Bacterial Pollution of Waters in Pristine and Agricultural Lands

R. M. Niemi* and J. S. Niemi

ABSTRACT

Concentrations of thermotolerant coliform bacteria, presumptive *E. coli*, and presumptive fecal streptococci were determined from ditches, brooks, and natural ponds in six agricultural areas and 22 uninhabited pristine areas in southern Finland in the summer of 1987. For comparison, the same fecal indicators were enumerated from the effluents of three wastewater treatment plants. The objective was to compare the importance of these waters as sources of fecal indicators in receiving waters. The numbers of bacteria in waters in agricultural areas often exceeded the limit of acceptable swimming water (1000 bacteria per 100 mL), especially during wet periods, which shows that diffuse loading can be a significant source of fecal pollution. Fecal indicators were detected in about half of the samples of pristine areas, sometimes in concentrations exceeding the limit of good swimming water (100 bacteria per 100 mL). This contamination was probably caused by wild animals, especially by elk (*Alces alces*) and deer (*Odocoileus virginianus*) living in the areas. The concentrations of bacteria were higher in running waters than in ponds. The reliabilities of routine enumeration methods for the bacteria were evaluated by carrying out confirmation tests for isolated strains. Thermotolerant coliforms were reliable indicators in waters contaminated by diffuse loading. The reliability of enumeration of fecal streptococci in these waters should be studied further.

Bacterial loads discharged to receiving waters from wastewater treatment plants appear to be decreasing in countries where purification processes have improved. Smith et al. (1987) studied water quality trends in the rivers of the USA in 1974–1981 and found that the concentrations of fecal indicator bacteria had decreased. Poikolainen (1988) observed a similar decrease of these bacteria in Finnish inland waters when analyzing 16 456 fecal coliform and 96 348 fecal streptococci measurements carried out from 1962 to 1984 from water samples of the national monitoring network. The decreases in these two examples are due to improved waste treatment. Because wastewater treatment plants discharge less bacteria to waters than earlier, the role of diffuse bacterial loading has simultaneously increased. For example, Smith et al. (1987) found that the occasionally observed increases in fecal coliform counts were positively associated with the density of cattle population and with feedlot activity in the drainage basins.

Bacterial pollution in runoff from agricultural lands has been reviewed, e.g., by Khaleel et al. (1980), Crane et al. (1983), and Baxter-Potter and Gilliand (1988). Gary et al. (1983) found that cattle (*Bos* sp.) grazing in pastures bisected by a small perennial stream had only minor effects on the water quality during a study of 2 yr. However, the concentrations of indicator bacteria in the stream increased when the number of grazing cattle was high. When the cattle were removed the bacterial concentrations decreased to the levels observed in adjacent ungrazed pasture. Jawson et al. (1982) monitored indicator bacteria for 3 yr in grazed and nongrazed watersheds and found that the concentrations of total coliforms and fecal streptococci in runoff from the two watersheds did not differ significantly but fecal coliforms were greater from the grazed watershed. Walter and Bottman (1967) found that the concentrations of coliforms and enterococci were affected by wildlife.

Coliforms have been enumerated from the waters of pristine areas affected only by wild animal populations. For example, Bohn and Buckhouse (1985) discussed the occurrence of coliforms and the problems of using them as indicators in wildland streams. Hazen (1988) and Fujioka et al. (1988) demonstrated the occurrence of high numbers of fecal coliforms and *E. coli* in pristine tropical environments. Niemelä and Niemi (1989) studied the species distribution and temperature relations of coliform populations from uninhabited watershed areas. They found that *Serratia fonticola* and *Hafnia alvei* were common among the species of total coliform bacteria isolated in pristine watersheds. Total coliform analysis therefore had no indication value in these waters. *E. coli* was the only coliform isolated from pristine areas that could grow at 44.5 °C, demonstrating the value of elevated temperature as a selective factor in monitoring fecal pollution of surface waters by coliform analysis.

Great temporal variation is observed in the concentrations of bacteria discharged from land to receiving waters. These variations are caused by characteristics of the individual drainage basins and by hydrological phenomena (Kunkle, 1970; Faust, 1976; Patni et al., 1985; Niemi and Niemi, 1988). The maximum bacterial concentrations that occur in water only for short periods of time may therefore remain undetected. Occasionally the concentrations of indicator bacteria in runoff from agricultural lands exceed the recommended water quality standards of point sources (Doran and Linn, 1979). This has led to discussions of whether the water quality standards developed for waters receiving point source pollution are applicable to waters under the influence of diffuse population (Doran and Linn, 1979; Bohn and Buckhouse, 1985; Milligan, 1987).

The objective of this work was to compare the concentrations of thermotolerant coliform bacteria, presumptive *E. coli*, and presumptive fecal streptococci in receiving waters of pristine and agricultural areas in southern Finland. For further comparison, the same bacteria were enumerated from treated wastewater. The reliabilities of routine enumeration methods for these bacteria were evaluated by carrying out additional tests for isolated strains.

MATERIALS AND METHODS

Sampling Sites

Twenty-two sampling sites were selected from uninhabited pristine upland regions. The regions studied were pristine

National Board of Waters and the Environment, Water and Environment Research Institute, P.O. Box 250, SF 00101 Helsinki, Finland. Received 27 Apr. 1990. *Corresponding author.

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Table 1. Sampling sites in pristine (1–22) and agricultural (A–E) areas.

Site designation	Description of sampling site	Description of drainage basin	pH
1	Ditch with humic water	mainly drained peatland	6.5
2	pond (2 ha) acidified	esker, heathy forest	4.4
3	pond (3 ha) acidified	heathy forest	4.9
4	pond (2 ha) acidified	peatland and heathy forest	4.6
5	pond (1.5 ha)	drained peatland and heathy forest	4.5
6	pond (2 ha)	drained peatland and heathy forest	4.8
7	pond (6 ha)	drained peatland and mixed forest	6.8
8	pond (5 ha)	drained peatland and mixed forest	4.9
9	ditch	drained peatland, mixed forest	6.7
10	pond (2 ha)	forest	5.8
11	ditch from peatland	drained peatland, forest, traces of elk	6.5
12	pond (1 ha)	drained peatland, mixed forest	6.5
13	ditch from pond no. 12	drained peatland, forest, traces of elk	6.5
14	ditch	drained peatland	4.4
15	pond (2 ha) acidified	heathy forest	4.9
16	pond (15 ha)	heathy forest	5.3
17	ditch	peatland and forest	4.8
18	ditch	drained peatland and forest	6.6
19	ditch	peatland and mixed forest	6.5
20	ditch	drained peatland	6.4
21	ditch with weir	0.68 km^2, forest of which 13 % peatland	7.5
22	brook	forest and peatland, traces of elk	7.1
A	ditch	cultivated field and pastures	7.6
B	brook with weir	4.04 km^2, cultivated field farms and forest	7.6
C	brook, turbid from clay	pasture and farms	7.4
D	ditch, standing water in later summer	summer houses, forest	6.8
E	ditch, little water	pasture and farms	7

with regard to input of fecal indicators. The only human impacts in the area were forestry and ditching, both in small scale. Sampling sites were ditches, brooks, and natural ponds with no human bacterial input (Table 1). The Sites 21 and 22 were situated in the proximity of agricultural areas and were therefore sampled according to the same sampling scheme as other sites in agricultural areas. The pH and color of the water in the sampling sites varied from brown humic waters to clear, acid waters.

Six sampling sites, including ditches and brooks, were selected from agricultural areas. The drainage basins of the sampling sites were forests, agricultural lands, pasture, and sparsely inhabited areas receiving diffuse bacterial load from farms and summer cottages (Table 1).

For comparison, the bacterial quality of treated wastewater was monitored in three municipal wastewater treatment plants. Ferrosulphate was used in the plants in simultaneous precipitation and lime was fed to the process to maintain the alkalinity required because of nitrification. Two plants employed primary sedimentation and one final precipitation.

Sampling

Water samples were taken into sterile borosilicate bottles. Samples were taken from treated wastewater and from ponds using a sampler as described by Niemelä and Niemi (1989) and from flowing waters aseptically by hand. Duplicate samples of half a liter were taken from the wastewater treatment plants and samples of 1 L without replicates from agricultural and pristine areas. Wastewater treatment plants were sampled six times in June and July, agricultural areas and Sites 21 and 22 of the pristine areas five times in June and July and the rest of the pristine watersheds twice, in June and August, during the summer of 1987. Site C in Table 1 was investigated more intensively in 1988 by determining presumptive *E. coli* from 25 water samples taken from April to December. Water samples were placed into insulating boxes, transported to the laboratory, and analyzed within a few hours of sampling.

Enumeration of Bacteria

Thermotolerant coliform bacteria were enumerated with the membrane filtration technique using Gelman GN-6 filters on m-FC agar (Difco) at 44.5 °C for 22 ± 2 h. Typical colonies were counted out and, when there were enough colonies, 30 strains from each sample were isolated in lactose mannitol tryptophane broth to determine gas and indole production at 44.5 °C, on the basis of which the concentrations of presumptive *E. coli* (SFS 4088; Finnish Standards Assoc., 1988) were calculated. When fewer colonies were available, all were isolated.

Fecal streptococci were enumerated on KF Streptococcus agar (Difco) by the membrane filtration technique with Gelman GN-6 filters at 35 ± 1 °C for 44 ± 4 h. Typical colonies were counted. When isolating colonies for further tests the same procedure was used as for coliforms. For the confirmation of fecal streptococci the isolated colonies were tested for growth, catalase production, and esculine hydrolysis on bile esculin azide agar at 44 ± 0.5 °C (ISO 7899/2; Int. Organization for Standardization, 1984).

Physical and Chemical Measurements

Temperature and pH of the water samples were measured in the field using a mercury thermometer and the field pH-meter (Hach). Temperature varied in pristine areas between 5 and 16.5 °C and in agricultural areas between 8 and 16 °C. Precipitation data were obtained from the Monthly Reviews of the Finnish Climate, Observation Station at Vihti, Maasoja (Finnish Meteorological Inst., 1987), from the files of the Finnish Meteorological Institute and from observations carried out at Helsinki-Vantaa Airport.

RESULTS AND DISCUSSION

Reliability of the Enumeration Methods for Bacteria

Thermotolerant Coliform Bacteria. In the pristine and agricultural areas thermotolerant coliform bacteria generally proved to be presumptive *E. coli* (Table 2). In treated wastewater the enumeration method gave inferior results as only 64% of the strains investigated were presumptive *E. coli.* There was a strong relationship between the thermotolerant coliform bacteria and presumptive *E. coli* in pristine and agricultural environments (Fig. 1a.).

Total and thermotolerant coliform bacteria multiply

Table 2. Proportions of presumptive *E. coli* of thermotolerant coliforms and confirmed fecal streptococci of presumptive fecal streptococci in samples from pristine and agricultural areas and from treated wastewater.

Source of samples	Coliforms: Thermotolerant	Coliforms: Presumptive E. coli		Fecal streptoccocci: Presumptive	Fecal streptoccocci: Confirmed	
	no.	no.	%	no.	no.	%
Pristine areas	215	206	96	267	118	44
Agricultural areas	391	355	91	392	171	44
Treated wastewater	529	339	64	166	137	82

in industrial wastewaters rich in organic matter, which limits the use of thermotolerant coliform bacteria as indicators of fecal pollution in these waters (Vlassoff, 1977). This interference was not observed in the samples of pristine or agricultural areas (Table 2). The deviations in the autocorrelation between thermotolerant coliforms and presumptive *E. coli* show that the cleaner the environment is, the better thermotolerant coliforms can be used to measure the occurrence of presumptive *E. coli* and thus indicate fecal contamination (Fig. 1a). Our results are in accordance with those of Niemelä and Niemi (1989).

Fecal Streptococci. The proportion of confirmed fecal streptococci in the enumeration of fecal streptococci was greater in treated wastewater than in water in pristine or agricultural areas (Table 2). In treated wastewater the percentage of confirmed fecal streptococci was 82%, whereas the corresponding figure in both the pristine and agricultural areas was only 44%. The relationship between presumptive fecal streptococci and confirmed fecal streptococci was strong in treated wastewater, weaker in agricultural areas and weakest in pristine areas (Fig. 1b). In contrast to coliform bacteria the correlation between presumptive and confirmed fecal streptococci was strongest in the areas where the total number of fecal streptococci were greatest.

Fecal streptococci are used as indicators of fecal pol-

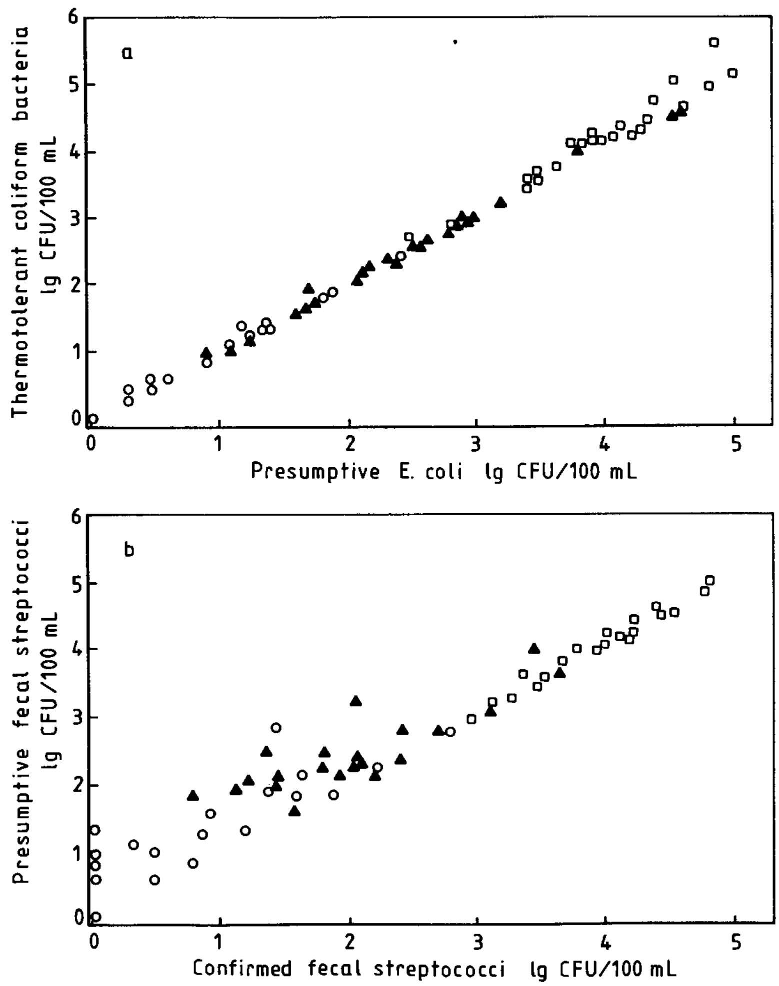

Fig. 1. Relationship between (*a*) thermotolerant coliform bacteria and presumptive *E. coli*, and (*b*) presumptive and confirmed fecal streptococci in different waters—○ = pristine areas, ▲ = agricultural areas, □ = treated wastewater.

lution, because they survive better in the water than thermotolerant coliforms and do not multiply significantly in industrial effluents (Beaudoin and Litsky, 1981), except in fish farms (Niemi, 1985). However, fecal streptococci multiply in vegetation and plant material, which limits their value as indicators. When investigating pristine and agricultural areas, confirmation tests are needed to obtain a reliable estimate of the fecal streptococci pollution of waters. As pointed by Doran and Linn (1979), the primary cultivation medium of fecal streptococci affects the results depending on the type of the sample.

The enumeration method and confirmation tests for fecal streptococci should be studied further. Recent changes in the taxonomy of these bacteria have been considerable. New genera have been established and new species described. It is not known whether the low proportion of confirmed fecal streptococci in the least contaminated waters is due to the fecal streptococci of wild animals being unable to give positive reactions in the confirmation tests or whether these bacteria have no value as fecal indicators. It is known, however, that not all strains of group D streptococci give a positive reaction in the esculin hydrolysis test on bile esculin azide agar at 44 °C (Havelaar et al., 1982). The importance of further studies on this topic is underlined by the result of epidemiological studies, which show that these bacteria are valuable indicators of health risks of swimmers in natural waters (Cabelli et al., 1982; Fattal et al., 1986; Ferley et al., 1989).

Occurrence of Bacteria

Treated Wastewater

Fecal indicators were enumerated from three wastewater treatment plants and the results were compared with the corresponding analyses of the pristine and agricultural areas. The diurnal fluctuation of the concentrations of fecal indicators was monitored in two of the plants in order to determine the best sampling time. Samples were taken at intervals of 2 h for 24 h for the determination of fecal indicators. The maximum concentrations were observed between 23.00 and 05.00 h. In order to level out the effects of diurnal variation, the five samples during the summer were taken outside these peak times. The loading from treated wastewater was therefore somewhat underestimated.

The concentrations of fecal indicators varied in three investigated plants. In treated wastewater the bacterial concentrations varied during the summer as follows: thermotolerant coliform bacteria 5×10^2 to 4×10^5, presumptive *E. coli* 3×10^2 to 1×10^5, presumptive fecal streptococci 9×10^2 to 2×10^5, and confirmed fecal streptococci 9×10^2 to 6×10^4 CFU/100 mL.

The quality of treated wastewater fluctuates widely. At its best the water could be acceptable swimming water (less than 100 thermotolerant coliforms in 100 mL) according to the Finnish criteria (National Board of Health, 1988; Table 3). However, when quality is poorest during the summer it would be necessary to dilute from 100 to 400 times before the standard of acceptable swimming water could be met. On the basis of the median values of the concentrations of fecal indicators, treated wastewater should be diluted at least from 10 to 20 times and on the basis of maximum daily concentrations about 40 times, before the standard of acceptable swimming water could be met. The decay of bacteria in receiving waters decreases the need of dilution but loading from diffuse sources may increase it considerably.

Table 3. Statistical parameters of the concentrations of indicator bacteria in pristine and agricultural areas and in treated wastewater (CFU/100 mL).

Bacterial group	Parameter	Sampling site: Pristine area	Agricultural area	Treated wastewater
Thermotolerant coliform bacteria	*n*	49	24	24
	mean	12	3 802	46 863
	median	0	307	17 800
	min	0	8	500
	max	268	38 200	414 300
Presumptive *E. coli*	*n*	47	24	23
	mean	12	3 610	20 639
	median	0	283	9 100
	min	0	7	300
	max	259	38 200	100 000
Presumptive fecal streptococci	*n*	50	24	24
	mean	48	1 629	35 092
	median	3.5	233	16 900
	min	0	41	900
	max	740	16 900	187 400
Confirmed fecal streptococci	*n*	39	21	20
	mean	26	495	15 970
	median	0	106	10 000
	min	0	5	900
	max	609	4 450	62 600

Agricultural Areas

Thermotolerant Coliform Bacteria. The concentrations of thermotolerant coliform bacteria and presumptive *E. coli* were substantially lower in agricultural areas than in treated wastewater (Fig. 2a). The highest concentrations of thermotolerant coliforms and presumptive *E. coli* in agricultural areas were observed in the sampling Site E, a ditch starting from a pond and flowing through a pasture. Bacterial concentrations were relatively high in the sampling Site B, a brook surrounded by an inhabited area and a field. The bacterial concentrations tended to increase toward the end of the summer.

Fecal Streptococci. The concentrations of presumptive and confirmed fecal streptococci were generally much lower in agricultural areas than in treated wastewater (Fig. 2b). The concentrations of fecal streptococci determined from different sampling sites varied, but less than those of thermotolerant coliform bacteria and presumptive *E. coli.* Highest concentrations were observed in the sampling Site C, a brook surrounded by an inhabited agricultural area. The concentrations of presumptive *E. coli* increased during the summer more clearly than the concentrations of confirmed fecal streptococci.

An intensive study of Site C carried out in April to

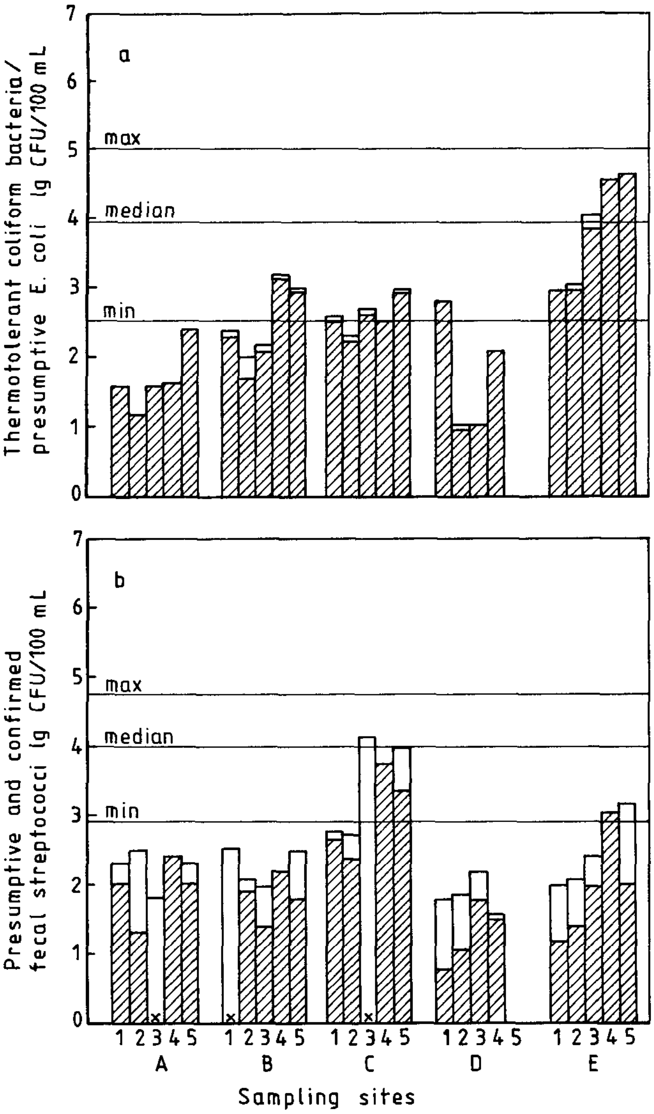

Fig. 2. Concentrations of fecal indicators of Sites A–E (Table 1) in agricultural areas in 1987 by sampling dates (1=6/29, 2=7/7, 3=7/13, 4=7/21, 5=7/27). Crossed bars show the proportion of presumptive *E. coli* of thermotolerant coliforms and the proportion of confirmed fecal streptococci of presumptive fecal streptococci, respectively. Confirmation tests not carried out = x. For comparison, the maximum, median, and minimum concentrations of presumptive *E. coli* (*a*) and confirmed fecal streptococci (*b*) in treated wastewater are shown by solid lines.

December 1988 revealed a different pattern of thermotolerant coliforms and fecal streptococci (Fig. 3). Thermotolerant coliforms were quantitatively confirmed as presumptive *E. coli* except on one occasion, 13 June, when only about 30% of typical colonies were presumptive *E. coli.* In early spring and winter a higher proportion of presumptive fecal streptococci was confirmed as fecal streptococci than in summer and autumn. Brooks were contaminated by thermotolerant coliforms and presumptive *E. coli* earlier than by fecal streptococci. In the autumn, however, high concentrations of fecal streptococci were observed when concentrations of presumptive *E. coli* had decreased. There was also more short term temporal variation between fecal streptococci than between presumptive *E. coli.* When the water quality in agricultural areas is at its worst, it is too polluted for swimming. Even on the basis of median values these waters were not fit for swimming (National Board of Health, 1988; Table 3). However, median values were lower than those reported by Doran et al. (1981) for grasslands.

Fecal pollution seems to be common in agricultural areas. This can be explained by land use, e.g., the drainage basins of the sampling Sites A, C, and E included pastures extending to the waterfront, swimming beaches, and summer cottages and houses. The highest concentrations of thermotolerant coliform bacteria and presumptive *E. coli* were found in the waters of inhabited areas. An exception was Site D, where an inhabited area was situated along the shore of the lake, upstream from the sampling site. Water pollution was probably low in Site D due to efficient waste management of summer cottages and due to inactivation and sedimentation of the bacteria in the lake.

Bacterial loads due to agricultural areas could not be distinguished from those due to cattle grazing. In the sampling Site A (Fig. 2), situated in an area of only occasionally grazed pasture, the concentrations of fecal indicators did not significantly differ from those observed in a brook flowing from pristine forest (Site 21, Fig. 4). All the sites sampled in the agricultural areas were flowing waters situated in the upland region. The contact between water and soil was therefore strong and sedimentation and decay of bacteria were less prominent than in larger watercourses rich in lakes, which are so typical of Finland. Rains that fell during the investigation clearly increased the concentrations of bacteria (Niemi and Niemi, 1990).

Pristine Areas

Thermotolerant Coliform Bacteria. The concentrations of thermotolerant coliforms and presumptive *E. coli* were low and these bacteria were found only in half of the investigated samples of 100/mL in the upland region (Sites 1–20, Fig. 4). By contrast the two uninhabited watersheds in the proximity of agricultural lands, Sites 21 and 22, yielded higher counts of these bacteria. In these two sites thermotolerant coliforms and presumptive *E. coli* increased during the investigation.

Fecal Streptococci. Presumptive fecal streptococci were found in half of the 100-mL samples from the pristine areas (Fig. 4). The concentrations of streptococci varied more than the concentrations of thermotolerant coliforms. In compliance with the concentrations of thermotolerant coliforms and *E. coli,* the concentrations of both presumptive and confirmed streptococci were higher in Sites 21 and 22 than in other sites.

In the pristine areas, the concentrations of thermotolerant coliforms and fecal streptococci were lower than in the agricultural areas. The sampling sites of the pristine areas, except Sites 21 and 22 (Fig. 4), were sampled during dry days only. Sites 21 and 22 and the sites in agricultural areas were sampled on both wet and dry days. Samples gathered on wet days yielded higher concentrations of bacteria than those taken on

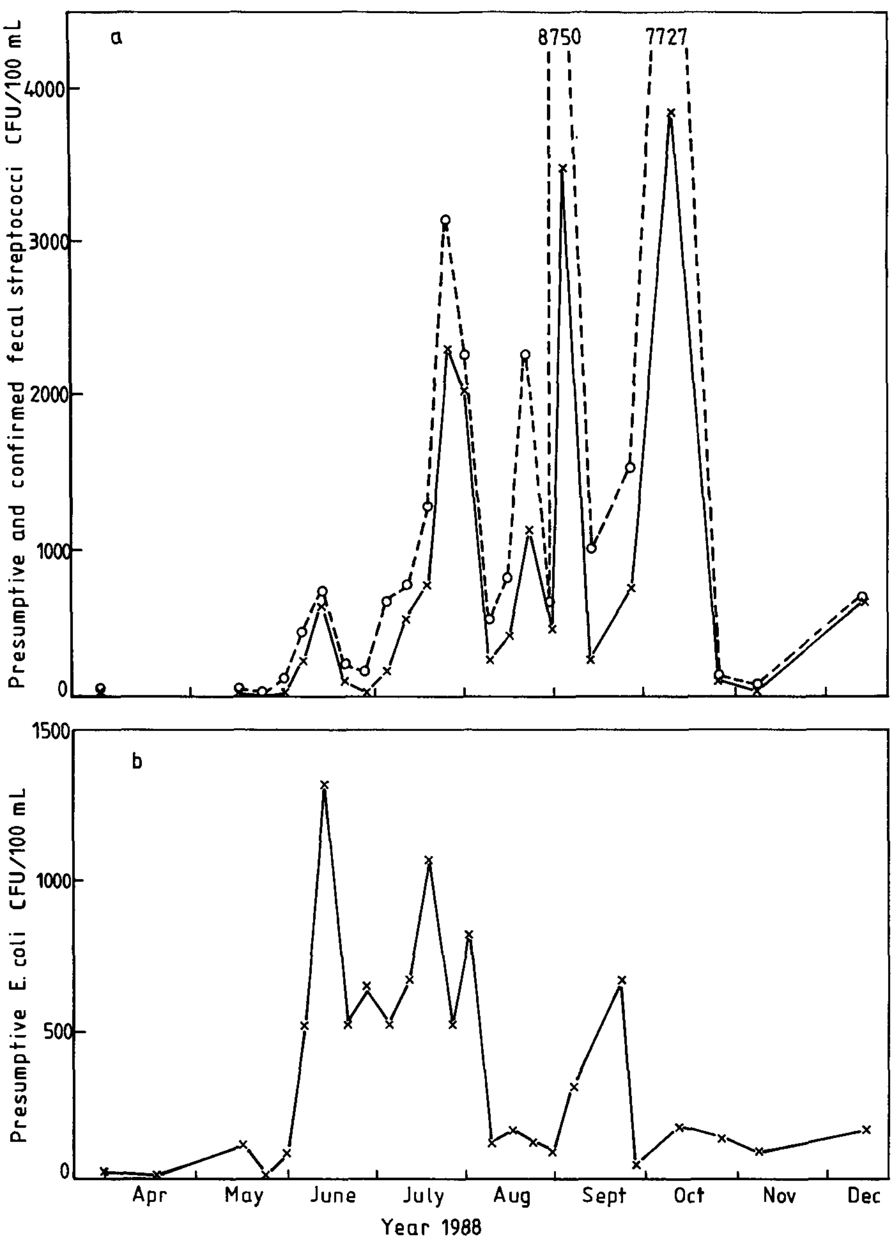

Fig. 3. A survey of the concentrations of (*a*) presumptive (O——O) and confirmed (x-x) fecal streptococci and (*b*) presumptive *E. coli* in a ditch (Site C, Table 1) discharging to Lake Kotojärvi from April to December 1988.

dry days. In addition to precipitation, the fauna of a region also affects the bacterial concentrations in receiving waters. Acid rain has affected the water and probably the soil of many pristine areas studied and is an additional factor that may decrease the survival of indicator bacteria. The results of this study show that the concentrations of fecal indicators in pristine areas occasionally exceeded the limit of good swimming water. Fecal pollution was much more common in flowing waters than in ponds (Fig. 4).

Comparison of Bacteria in Different Environments

Statistical parameters were calculated for the combined data of all samples of the pristine and agricultural areas and treated wastewater (Table 3). As might be expected, the median concentrations of fecal indicators were highest in treated wastewater. However, the highest bacterial concentrations observed in the agricultural areas were relatively high in comparison with the concentrations in wastewater. The highest concentrations observed in the pristine areas were close to the median concentrations observed in the watersheds affected by agriculture.

Treated wastewater was the most significant source of bacteria, especially because bacteria are discharged from treatment plants continuously. On the other hand, the medians of the bacterial concentrations in waters from agricultural areas exceeded the limit of good swimming water. The total bacterial load discharged from all agricultural areas of the country is a significant source of pollution because the volume of

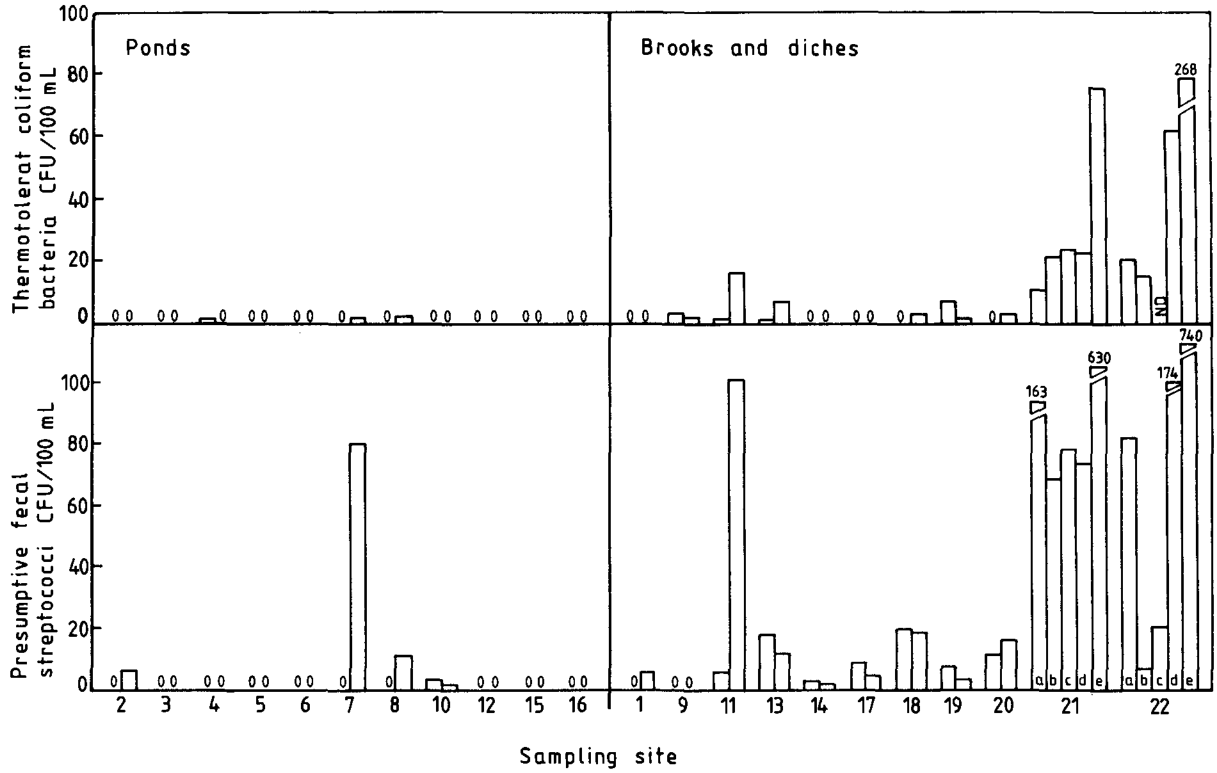

Fig. 4. Concentrations of fecal indicators in ponds, brooks, and ditches of sampling sites 1–22 in pristine areas. Sites 1–20 were sampled twice (8 June and 17 August). Sites 21 and 22 were sampled five times (*a*=6/29, *b*=7/7, *c*=7/13, *d*=7/21, and *e*=7/27). Bacteria not detected=0. ND= enumeration not done.

water discharged from these areas vastly exceeds that of treated wastewater. Although the waters in the pristine areas sometimes exceeded the limit of good swimming water, the total bacterial load discharged from these areas is small in comparison with the loads from wastewater treatment plants or from agricultural areas. Diffuse load from pristine areas may, however, have temporary local importance.

CONCLUSIONS

Fecal indicators were common in the waters of agricultural areas. The concentrations generally exceeded the limit of good swimming water (100 bacteria per 100 mL) and occasionally the water was unsuitable for bathing (more than 1000 bacteria per 100 mL). Highest counts were observed during wet periods. This shows that diffuse bacterial loading from agricultural areas can be significant and must be taken into account as a source of fecal pollution.

Fecal indicators were found in about half of the samples in the waters of pristine areas, sometimes in high concentrations. Contamination was probably caused by wild animals, especially by elk (*Alces alces*) and deer (*Odocoileus virginianus*) that live in such areas. Occasionally the waters did not fulfill the criteria of good swimming water. The total output of bacteria from pristine areas is, however, insignificant in comparison with the bacterial load of agricultural areas, not to mention wastewaters.

Natural ponds seemed to decrease the concentrations of fecal bacteria. The detention time in ponds is longer than in flowing waters, which increases the decay and sedimentation of bacteria and thus decreases bacterial concentrations.

Thermotolerant coliforms were reliable indicators in waters contaminated by diffuse loading. The reliability of fecal streptococci as indicators in these waters should be further studied.

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