Particle size, water-stable aggregates, and bacterial populations in lake sediments

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Sediments from 10 lakes for which bacterial counts were available were submitted to particle size analysis (Coulter Counter), both before and after removal of organic material. While these sediments were not so highly aggregated as soils, they contained water-stable aggregates and differed from one another in this characteristic. The counter failed to detect some apparently unstable aggregations visible in the sediment of one lake. The median sizes of the untreated sediment were observed to be inversely proportional to the median sizes of the sediment from which the organic component had been removed. There was good correlation between logarithms of the heterotrophic bacterial population (culture counts) and the median sizes of the particles in untreated samples of the sediment. Estimations of bacterial density, assuming a specific gravity of 1.6 for these sediments indicated that, for the four lakes for which direct counts of bacteria were available, there were from 3000 to 15 000 bacteria per square millimeter of sediment particle surface, or about 1 bacterium for every 70 to 300 μ^2.

Introduction

The ultimate particles, such as clays or sands, from which natural soils derive their basic textures are commonly observed to be bound into aggregates, sometimes called crumbs (Teuscher and Adler 1960; Waksman 1932, 1952). The crumb structure of soil is largely due to the activities of its microflora (Griffiths 1965). The particulate nature of sediments is similar to that of soils, but the aggregation of sediment particles is an aspect of aquatic ecology that has not received much attention.

Twenhofel and Broughton (1939) observed both the bacterial population and the particle size of sediment from Crystal Lake (Wisconsin), but they did not examine the possibility of a correlation between these two features, nor do their published data provide for such analysis. ZoBell (1938) found correlation between his own bacterial counts in the Channel Island region and the median particle diameter of the sediment from Trask's (1932) data. Wigley (1961) found a good correlation between median particle size and organic content of sediments from George's Bank. This relationship has been reported by others (Nota 1958; Reuszer 1933).

Anthony and Hayes (1964) examined the possibility that the general level of the bacterial population in the sediment might "reflect the integrated performance of the overlying water in the recent past." Among the relationships examined, good correlation was detected between the number of heterotrophic bacteria in lake sediment and two features of the water above: color (in Pt units) and methyl orange alkalinity. The relevant equation was log bacteria $= 0.568 + 0.513$ log color $+ 0.019$ methyl orange alkalinity. No significant single or multiple regression was found to describe the relationship between the bacteria in the sediment and its organic content measured by loss upon ignition. That the floral level might be related to particle size of the sediment was considered, but the size range of the particles comprising the sediments then being studied was not known.

We have now examined the particulate nature of the sediments of 10 lakes and ponds previously studied by this laboratory. In this investigation, we have (1) examined the particle size structure of the sediment after it had been diluted and agitated in accordance with procedure used in preparation for counting the bacteria by agar plate or membrane filter method, (2) examined the ultimate particle structure of the sediment after removal of its organic component, and (3) compared these observations with the organic content and bacterial population of the sediments.

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Materials and Methods

Figure 1 (map) of Hayes and Anthony (1958) shows the location of these lakes with the exception of Amherst Pond, and Table I of that paper provides the limnological data available for the other nine lakes. Amherst Pond (45°52′ N, 64°11′ W) is in Nova Scotia, near the junction of that province with the province of New Brunswick. It is formed by an old dam on a small stream. It served in the past as a source of ice. Area less than 2 acres. Mean depth about 1.5 m. Not stratified. Color = 28 Pt units. The pH = 7.7. Methyl orange alkalinity = 41. Conductivity at 20°C = 148 megamhos. Cations in ppm = 17.4 Ca, 0.9 Mg. The organic content of the sediment = 23.5% dry weight.

Samples of the uppermost layers of sediment were obtained from the same profundal regions of the 10 lakes as had been sampled previously for the quantitative estimates of the heterotrophic bacterial flora published by Hayes and Anthony (1959). Sampling of the sediment was carried out as described by those authors.

The range of size of the particles comprising the sediment was measured by a Coulter Counter model A (Coulter Electronics, Chicago). Both the principles underlying the operation of this instrument and the practical aspects of its application have been described in numerous publications, hence we shall limit our description to three references: Brecher *et al.* (1956), Mattern *et al.* (1957), Kubitschek (1960). Sizing of the sediment particles was carried out both before (untreated) and after (treated) removal of organic material by 6% H_2O_2 as described by Russell (1932, p. 575).

About 0.5 g (dry weight) of untreated sediment was added to 100 ml of water and a few glass beads in a flask, and shaken by hand for 5 min. One milliliter of the resulting suspension was added to 200 ml of 0.9% NaCl solution and submitted to the counting procedure. Any interfering particles that may have been present in the 0.9% NaCl suspending fluid had been removed previously by membrane filtration (HA 0.45 μ Millipore Corp., Watertown, Mass.). The treated samples of sediment went readily into suspension without shaking with glass beads.

TABLE 1

Example of Coulter Counter results and corresponding surface area calculations for a given sample of lake sediment (Copper Lake, treated sediment)

		Particle size						Relative surface area		
TD	ACS	Microns	Φ-Units	Mean count	Corrected count	% total count	Δ count	Mean μ^2	Total 1000 μ^2	%
							1	2171	2	0.07
100	1	43.21	4.53	1	1	0.000	3	1520	7	0.22
100	2	34.32	4.87	4	4	0.002	6	948	12	0.41
100	3	27.26	5.20	10	10	0.004	24	599	27	0.88
100	4	21.68	5.53	34	34	0.014	44	379	43	1.44
100	5	17.28	5.86	78	78	0.032	143	242	78	2.58
100	6	13.82	6.18	221	221	0.090	676	156	183	6.05
100	7	11.14	6.49	892	897	0.366	2082	102	397	13.1
100	8	9.10	6.78	2921	2979	1.21	3121	69.9	615	20.3
100	8	(Dilution run)		(624)	(627)					
Dilution factor = 2979 ÷ 626.6 = 4.754										
100	9	7.62	7.04	1276	6100	2.49				
							7250	46.4	951	31.4
49	9	6.00	7.38	2750	13350	5.44	6940	30.3	1160	38.4
28.5	9	5.01	7.64	4150	20290	8.27	13750	20.3	1439	47.6
14.5	9	4.00	7.97	6749	34040	13.9	45350	12.3	1995	66.0
9	10	3.01	8.38	51140	79400	32.4	72250	7.5	2537	83.9
5	10	2.47	8.66	26935	151650	61.8	93650	4.2	3024	100.
3	10	2.09	8.90	40407	245300	100.				

The percentage of organic matter in the sediment was determined in the manner described by Atkinson *et al.* (1958).

The manner by which the bacteria in the sediments were counted is described by Hayes and Anthony (1959), whence data for nine of the lakes are taken. The bacterial population of Amherst Pond sediment was also estimated in the same fashion in this laboratory, but the results have not been published before. Professor F. R. Hayes kindly furnished results of direct counts of bacteria made by students using Strugger's (1944) fluorescent microscopy technique in studying the sediment of lakes, and also with the limnological data for Amherst Pond.

Results

We used the 140-μ aperture with the counter and set it to aspirate 0.5 ml for each count. The entire sampling assemblage was surrounded with copper screening to protect against interference from fluorescent lighting. The effective measuring range of the instrument was divided into 15 steps through combinations of threshold dial (TD) and aperture current selector (ACS) settings as indicated in Table 1. We started counting each sample at the upper end of the size range, where the smallest number of particles were encountered. At least four counts were made at each of the 15 steps to check reproducibility and the slight change in count with change of polarity made an even number of counts most suitable. We found the counter to be remarkably precise. Only the mean count is recorded in column 5 Table 1. When dilution of the sample became necessary to avoid high corrections for coincidence, we simply diluted the sample *in situ* with an appropriate quantity of suspending fluid. From the ratio of the coincidence-corrected mean counts at the same settings before and after dilution, an accurate dilution factor could be calculated as shown in Table 1.

Ragweed pollen was used in setting up and checking calibration curves for the TD and ACS settings used with the 140-μ aperture. Table 1, column 3, shows that the actual size range covered was from 2 to 43 μ diameter. Column 6 records the counts corrected for coincidence; each count is the number of particles exceeding the stated size. The percentage count is shown in column 7.

The relative proportions of the surface of the sediment particles were calculated as indicated in the last four columns of Table 1. Column 8 records the difference between successive counts in column 6. This Δ count gives the number of particles falling between two different sizes. To emphasize this subtraction, the rows of these four columns alternate with the rows of the rest of the table. Taking for example the first two rows of counts, the Δ count $= 4 - 1 = 3 =$ the number of particles for which $43.21 > D > 34.32$, where $D =$ diameter in microns. The average diameter of these particles is 38.77 μ and their average relative area $= 38.77^2 = 1520\ \mu^2$, which is entered in column 9. Since only relative area is required, the constant π is omitted. The total relative area for these particles $= 1520 \times 3 = 4750\ \mu^2$. These products are divided by 1000 to conserve table space, rounded up, and accumulated in column 10, i.e. $4750 + 2171 = 6739 \div 1000 = 7$. Finally, column 11 records the accumulated relative surface area as percentage. In a similar manner, the corresponding percentage relative volume of the particles was calculated, but is not shown in Table 1. If we assume that the

TABLE 2

Example of Coulter Counter results and corresponding surface area calculations for a sample of untreated lake sediment (Copper Lake). Certain columns inserted in Table 1 for explanatory purposes are omitted here for brevity

Particle size, μ	Corrected count	% total count	Relative surface area: Mean 1000 μ²	Relative surface area: %
43.21	15	0.24		
			0.35	5.2
34.32	23	0.33		
			0.60	9.1
27.26	50	0.72		
			0.99	14.8
21.68	114	1.65		
			2.13	32.0
17.28	414	6.01		
			4.51	68.0
13.82	1400	20.3		
			5.78	87.1
11.14	2500	36.2		
			5.88	88.5
9.10	2590	37.6		
			5.91	89.1
7.62	2640	38.3		
			6.18	93.1
6.00	3220	46.7		
			6.31	95.1
5.01	3650	52.9		
			6.36	95.8
4.00	3900	56.5		
			6.55	98.7
3.01	5425	78.6		
			6.59	99.3
2.47	5970	86.5		
			6.64	100.0
2.09	6900	100.0		

particles tend to be spherical, it may be seen that the "absolute" area of sediment surface corresponding to any given level can be obtained by multiplying the relative surface area by 3.14; for example, the total surface is $3.02 \times 3.14 \times 10^6 = 9.48 \times 10^6$ μ^2. In this manner, it may also be estimated that their volume is 8.48×10^6 μ^3. The example in Table 1 is from treated sediment. Comparable results for untreated sediment are shown in abbreviated form in Table 2.

A variety of methods have been used to plot particle size distribution. Most European students appear to prefer arithmetic probability paper (e.g. Nota 1958, Fig. 30), whereas those in North America seem to favor geometric probability paper (e.g. Pettijohn 1957, Fig. 20). The latter type of plot can be achieved by plotting logarithms of particle size on arithmetic probability paper. This we have done, but instead of common logarithms, we have used Φ-units (Krumbein 1934) which are defined by the equation $d = 2^{-\Phi}$, where d is the diameter in millimeters. We have reversed the usual method of plotting the Φ values along the ordinate so that, in our figures, particle size is increasing as one moves away from the origin of the graph.

The probability that flocculation might interfere with proper sizing of the sediment particles was examined in two ways. First of all, recovery tests were carried out as follows. Counts were recorded at the various levels with two suspensions containing different concentrations of sediment. Then equal volumes of these two suspensions were blended and counted. This third series of counts usually did not differ markedly from the arithmetic mean of the first two series. The only exceptions of this observation occurred at TD settings very near to zero, where the counts of the third series were lower than predicted. Related to this is a further observation that if the "clean" suspending fluid were counted at this low setting and then the count repeated after adding a small volume of sediment, the second count was lower. This is probably evidence of aggregation of very small particles to one another and (or) larger particles. Kubitschek (1960) discusses the use of the counter for studying this problem. However, such low TD settings were avoided in our routine sediment counts.

Secondly, we were prompted by the foregoing observations to test the effect of a dispersing agent, sodium oxalate, that is commonly used in sedimentary analyses. It had no effect within the size range we measured routinely. Hence we did not use any deflocculating agent. The sizes we have recorded may be in some error because of the adherence of very small particles, but even at the lower end of the range the error is likely quite small.

Plots were made of the accumulative percentage for number, surface area, and volume of the particles from the sediment of each of the 10

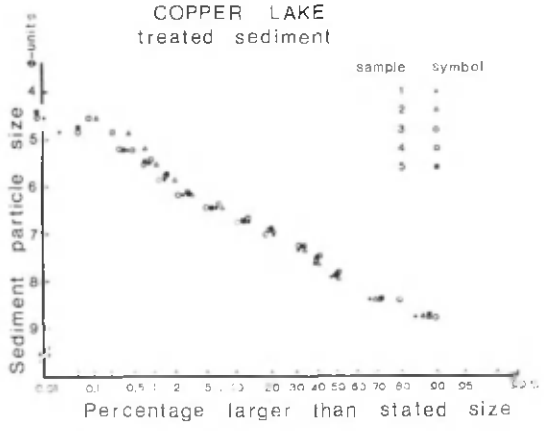

FIG. 1. Reproducibility of Coulter Counter particle size analysis for samples from a given lake illustrated by a plot of surface area corresponding to given diameters. Samples 1 to 4 are from different profundal stations in Copper Lake. Number 5 is a re-run of sample 4. All samples had been treated with H_2O_2 to remove organic material.

TABLE 3

Reproducibility of sediment particle analysis. First four numbered columns show results from four different samples from profundal regions of Copper Lake. Column 5 shows the results of recounting sample 4. The results are expressed as percentage surface area of particles above stated size

Φ	1	2	3	4	5
4.53	0.01	0.11	0.00	0.08	0.00
4.87	0.02	0.40	0.05	0.21	0.05
5.20	0.41	0.72	0.28	0.48	0.33
5.53	0.88	1.13	0.72	0.86	0.73
5.86	1.44	1.91	1.22	1.48	1.49
6.18	2.58	3.27	2.42	2.69	2.82
6.49	6.05	7.14	4.86	6.36	5.72
6.78	13.1	12.5	10.1	12.8	11.6
7.04	20.3	20.4	18.0	19.6	20.2
7.38	31.4	34.2	31.2	33.1	32.1
7.64	38.4	41.4	39.0	41.2	39.8
7.97	47.6	50.3	49.5	50.9	50.0
8.38	66.0	68.1	79.0	71.0	71.6
8.66	83.9	86.5	89.7	87.7	87.7
8.90	100.0	100.0	100.0	100.0	100.0

lakes. Figure 1 is a representative plot. The surface area was the feature most closely investigated in the present work because we thought it likely to be closely associated with the activity of the sediment flora. It also happens to be the measurement whose median can be most easily and accurately picked from graphs such as Fig. 1; the median of particle numbers is close to the minimum size and volume is more affected by any inaccuracies of counting the larger size particles.

The reproducibility of the sizing measurements on the sediment of a given lake was tested by counting four treated samples from four different profundal stations of the same lake (Copper). The results are shown in Table 3 and in Fig. 2. Column 5 of Table 3 presents the

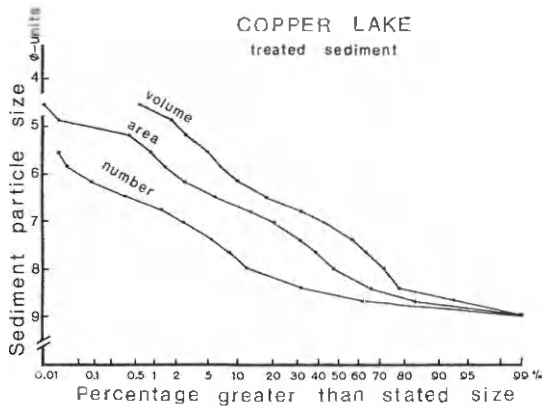

FIG. 2. Comparative plot of number, surface area, and volume of particles comprising a sample of sediment after treatment with H_2O_2 to remove organic material.

TABLE 4

Percentage surface area above stated size for untreated lake sediments

Size, Φ	Amherst Pond	Black Brook	Bluff	Boar's Back	Copper	Grand	Jesse	Lily	Montague Pond	Tedford
4.87	6.37	13.2	13.8	13.9	5.20	8.09	12.5	13.2	4.80	12.1
5.20	6.99	18.2	20.0	19.8	9.20	14.0	20.8	20.9	11.2	18.1
5.53	20.3	26.3	26.3	29.0	14.8	20.9	26.3	29.4	16.5	24.8
5.86	32.8	34.8	36.0	39.2	32.0	28.3	34.3	39.3	33.8	32.0
6.18	45.7	52.2	41.1	46.5	68.0	34.4	41.5	46.2	44.2	39.8
6.49	54.7	53.3	45.3	50.4	87.1	41.7	46.9	49.3	51.7	44.6
6.78	63.7	58.0	51.6	55.3	88.5	42.4	52.7	54.1	58.1	49.7
7.04	66.7	61.8	55.8	59.3	89.1	52.6	57.4	62.9	62.6	54.3
7.38	79.9	69.8	64.5	67.3	93.1	62.3	72.8	72.7	74.5	64.8
7.64	86.8	74.6	70.6	73.3	95.1	69.4	77.4	77.8	81.4	72.8
7.97	93.5	81.6	78.0	80.1	95.8	77.9	84.8	83.3	88.9	81.8
8.38	98.4	90.4	87.8	89.1	98.7	89.2	93.2	91.6	95.6	92.1
8.66	99.4	96.3	94.6	94.8	99.3	96.1	97.9	96.1	97.5	97.0
8.90	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

TABLE 5

Percentage surface area above stated size for treated lake sediments

Size, Φ	Amherst Pond	Black Brook	Bluff	Boar's Back	Copper	Grand	Jesse	Lily	Montague Pond	Tedford
4.53	0.15	0.38	0.00	3.20	0.07	3.74	0.20	0.00	0.00	0.60
4.87	0.37	0.64	0.02	4.22	0.22	7.63	0.81	0.23	0.16	1.99
5.20	1.09	0.98	1.60	6.05	0.41	13.1	2.89	0.52	0.06	4.23
5.53	2.59	1.71	5.36	8.77	0.88	20.0	6.24	1.30	1.04	8.41
5.86	5.58	2.64	10.7	15.0	1.44	27.1	11.2	2.51	1.95	11.3
6.18	9.91	5.48	17.3	20.1	2.58	32.9	16.7	3.95	3.75	17.3
6.49	14.0	8.62	23.3	25.6	6.05	37.9	22.4	5.53	6.12	21.7
6.78	18.9	12.4	31.2	30.8	13.1	42.7	27.4	7.73	10.3	27.4
7.04	24.1	16.3	35.5	34.3	20.3	47.1	31.6	10.1	14.0	31.6
7.38	33.3	24.0	44.5	41.4	31.4	53.9	39.6	14.7	22.7	39.6
7.64	40.6	30.8	51.2	47.8	38.4	60.2	46.7	19.6	31.7	46.7
7.97	52.1	41.6	62.7	63.3	47.6	71.0	58.2	28.5	44.2	58.9
8.38	70.4	64.9	80.8	76.2	66.0	79.5	80.3	48.9	69.7	79.6
8.66	87.8	80.2	92.4	89.9	83.9	90.0	91.0	74.6	85.0	92.3
8.90	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

results of a second count of sample 4. The symbol for sample 1 is plotted accurately throughout Fig. 2, but some of the other symbols have been off set vertically so that they are legible where coincidence would have obscured the results. It appears that reproducibility for a given sample is good and that surprisingly little variation exists between samples from different stations of the lake.

The variation of the surface area median in the sample given above ranges from 3.9 to 4.0 μ and indicates that when the median of a treated sample is estimated to the nearest 0.1 μ, which is about as close as can be done with our results, it probably will not differ by more than 0.1 to 0.2 μ from the median of a second sample. In practice, one sample was examined and worked out in detail. A second run was then performed on the

TABLE 6

Heterotrophic bacterial population, organic content, median surface area sizes, and surface area per microgram of lake sediment. Values in parentheses are loss upon ignition (from Hayes and Anthony 1958)

Lakes	Bacteria per gram dry weight (thousands)	Organic matter, %	Diameter, μ, at median surface area level		Surface area, mm², per microgram	
			Untreated	Treated	Untreated	Treated
Amherst Pond	1312	11.5	12.5	4.2	0.258	0.588
Black Brook	1409	32.0 (32.4)	12.0	3.6	0.227	0.702
Bluff	154	38.7 (37.6)	9.5	5.2	0.237	0.470
Boar's Back	218	37.3 (33.9)	11.0	4.8	0.224	0.408
Copper	645	12.7 (17.9)	15.0	3.9	0.220	0.697
Grand	211	17.6 (21.0)	8.5	6.5	0.273	0.302
Jesse	74	22.4 (17.9)	10.0	4.7	0.412	0.450
Lily	407	32.5 (37.4)	11.5	3.0	0.187	0.701
Montague Pond	1782	10.1 (13.9)	11.5	3.7	0.265	0.737
Tedford	115	17.7 (21.6)	9.0	4.7	0.246	0.453

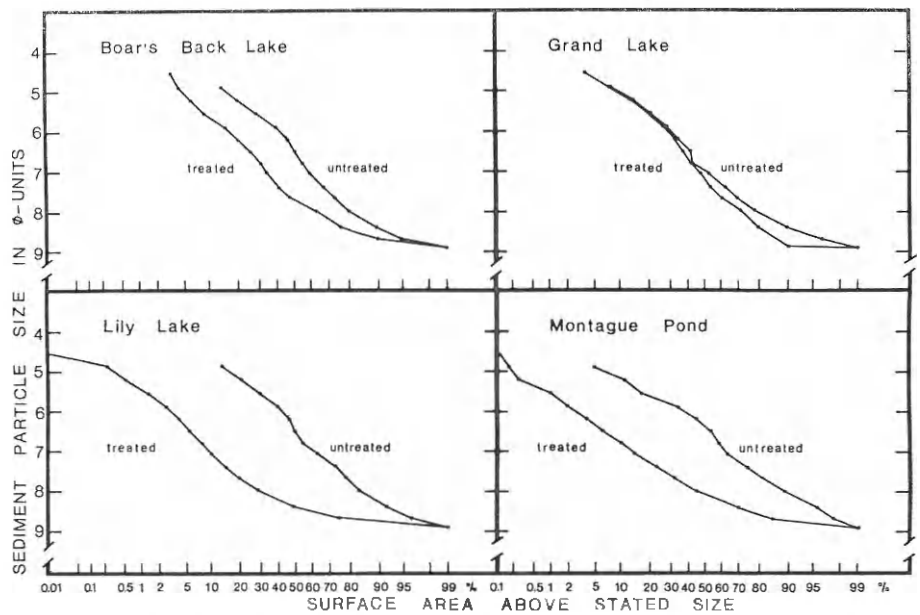

FIG. 3. Representative plots of size distribution in sediments from 4 of the 10 lakes sampled, both before and after removal of organic material by H_2O_2. Type and location of lake are as follows.

Boar's Back Lake	Dystrophic	Nova Scotia
Grand Lake	Oligotrophic	Nova Scotia
Lily Lake	Mesotrophic	Nova Scotia
Montague Pond	Eutrophic	Prince Edward Island

same sample to check reproducibility. The latter procedure was then extended to two different samples. This procedure provided reasonable assurance that the analytical results of counting were representative of those parts of the lakes concerned from which estimates of the bacterial population have previously been made. Untreated samples were examined in a similar manner. Table 2 provides an example of counts on such samples. Here the probable error is about 0.5 μ, the range in median values being on the average about three times higher than those of the treated samples.

Results of the sediment size analyses for all 10 lakes are presented in terms of surface area in Tables 4 (untreated) and 5 (treated). Median surface area sizes from these analyses are shown in Table 6 together with the percentage organic

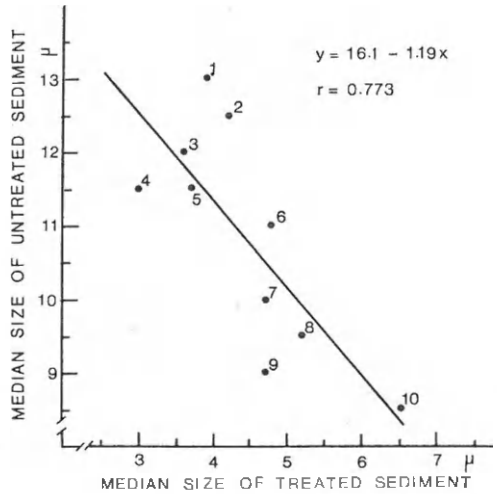

FIG. 4. Regression of the median size of sediment particles from 10 lakes, before vs. after removal of organic material with H_2O_2. 1. Copper Lake. 2. Amherst Pond. 3. Black Brook Lake. 4. Lily Lake. 5. Montague Pond. 6. Boar's Back Lake. 7. Lake Jesse. 8. Bluff Lake. 9. Tedford Lake. 10. Grand Lake. The absolute value of the correlation coefficient is shown. It is negative as indicated by the slope.

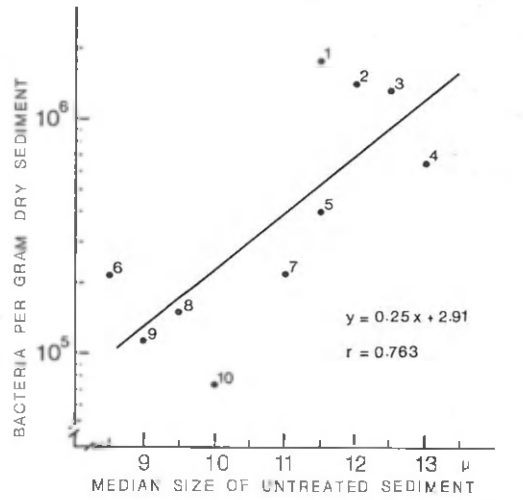

FIG. 5. Regression of median size of the particles of the sediment from 10 lakes versus the bacterial population of the sediment. 1. Montague Pond. 2. Black Brook. 3. Amherst Pond. 4. Copper Lake. 5. Lily Lake. 6. Grand Lake. 7. Boar's Back Lake. 8. Bluff Lake. 9. Tedford Lake. 10. Lake Jesse.

TABLE 7

Density of bacteria on sediment particle surface compared with similar measurements of others on sediments, soils, and pebbles

Sources	Bacteria/μg dry weight	Surface in mm²/μg of sediment		Bacteria/mm²	
		Untreated	Treated	Untreated	Treated
Present work					
Amherst Pond	3780	0.258	0.588	14700	6410
Copper Lake	2240	0.220	0.697	10200	3210
Grand Lake	1420	0.273	0.302	5180	4690
Jesse Lake	3730	0.412	0.450	9090	8260
Batoosingh (1964)					
Marine pebbles				16000	
Anderson and Meadows (1965)					
Marine sand				60000*	
Gray *et al.* (1968)					
Sand dune					
Horizon A_1	1850	0.722		2570	
Horizon C	2390	0.725		3300	

*Median of the published range of values.

content and the mean bacterial population. The present method of measuring the amount of organic material in the sediment was by a wet (chromic acid) ashing method. The values in parentheses derive from measurements of loss on ignition published by Hayes and Anthony (1959). The estimates of the bacterial population are from the same publication. They are based upon aerobic agar plate and filter culture (mainly the latter) counts of heterotrophic bacteria.

A regression of the median surface area values for the sediment samples, untreated versus treated, is shown in Fig. 4. The correlation coefficient ($r = -0.773, n = 10$) suggests a high level of significance for the relationship ($p < 0.01$). Figure 5 illustrates that a regression logarithm of the bacterial count versus median surface area of the untreated sediment particles has about the same level of significance ($r = 0.763, n = 10$). A similar regression involving the treated sediment samples did not show this level of correlation ($r = 0.576, n = 10$). There does not seem to be any simple relationship between the organic content of the sediment and either its bacterial population or its particle size.

We have attempted to estimate the density of the bacterial population in terms of the number bacteria per square millimeter and also the number of square microns of sediment surface available per bacterium. The results are shown in Table 7 and they are limited to the sediments of those four lakes for which direct counts of the bacteria were available. The assumption underlying the calculations and the manner in which they were made is explained in discussion. Estimates of surface area per microgram for both treated and untreated samples of sediment are recorded for all of the lakes in Table 6.

Discussion

Both sediments and soils have been widely subjected to particle size analysis, but aggregation of the component particles has been much more thoroughly studied in soils. Most soils are subject to periodic drying and are usually more thoroughly aerated than sediments. Soil-aggregating activities are promoted by drying and by the enhancement of oxidative processes through better aeration. Thus the fact that sediments are not so highly aggregated as soils is probably due primarily to the overlying water, which prevents drying and inhibits aeration.

Our method of preparing untreated sediment samples for particle size analysis is somewhat more vigorous than the wet sieving commonly used to estimate aggregation of soils; hence our results tend to underestimate comparatively the extent to which the sediments were aggregated. Conversely, those aggregates that we have observed may be said to be highly water-stable. The lake sediments we have studied not only contain water-stable aggregates, but they differ from one another in the degree of that aggregation.

Figure 3 shows that little or no aggregation of Grand Lake sediment was detected by Coulter Counter analysis, a surprising result in view of the distinctly granular appearance of its upper layers. As seen through the walls of glass coring devices, the individual grains (crumbs?) have an angularity reminiscent of coarser grades of grinding powders, but the sediment is much more colorful, showing various shades of gray, yellow, brown, and red. From its appearance, this sediment would be expected to feel gritty, but does not. Furthermore, if stirred gently for even a brief period, its appearance changes rapidly and takes on the smooth consistency of chocolate sauce.

The disparity between analysis and gross appearance of Grand Lake sediment leads us to three conclusions. First, the ultimate particles are much too small to account for the visible particles. Secondly, the visible particles failed to survive the preparation for counting the untreated samples. Thirdly, there are levels of aggregation in the sediments that we have failed to detect with the counter method of analysis. They may be regarded as unstable aggregations. We have no information on their nature, mode of formation, or importance to what may be called the physiology of the sediment. Nor do we have any estimate of the extent to which these unstable aggregates occurred in the other sediments we studied, except that it was at least below the visible level that attracted attention to the sediment of Grand Lake.

From his observations in marine environments, Riley (personal communication 1966) finds that the uppermost layers of sediment consist largely of organic aggregates similar to those he has observed in the overlying water. The chief difference between water and sediment in this respect is a greater concentration of aggregates in the sediment where they also contain more in-

organic (silt, clay) material. These observations indicate that some proportion of the aggregates in sediments arrive there as such. This view extends the site of aggregation throughout the overlying water. Sieburth (1965) has suggested a possible mechanism for the formation of sedimenting organic aggregates by bacteria that could account for the unstable aggregations in the upper layers of Grand Lake sediment.

Aggregation of soil particles results from complex activities of various components of the soil microflora (Griffiths 1965; Swaby 1949; Waksman 1952, Chap. 15). No pure culture or single group of organisms is as effective as a mixed flora in producing the level of aggregation commonly observed in a given soil. Fungi appear to be most effective in initiating the aggregation of soil particles, but the gums or cementing substances arising from bacterial activity may be more effective in producing water-stable aggregates (Geltser 1936, 1937; Martin 1945, 1946; Martin and Richards 1963; McCalla 1945; Sieburth 1965). The most rapid restoration of crumb structure in soil appears to be favored by the more readily decomposable substances, e.g. glucose. All of these observations emphasize the role of the heterotrophic component of the flora in aggregating processes. Consequently the association we have observed between the indirect counts of bacteria and sediment particle aggregation (Fig. 5) is not entirely unexpected.

Even though the heterotrophic microflora and their metabolic by-products may account for most of the biological aspect of soil or sediment aggregation, it does not follow necessarily that the particle-aggregating activity is limited to the more easily cultured and counted members of the flora. A comparison of the direct and indirect counts recorded here recalls the well-known fact that direct counts reveal a much larger microflora than is ever detected by culture methods. It is likely that many, if not most, of the additional organisms revealed by direct counts are also heterotrophic, but, regardless of their nutritional affinities, they well may share in the aggregating activities taking place in their environment.

In normal sedimentary environment, bacteria find themselves living amid an array of particles whose size varies quite widely in comparison to the dimensions of a bacterium. At one end of the range, we may think of bacteria associated with or adhering to sediment particles; at the other extreme, we may think of particles associated with or maybe even adhering to bacteria (Lahav 1962). These two relative situations may constitute quite different environments for members of the sediment flora.

Microorganisms adhering to sedimentary sand grains have recently been observed by Meadows and Anderson (1966) and somewhat similar observations for soil particles have been made by Seifert (1958), Zvyagintsev (1962), and Gray *et al.* (1968). Meadows and Anderson (1966) fixed and stained sand grains from both freshwater and marine environments. They found less difference in their observations between the two environments than between geographical locations within each environment. They found microorganisms were always present in aggregated or overdispersed patterns and concluded that this distribution reflected the heterogeneity of the underlying microhabitats.

Batoosingh (1964) examined the bacterial flora on the surface of marine pebbles by adapting Strugger's (1944) technique to observations via incident light microscopy in a manner similar to that of Zvyagintsev (1962). He observed the bacterial population on the pebbles to be surprisingly sparse, about 1 bacterium per 300 μ^2. He did observe colonies of bacteria on the pebbles, but they were few, widely separated, and rarely comprised of more than a dozen cells. Potter's (1964) observations suggest that the bacterial population on the surfaces of pebbles from freshwater environments may have the same low density. Although the largest particle in the present study was very much smaller than the smallest pebble examined by Batoosingh (1964), his observations enhanced our interests in the relationship between bacteria and the surface presented by the sediments they occupy.

We have attempted estimates of the bacterial density for the surfaces of the sediments from four of the lakes included in the present study for which we also have direct counts of bacteria. Since the counts of bacteria were based upon weight, and since the quantity of sediment submitted to size analysis was not accurately measured, we have had to assume a specific gravity for the sediment in order to proceed.

Taking Copper Lake sediment (treated) as an example, and referring to the discussion of Table 1 in methods, the total surface area and total volume of particles aspirated per count were

$9.48 \times 10^6\ \mu^2$ and $8.48 \times 10^6\ \mu^2$ respectively. If we assume for this sediment the specific gravity of 1.6 given by Kaye and Laby (1956) for sand, the weight of sediment is

$$(0.5 \times 0.5)/(1 \times 10^{12}) = 13.6 \times 10^{-6}\ \text{g}.$$

(This may be compared with the quantity expected if 0.5 g of sediment were diluted as described:

$$(0.5 \times 0.5)/(100 \times 200) = 12.5 \times 10^{-6}\ \text{g}).$$

Then the surface area per microgram of sediment is

$$(9.48 \times 10^6)/13.6 = 0.96 \times 10^6\ \mu^2/\text{g} = 0.696\ \text{mm}^2/\mu\text{g}.$$

The number of bacteria per microgram (direct counts, fluorescent microscopy) : 2240, giving $2240 \div 0.696 = 3210$ bacterium/mm^2, or about 1 bacterium per 311 μ^2.

It is likely that the assumptions behind the foregoing calculations are more applicable to treated samples of sediment, but both sets of results are shown in Table 7. It may be seen that present estimates run from roughly 3000 to 15 000 bacteria per square millimeter, depending upon the source of the sediment and whether or not it was treated. In other words, there is one bacterium for every 70 to 300 μ^2 of sediment surface. Considering the average size of the bacterial cell, this means that only a small fraction, about 0.2%, of the surface is colonized (Gray *et al.* 1968). These rather crude calculations lead to results that are indicative of what more adequate technique is beginning to reveal. They imply an intimate relationship between the flora and the surface of the sediment, but the extent to which the flora is attached or free in the interstices is unknown to us.

Although fluorescent microscopy was used in both the direct counts reported here and in those made by others to which we have referred (Batoosingh 1964; Gray *et al.* 1968; Zvyagintsev 1962), the latter counts were more truly "direct" than is usually meant by the term, since, in those instances, the bacteria were both stained (fluorochromed) and examined *in situ*, using incident illumination. The counts upon which we have based our present estimates were made via Strugger's (1944) technique whereby the sediment, or dilutions thereof, were suspended in fluorochrome and then examined in a Petroff-Hauser counting chamber by transmitted light.

The inverse relationship between the median size of the sediment particles before and after removal of the organic component from the sediment (Fig. 4) seems to imply that the smaller the particles of a sediment, the more readily they are assembled into crumbs. This may indicate some non-biological aggregation; the sorption reactions of clays come to mind. It may be recalled that the "background" count observed in the suspending fluid at levels below those routinely counted, i.e. near the lower limit for the aperture used, was actually reduced by adding a small amount of sediment. Presumably this indicated aggregation independent of biological activity. However, it is clear that the untreated samples tended to show greater aggregation and that those aggregations displayed considerable stability in surviving the counting procedure. We think this stability is conferred by the organic component of the sediment. If the soil analogy is not completely misleading, the production of the more binding fractions of the material stems from metabolic products of the associated microflora. Even if the initial aggregation of the very small particles of a sediment occurs independently of that flora, its activities subsequently confer upon these unions greater stability than would be observed in a sterile sediment.

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