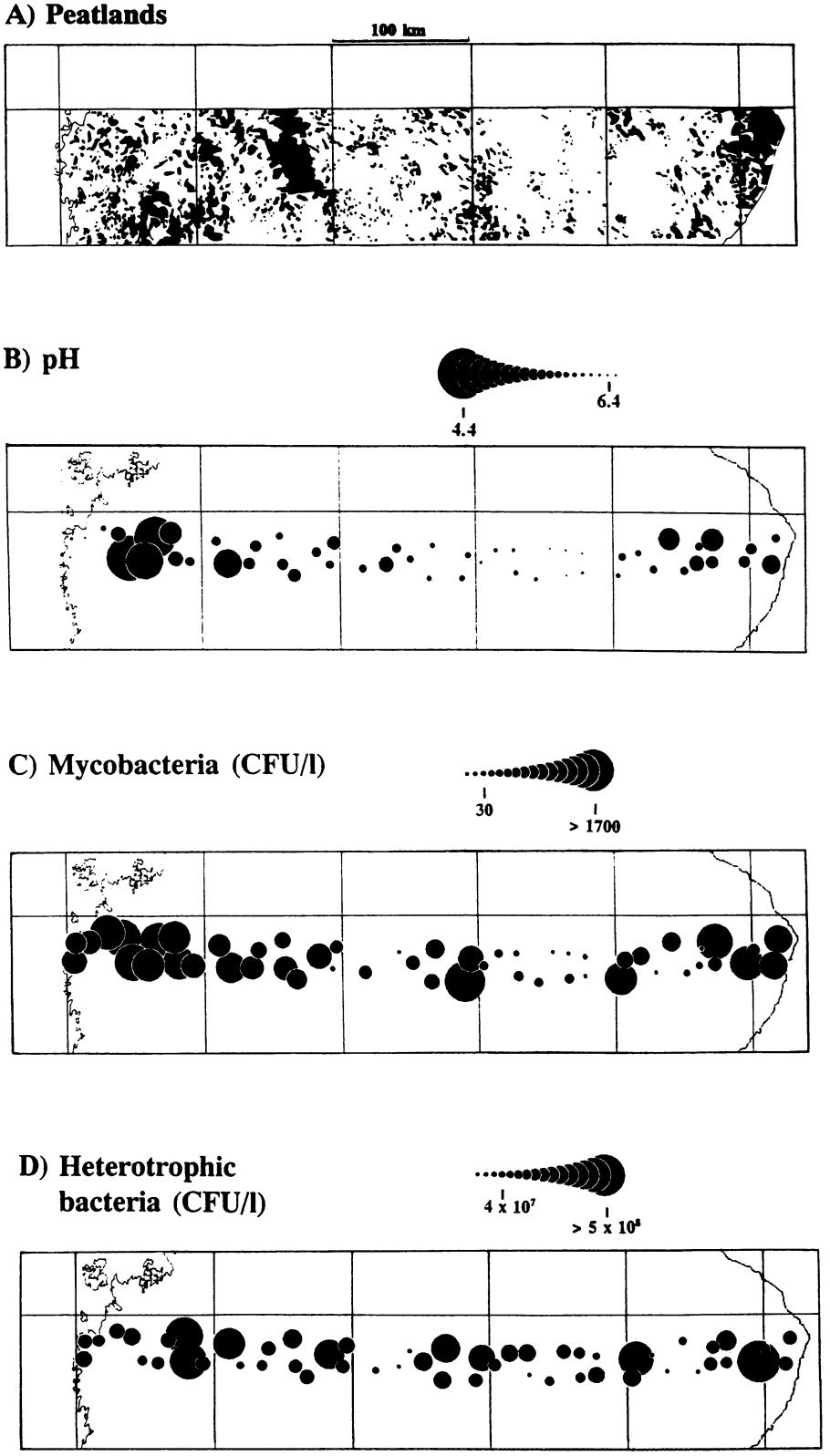

FIG. 3. Geographical variation in the occurrence of peatlands (A) (black areas on the map) (38), water pH (B), numbers of mycobacteria (C), and numbers of heterotrophic bacteria (D). The samples (each spot represents one sample) were taken from brooks located along a 550-km-long linear belt crossing Finland at 63° north latitude.

TABLE 3. Correlation coefficients between the numbers of mycobacteria and heterotrophic bacteria and environmental, climatic, physical, and chemical variables

Variable	Mycobacteria (n = 52)[a]		Heterotrophic bacteria (n = 53)	
	r[b]	P	r	P
Peatlands	0.66	<0.001	0.24	<0.05
Water temp	−0.16		0.07	
Air temp	0.13		−0.02	
Precipitation (3 days)[c]	0.23		0.21	
Precipitation (2 weeks)[c]	0.42	<0.01	0.13	
Precipitation (3 weeks)[c]	0.44	<0.001	0.01	
pH	−0.47	<0.001	−0.004	
Alkalinity	−0.20		0.09	
Electrical conductivity	0.25	<0.05	0.36	<0.01
Color	0.79	<0.001	0.50	<0.001
COD	0.73	<0.001	0.42	<0.01
Concn of:				
NO_3^-	0.38	<0.01	0.19	
Cl^-	0.27	<0.05	0.26	<0.05
F^-	0.36	<0.01	0.32	<0.01
SO_4^{2-}	0.01		0.08	
SiO_2	0.44	<0.01	0.21	
Fe	0.72	<0.001	0.46	<0.001
Mn	0.25	<0.05	0.25	<0.05
Na	0.38	<0.01	0.33	<0.01
K	0.14		0.30	<0.05
Mg	0.30	<0.05	0.39	<0.01
Ca	0.25	<0.05	0.38	<0.01
Al	0.70	<0.001	0.36	<0.01
Cu	0.50	<0.001	0.23	
Co	0.63	<0.001	0.31	<0.05
Ni	0.41	<0.01	0.17	
Cr	0.64	<0.001	0.41	<0.01
Heterotrophic bacteria	0.40	<0.01		
Mycobacteria			0.40	<0.01

[a] n is the number of samples tested. One mycobacterial sample was lost during filtration.
[b] r, Spearman rank correlation coefficient.
[c] Cumulative precipitation for the 3 days or 2 or 3 weeks before each sampling.

(about 1 to 32°C) in the study of Kirschner et al. Our samples were collected during a rather short period in summer when water temperatures varied from 11 to 19°C. In these areas, the water only occasionally reaches temperatures over 20°C, which most mycobacterial species require for replication (39). However, Kazda (25) has shown that temperatures over 30°C may be reached easily for extended periods in sphagnum vegetation under climatic conditions similar to those in Finland. By providing the necessary growth temperature for mycobacteria, sphagnum vegetation makes replication of mycobacteria possible despite low water temperatures.

TABLE 4. Linear regression model for numbers of mycobacteria[a]

Independent variable	Regression coefficient	SE	P
Peatland[b]	0.014	0.003	0.0000
COD[c]	0.999	0.210	0.0000
K concn[c]	1.033	0.180	0.0000
pH	−0.384	0.094	0.0002

[a] The constant was 2.183, and the R^2 value was 0.83.
[b] Nonlogarithmic form of the variable.
[c] Logarithmic form of the variable.

Our results show that several environmental factors affect the occurrence of mycobacteria in brook waters. The regression model, which included the percentage of peatland in the drainage area, COD, potassium concentration, and pH as independent variables, emphasized the importance of acidity and organic matter for the occurrence of mycobacteria. Potassium is a common element in acidic granitoid and mica schist rocks. The presence of this element in the model indicated that the mineralogy of the drainage area also affected the occurrence of mycobacteria. In this study, high concentrations of mycobacteria were found in areas where acidic sulfide soils are common. More studies will be needed to draw conclusions about the effect of environmental acidification on the occurrence of mycobacteria, especially potentially pathogenic species. However, our results indicate that acidic waters with high organic matter contents are potential sources of mycobacteria. Such waters are common at northern latitudes, where the most extensive peatlands in the world occur (12).

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