THE ACCURACY OF THE PLATE COUNT OF SUSPENSIONS OF PURE CULTURES OF BACTERIA IN STERILE SOIL.

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THE ACCURACY OF THE PLATE COUNT OF SUSPENSIONS OF PURE CULTURES OF BACTERIA IN STERILE SOIL

INTRODUCTION AND HISTORICAL.

In Soil Microbiology some practical method of obtaining estimates of the individual populations in soils of various types is needed. The accurate estimation of numbers of microorganisms may be of value in the measurement of soil potentialities or the effect of soil treatment. Present available methods are used with little knowledge of their accuracy. There is not general agreement on a test to determine how much of the variation among replicates prepared from a single sample is due to random sampling and how much is due to characters inherent in the population.

The plate method has been the most popular means of counting organisms in soil or elsewhere since Koch's development of the liquefiable solid medium in 1881. Certain limitations of the method are recognized. The result obtained does not represent a count of the actual numbers in the original sample but only such organisms or clumps of organisms as grow to form colonies under the conditions provided. Many investigators have found numerous sources of error in the plating technique. In spite of the shortcomings of the method, a large part of the advance made in Bacteriology is based upon plate counts. Working with milk and cultures of the colon organism, Breed and Stocking (1) found that skilled analysts using proper technique usually make reasonably accurate estimates of the number of living bacteria in milk. More experience and improved practices result in more regular and supposedly more accurate counts.

One of the more recent approaches in testing the value of Bacteriological data is based upon statistical methods. Fisher, Thornton and MacKenzie (4) suggested the X^2 (Chi square) test for use on large numbers of counts of series of parallel plates. The object of this test is to determine whether the variation found in a series of replicate plates is due to random sampling. ~~from a normally distributed population.~~ Harmsen and Verweel (6) give a brief summary of the reasoning involved in the development of the use of the X^2 test.

In the test a value of X^2 appropriate to the Poisson distribution is worked out for each set of parallel plates by the equation

$$X^2 = \frac{S(x - \bar{x})^2}{\bar{x}}$$

where x is the number of colonies counted on a plate and $\bar{x}$ is the mean of the set. Fisher's table of X^2 gives the probability of the occurrence of given X^2 values in an infinite population for selected degrees of freedom. The P values range from .99 to .01. The class boundaries are the X^2 for these selected values of P. On the basis of one hundred samples, there should be one X^2 value with a P greater than .99, one between .99 and .98, three between .98 and .95, five between .95 and .90, etc., until finally one with a X^2 value less than .01. The X^2 values for class boundaries are chosen for degrees of freedom equal to one less than the number of plates in the series. The observed values are placed in their respective classes and the distribution compared with the theoretical or expected.

A goodness of fit test (3) is used to ascertain the extent of this agreement. The observed and theoretical values for each class in the distribution are set up. From the formula $\frac{(\text{actual} - \text{theoretical})^2}{\text{theoretical}}$ a value is calculated for each class. These are totalled and the probability of the occurrence of the final X^2 value, obtained in this manner, is determined by finding the corresponding P value. A final X^2 value with a P of .50 is accepted as indicating a perfect fit. A close agreement with the theoretical is accepted as indicating that the means of the series are reliable, and that the data give no reason for questioning the hypothesis tested. Fisher, Thornton and MacKenzie (4) believe that close agreement with the theoretical distribution is rare but possible, and that the conditions may be satisfied with simple flora or certain mixtures of organisms.

Harmsen and Verweel (6) carried out experiments with soil platings, making parallel sets of ten each. They state that their results for the total counts of bacteria and actinomycetes show too many high X^2 values. Technique was eliminated as the cause, since their results with starch disintegrating organisms, protein disintegrating organisms and actinomycetes alone show reasonably good conformity with the theoretical distribution. Next, they grouped their results on the basis of numbers on the plates and tested the X^2 distribution for these sets. An equally bad distribution resulted. As a last resort, this test was applied to similar data published from another laboratory. Material collected by Waksman (10) in 1920 - 21 was used and found to give a similar picture when submitted to the X^2 test. In unpublished results of work done in the Bacteriology Laboratory at the University of Manitoba, three sets of data were obtained. One represents the total count of bacteria and actinomycetes, the second the count of actinomycetes selected from the same plates after longer incubation, and the third the counts of fungi on a lower dilution of the same samples. The results obtained for the counts of bacteria and actinomycetes confirm the finding referred to above, while the counts for the fungi and for the actinomycetes alone agree very well with the theoretical distribution.

Wilson and Kullman (11) sought to overcome this difficulty, also found in plating pure cultures of rhizobia, by discarding a X^2 value over ten where the variation was due chiefly to one plate. In this way they obtained a very good fit. Another method used by the same investigators was to pour a set of five plates and eliminate one, resulting in a four plate set. Any plate showing marked deviation from the others was eliminated: otherwise the third plate counted was discarded arbitrarily. It is difficult to understand how this can be done without bias.

SCOPE OF PROBLEM.

From an examination of the literature it is evident that, before the means of replicate sets of bacterial counts may be used with confidence, it is necessary to determine the cause of the too frequent occurrence of high X^2 values in the reports referred to above.
Three points were selected for study.

1. It appeared proper to consider first whether the method of dilution and preparing the plates, as uded in this laboratory, gives reliable results when a pure culture of bacteria is used.

2. At the same time it seemed desirable to determine whether there is a change in accuracy when dilutions yielding high or low counts are used.

3. The effect of mixing two pure cultures of common soil organisms was suggested as the next logical procedure.

EXPERIMENT 1.

This experiment was designed to cover the first two points listed. It is known that non spore forming bacteria are more representative of the general types found in soil than the spore formers. Waksman (8) lists the common heterotrophic non spore formers as "Bact. fluorescens, Bact. caudatum, Bact. radiobacter, etc." Pseudomonas fluorescens was chosen as the test organism for this study. A stock culture was used. The culture was grown on agar slants for one to three days at 26° C. This time variation was used in order that the counts would not be influenced by growth phase phenomena.

In preparing the first dilution, sterile soil was added in an attempt to duplicate the effect of shaking the natural soil sample. There may be some physical or chemical condition in soil, not usually found in pure cultures from agar slants, that affects the count obtained. The dilution blanks were prepared so that after sterilizing there was approximately 245 ml. of water in the first and 99 ml. in the others.

To prepare one set of plates, a streak culture of Pseudomonas fluorescens was flooded with five ml. of sterile water, the growth scraped from the agar and the suspension added to a 245 ml. blank, after which twenty-five grams of sterile soil were introduced. This dilution was shaken for five minutes in an automatic shaker and dilutions made to 1:2,000,000 and 1:10,000,000. Four plates were made from each final dilution using one pipette for each dilution. About ten ml. of standard nutrient agar were added to each plate.

Nutrient agar was used for plating because of its general nature. Since Pseudomonas fluorescens normally is not a spreading type there was little need for a medium designed to control spreaders. Bottom spreaders developed sometimes when the agar was not added immediately after the delivery of the sample to the plate. When this occurred the entire set of four plates from each dilution of the sample was discarded, as it was impossible to secure an accurate count or estimate of the original number of organisms. Contamination was considered a reason for discarding sets of plates but excessive variation in numbers of colonies was not. The plates were incubated three to five days, the colonies counted and the count checked with a hand lens. At three days some of the colonies were missed with the unaided eye but were found when checked with the hand lens. An increase in the number of colonies was not noted when the longer period of incubation was used, but the count was made more readily at the end of the five day period.

RESULTS OF EXPERIMENT 1.

The results on the 1:2,000,000 dilution of the two hundred samples are given in table 1. Table 2 represents the results of the 1:10,000,000 dilution.

The figures were summarized by calculating the X^2 value of each set of four counts. The actual and theoretical distributions of the X^2 values are presented in histograms 1 and 2.

These results show definitely that there is very good agreement of the observed with the theoretical results when a pure culture of *Pseudomonas fluorescens* is used under the conditions of this experiment. The close agreement of the two final X^2 values in the goodness of fit test shows that, in so far as this statistical method is concerned, there is nothing to be gained by using dilutions yielding counts of one hundred or more colonies per plate.

Table 1

Counts of 1:2,000,000 Dilution of *Pseudomonas fluorescens*.

	plate 1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	χ^2
1.	278	254	276	285	273.25	538.75	1.9716
2.	288	306	329	311	308.50	853.00	2.7650
3.	293	315	307	334	312.25	878.75	2.8088
4.	296	254	223	230	250.75	3258.75	12.9960
5.	181	162	156	171	167.50	357.00	2.1313
6.	122	163	113	155	138.25	1794.75	12.9819
7.	102	100	106	107	103.75	32.75	.3157
8.	139	152	174	147	153.00	674.00	4.4052
9.	163	141	148	163	153.75	366.75	2.3854
10.	152	137	153	142	146.00	182.00	1.2466
11.	157	137	141	136	141.25	140.75	.9965
12.	191	196	178	193	189.50	189.00	.9974
13.	146	161	151	172	157.50	397.00	2.5206
14.	146	143	132	138	139.75	112.75	.8068
15.	138	132	146	141	139.25	102.75	.7379
16.	132	132	133	134	132.75	2.75	.0207
17.	132	148	131	146	139.25	242.75	1.7433
18.	169	152	179	162	165.50	389.00	2.3505
19.	167	156	169	189	170.25	566.75	3.3289
20.	146	168	178	165	164.25	536.75	3.2679
21.	125	142	139	151	139.25	348.75	2.5045
22.	147	169	168	163	161.75	310.75	1.9212
23.	163	169	211	158	175.25	1764.75	10.0699
24.	166	154	183	177	170.00	490.00	2.8824

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	X^2
25.	160	166	161	167	163.50	37.00	.2263
26.	182	173	192	199	186.50	389.00	2.0858
27.	148	177	222	201	187.00	3042.00	16.2674
28.	167	182	166	169	171.00	166.00	.9708
29.	195	174	196	179	186.00	374.00	2.0108
30.	201	211	215	222	212.25	203.75	.9600
31.	195	195	207	243	210.00	1548.00	7.3714
32.	196	194	203	216	202.25	296.75	1.4672
33.	179	179	195	206	189.75	522.75	2.7549
34.	191	182	189	198	190.00	130.00	.6842
35.	214	207	184	211	204.00	558.00	2.7353
36.	223	229	271	227	237.50	1506.00	6.3410
37.	178	209	230	223	210.00	1594.00	7.5905
38.	228	223	235	261	236.75	856.75	3.6188
39.	270	329	296	271	292.25	2192.75	7.5030
40.	244	251	259	276	257.50	569.00	2.2076
41.	227	218	237	247	232.25	470.75	2.0269
42.	263	307	272	302	286.00	1489.00	5.1972
43.	207	176	183	206	193.00	754.00	3.9067
44.	219	218	233	245	228.75	492.75	2.1541
45.	237	241	247	266	247.75	494.75	1.9970
46.	231	209	203	181	206.00	1268.00	6.1553
47.	213	252	243	254	240.50	1077.00	4.4782
48.	265	263	240	266	258.50	461.00	1.7834
49.	189	209	154	189	185.25	1568.75	8.4383
50.	279	239	244	238	250.00	1142.00	4.5680

	plate						
	1	2	3	4	$\bar{x}$	$S(x-\bar{x})^2$	X^2
51.	271	271	276	263	270.25	86.75	.3210
52.	221	237	273	236	241.75	1462.75	6.0510
53.	314	304	300	262	295.00	1556.00	5.2746
54.	208	206	211	222	211.75	152.75	.7214
55.	221	226	243	246	234.00	458.00	1.9573
56.	248	232	247	253	245.00	246.00	1.0041
57.	256	253	248	229	246.50	441.00	1.9470
58.	228	231	222	222	225.75	60.75	.2698
59.	184	196	185	178	185.75	168.75	.9085
60.	188	168	182	197	183.75	444.75	2.4204
61.	193	209	205	203	202.50	139.00	.6764
62.	181	173	192	181	181.75	182.75	1.0055
63.	233	231	225	202	222.75	608.75	2.7329
64.	221	228	241	188	219.50	1529.00	6.9658
65.	162	175	168	184	172.25	268.75	1.5602
66.	279	281	299	296	288.75	312.75	1.0831
67.	236	248	244	263	247.75	384.75	1.5530
68.	319	321	304	286	307.50	789.00	2.5658
69.	265	251	256	244	254.00	234.00	.9213
70.	255	263	233	235	246.50	659.00	2.6734
71.	242	225	252	213	233.00	906.00	3.8884
72.	174	197	180	210	187.75	1084.75	5.7776
73.	193	192	196	202	195.75	60.75	.3103
74.	256	285	264	265	267.50	457.00	1.7084
75.	229	242	238	263	243.00	622.00	2.5597

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	X^2
76.	322	307	306	331	316.50	441.00	1.3934
77.	269	261	286	283	274.75	416.75	1.5168
78.	186	246	221	197	212.50	2137.00	10.0565
79.	360	328	345	350	345.75	536.75	1.5524
80.	271	260	274	279	271.00	194.00	0.7159
81.	240	212	231	221	226.00	442.00	1.9558
82.	229	240	226	237	233.00	130.00	.5579
83.	264	298	303	300	291.25	1002.75	3.4429
84.	202	205	210	215	208.00	98.00	.4712
85.	325	320	322	313	320.00	78.00	.2438
86.	297	274	294	313	294.50	769.00	2.6112
87.	311	329	324	366	332.50	1669.00	5.0194
88.	283	330	301	288	300.50	1333.00	4.4359
89.	235	256	231	240	240.50	361.00	1.5010
90.	59	50	58	51	54.50	65.00	1.1927
91.	93	83	96	74	86.50	301.00	3.4798
92.	80	56	71	60	66.75	354.75	5.3146
93.	121	136	131	118	126.50	213.00	1.6838
94.	152	114	141	130	134.25	788.75	5.8752
95.	76	70	92	82	80.00	264.00	3.3000
96.	110	126	111	92	109.75	580.75	5.2916
97.	96	64	72	65	74.25	668.75	9.0067
98.	35	40	43	51	42.25	134.75	3.1893
99.	144	135	132	138	137.25	78.75	.5738
100.	148	132	127	130	134.25	264.75	1.9721

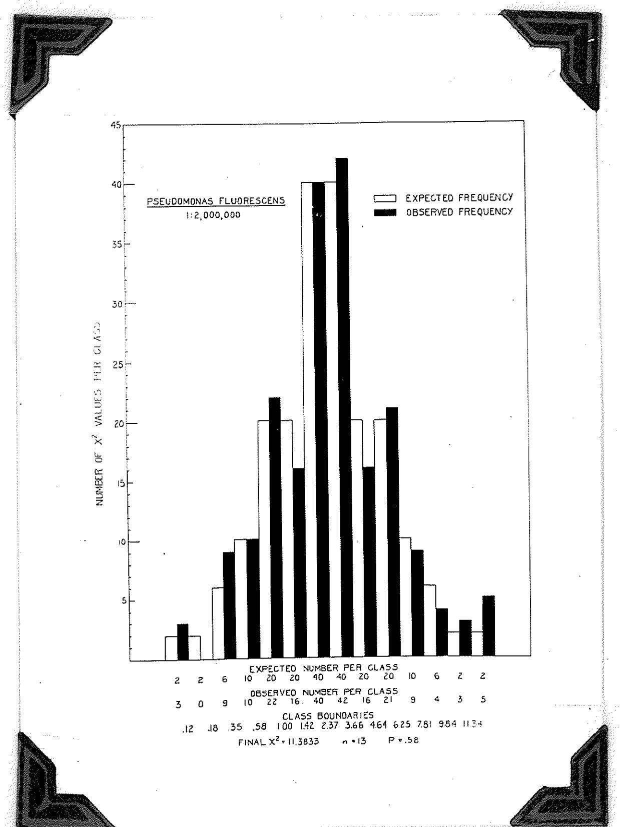
	plate						
	1	2	3	4	$\bar{\bar{x}}$	$S(x - \bar{x})^2$	X^2
101.	33	44	40	49	41.50	137.00	3.3012
102.	288	293	301	274	289.00	386.00	1.3356
103.	174	178	170	156	169.50	275.00	1.6224
104.	270	276	292	281	279.75	260.75	.9321
105.	103	133	120	132	122.00	586.00	4.8033
106.	242	244	216	264	241.50	1163.00	4.8157
107.	199	173	201	190	190.75	488.75	2.5622
108.	222	256	253	232	240.75	810.75	3.3676
109.	234	203	231	254	230.50	1321.00	5.7310
110.	229	237	251	253	242.50	395.00	1.6289
111.	246	226	217	215	224.50	389.00	1.7327
112.	228	206	233	220	221.75	416.75	1.8794
113.	253	228	263	265	252.25	866.75	3.4361
114.	121	112	114	104	112.75	146.75	1.3016
115.	112	127	108	114	115.25	202.75	1.7592
116.	117	94	116	134	115.25	806.75	7.0000
117.	112	109	102	99	105.50	109.00	1.0332
118.	96	79	96	85	89.00	214.00	2.4045
119.	92	105	100	81	94.50	329.00	3.4815
120.	72	69	61	58	64.00	130.00	2.0312
121.	81	88	88	82	84.75	42.75	.5044
122.	155	154	171	153	158.25	218.75	1.3823
123.	139	121	127	127	128.50	171.00	1.5307
124.	153	154	122	129	139.50	809.00	5.7993
125.	193	199	179	197	192.00	244.00	1.2708

	plate 1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	χ^2
126.	78	66	65	68	69.25	106.75	1.5415
127.	95	95	91	97	94.50	19.00	.2011
128.	90	94	67	78	82.25	448.75	5.4559
129.	81	99	82	97	89.75	275.75	3.0724
130.	78	58	63	77	69.00	302.00	4.3768
131.	123	143	116	112	123.50	569.00	4.6073
132.	105	125	126	134	122.50	457.00	3.7306
133.	89	92	119	97	99.25	552.75	5.5693
134.	72	91	84	101	87.00	446.00	5.1264
135.	87	131	103	92	103.25	1160.75	11.2421
136.	94	82	81	106	90.75	414.75	4.5702
137.	127	116	128	132	125.75	140.75	1.1193
138.	157	186	154	165	165.50	625.00	3.7764
139.	89	89	114	90	95.50	502.00	5.2565
140.	173	162	147	144	156.50	549.00	3.5080
141.	127	131	139	134	132.75	76.75	.5782
142.	184	164	159	167	168.50	353.00	2.0950
143.	126	124	121	132	125.75	64.75	.5149
144.	143	113	127	127	127.50	451.00	3.5372
145.	114	87	96	98	97.25	438.75	4.5116
146.	174	152	153	179	164.50	589.00	3.5805
147.	147	158	145	156	151.50	125.00	.8251
148.	251	265	259	256	257.75	102.75	.3986
149.	124	128	125	137	128.50	105.00	.8171
150.	71	92	73	78	78.50	269.00	3.4268

	plate 1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	χ^2
151.	72	80	81	81	78.50	57.00	.7261
152.	31	48	30	38	36.75	207.75	5.6531
153.	72	63	31	44	52.50	1025.00	19.5238
154.	53	40	48	47	47.00	86.00	1.8298
155.	64	51	65	70	62.50	197.00	3.1520
156.	30	55	41	59	46.25	530.75	11.4757
157.	23	41	30	34	32.00	170.00	5.3125
158.	43	50	45	36	43.50	101.00	2.3218
159.	53	55	52	48	52.00	26.00	.5000
160.	68	76	67	65	69.00	96.00	1.3913
161.	58	46	45	44	48.25	129.75	2.6891
162.	207	159	209	176	187.75	1786.75	9.5166
163.	63	95	78	85	80.25	542.75	6.7632
164.	184	161	187	185	179.25	448.75	2.5035
165.	192	183	186	185	186.50	45.00	.2413
166.	196	182	203	202	195.75	280.75	1.0664
167.	149	152	153	128	145.50	417.00	2.8660
168.	177	189	201	164	182.75	756.75	4.1409
169.	107	125	128	100	115.00	558.00	4.8522
170.	108	106	112	115	110.25	48.75	.4432
171.	125	136	119	123	125.75	158.75	1.2624
172.	112	96	118	136	115.50	819.00	7.0909
173.	173	163	188	176	175.00	318.00	1.8171
174.	58	65	61	67	62.75	48.75	.7769
175.	63	84	73	71	72.75	224.75	3.0893

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	χ^2
176.	53	61	58	56	57.00	34.00	.5965
177.	85	92	75	69	80.25	314.75	3.9221
178.	118	118	114	102	113.00	172.00	1.5221
179.	65	58	52	35	52.50	493.00	9.3905
180.	29	25	25	24	25.75	14.75	.5728
181.	47	59	47	51	51.00	96.00	1.8824
182.	79	84	77	74	78.50	53.00	.6752
183.	118	143	124	122	126.75	370.75	2.9250
184.	101	105	103	108	104.25	26.75	.2566
185.	91	83	72	94	85.00	290.00	3.4118
186.	129	132	120	117	124.50	153.00	1.3289
187.	166	129	148	145	147.00	690.00	4.6939
188.	138	150	150	125	140.75	426.75	3.0320
189.	127	135	133	117	128.00	196.00	1.5312
190.	134	156	142	123	138.75	578.75	4.1712
191.	119	149	130	131	132.25	462.75	3.4990
192.	108	111	146	121	121.50	893.00	7.3498
193.	141	143	143	146	143.25	12.75	.0887
194.	158	161	167	162	162.00	42.00	.2592
195.	143	156	156	154	152.25	116.75	.7668
196.	150	151	152	147	150.00	14.00	.0933
197.	149	148	170	149	154.00	342.00	2.2208
198.	141	163	159	143	151.50	371.00	2.4488
199.	136	104	116	124	120.00	544.00	4.5333
200.	108	117	106	116	111.75	95.00	.8085

HISTOGRAM 1.



PSEUDOMONAS FLUORESCENS
1:2,000,000
EXPECTED FREQUENCY
OBSERVED FREQUENCY
NUMBER OF X^2 VALUES PER CLASS
45
40
35
30
25
20
15
10
5
EXPECTED NUMBER PER CLASS
2 2 6 10 20 20 40 40 20 20 10 6 2 2
OBSERVED NUMBER PER CLASS
3 0 9 10 22 16 40 42 16 21 9 4 3 5
CLASS BOUNDARIES
.12 .18 .35 .58 1.00 1.42 2.37 3.66 4.64 6.25 7.81 9.84 11.34
FINAL X^2 = 11.3833 n = 13 P = .58

Table 2

Counts of 1:10,000,000 Dilution of Pseudomonas fluorescens.

	plate 1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	X^2
1.	43	44	36	37	40.00	50.00	1.2500
2.	45	52	32	41	42.50	209.00	4.9176
3.	43	36	31	46	39.00	138.00	3.5385
4.	46	32	52	51	45.25	254.75	5.6298
5.	28	38	39	45	37.50	149.00	3.9733
6.	16	24	18	21	19.75	36.75	1.8608
7.	40	27	35	41	35.75	122.75	3.4336
8.	35	34	41	31	35.25	52.75	1.4964
9.	42	35	33	31	35.25	68.75	1.9504
10.	36	42	45	28	37.75	168.75	4.4702
11.	19	37	41	34	32.75	276.75	8.4539
12.	29	48	35	53	41.25	372.75	9.0364
13.	42	50	46	37	43.75	92.75	2.1200
14.	34	34	38	41	36.75	34.75	.9456
15.	43	38	33	28	35.50	125.00	3.5211
16.	25	27	27	24	25.75	6.75	.2621
17.	20	20	21	26	21.75	24.75	1.1379
18.	27	02	18	15	15.50	321.00	20.7097
19.	44	41	37	42	41.00	26.00	.6341
20.	31	38	40	47	39.00	130.00	3.3333
21.	32	42	36	31	35.25	74.75	2.1206
22.	38	24	27	31	30.00	110.00	3.6667
23.	28	46	31	38	35.75	192.75	5.3916
24.	28	35	31	26	30.00	46.00	1.2778
25.	35	30	41	36	35.50	61.00	1.7183

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	χ^2
26.	24	36	40	35	33.75	140.75	4.1704
27.	31	38	39	41	37.25	56.75	1.5235
28.	51	43	52	41	46.75	92.75	1.9840
29.	30	25	40	32	31.75	116.75	3.6772
30.	34	36	37	37	36.00	6.00	.1667
31.	39	43	50	52	46.00	110.00	2.3913
32.	31	40	40	47	39.50	129.00	3.2658
33.	35	35	36	40	36.50	17.00	.4658
34.	46	33	33	48	40.00	198.00	4.9500
35.	57	47	42	46	48.00	122.00	2.5417
36.	41	42	47	53	45.75	90.75	1.9836
37.	35	38	47	56	44.00	270.00	6.1364
38.	37	71	55	45	52.00	644.00	12.3846
39.	60	49	43	57	52.25	178.75	3.4210
40.	43	52	46	54	48.75	78.75	1.6154
41.	73	47	51	62	58.25	410.75	7.0515
42.	43	39	51	38	42.75	104.75	2.4503
43.	44	38	48	41	42.75	54.75	1.2807
44.	33	44	58	52	46.75	350.75	7.5027
45.	40	40	46	41	41.75	24.75	.5928
46.	44	48	48	46	46.50	11.00	.2366
47.	42	13	56	31	35.50	989.00	27.8592
48.	39	53	44	35	42.75	100.75	2.3576
49.	48	53	81	62	61.00	634.00	10.3934
50.	55	54	55	55	54.75	.75	.0137

	plate 1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	χ^2
51.	22	37	47	41	36.75	340.75	9.2721
52.	59	54	51	51	53.75	42.75	.7953
53.	55	39	60	64	54.50	361.00	6.6239
54.	65	65	66	48	61.00	226.00	3.7049
55.	44	46	55	56	50.25	92.75	1.8458
56.	62	68	62	71	65.75	60.75	.9240
57.	43	44	56	40	45.75	148.75	3.2514
58.	53	51	48	54	51.50	21.00	.4078
59.	62	53	54	43	53.00	182.00	3.4340
60.	41	37	46	55	44.75	180.75	4.0391
61.	53	49	55	41	49.50	115.00	2.3232
62.	45	43	31	33	38.00	148.00	3.8947
63.	39	38	22	42	35.25	242.75	6.8865
64.	47	43	44	39	41.25	148.75	3.6061
65.	42	40	47	38	41.75	44.75	1.0719
66.	47	51	62	60	55.00	154.00	2.8000
67.	41	48	55	51	48.75	104.75	2.1487
68.	27	32	26	34	29.75	44.75	1.5042
69.	64	71	54	67	64.00	158.00	2.4688
70.	61	43	35	54	48.25	398.75	8.2642
71.	51	64	66	67	62.00	166.00	2.6774
72.	53	56	56	46	52.75	66.75	1.2654
73.	51	59	50	56	54.00	52.00	.9630
74.	49	40	45	43	44.25	42.75	.9503
75.	35	39	40	44	39.50	41.00	1.0380

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	x^2
76.	44	51	45	41	45.25	52.75	1.1657
77.	63	53	56	66	59.50	109.00	1.8319
78.	38	48	46	56	47.00	164.00	3.4894
79.	64	67	56	60	61.75	68.75	1.1134
80.	44	58	62	69	58.25	332.75	5.7124
81.	39	43	47	39	42.00	44.00	1.0476
82.	71	69	62	69	67.75	46.75	.6900
83.	61	63	60	61	61.25	4.75	.0661
84.	55	53	50	55	53.25	16.75	.3146
85.	36	35	28	61	40.00	626.00	15.6500
86.	70	58	59	73	65.00	173.00	2.6769
87.	56	46	42	44	47.00	116.00	2.4681
88.	67	44	63	60	58.50	305.00	5.2137
89.	60	58	60	47	56.25	116.75	2.0756
90.	63	77	69	67	69.00	104.00	1.5072
91.	66	64	69	55	63.50	109.00	1.7165
92.	53	60	47	57	54.25	94.75	1.7465
93.	12	10	09	13	11.00	10.00	0.9091
94.	24	18	20	23	21.25	22.75	1.0706
95.	20	14	15	23	18.00	54.00	3.0000
96.	31	26	34	31	30.50	33.00	1.0820
97.	30	33	36	34	33.25	18.75	.5639
98.	31	21	17	19	22.00	89.00	4.0455
99.	19	26	18	27	22.50	65.00	2.8889
100.	21	17	19	24	20.25	26.75	1.3210

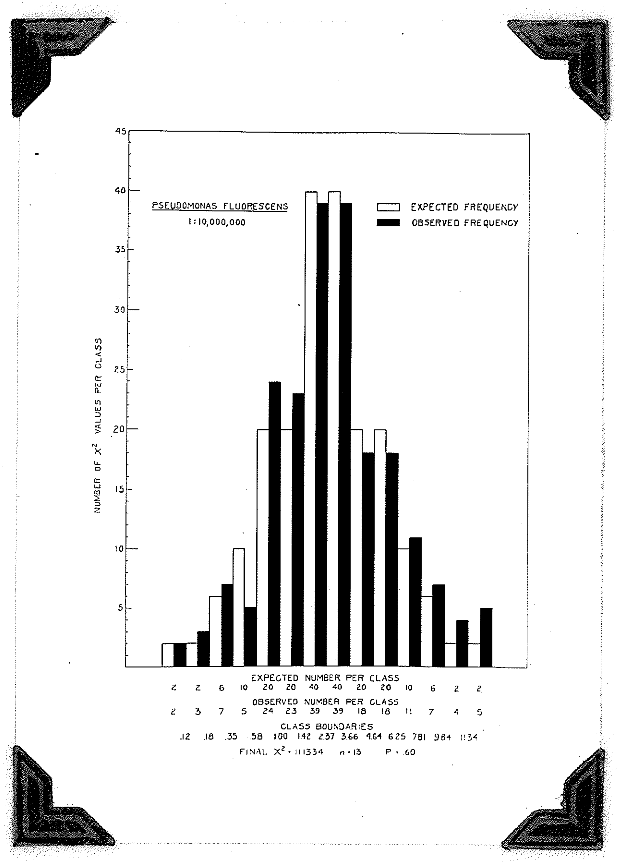
	plate 1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	x^2
101.	12	09	11	04	09.00	38.00	4.2222
102.	32	30	26	34	30.50	35.00	1.1476
103.	06	04	10	06	06.50	19.00	2.9231
104.	66	42	69	75	63.00	630.00	10.0000
105.	49	42	36	54	45.25	186.75	4.1271
106.	65	66	71	69	67.75	22.75	.3358
107.	25	30	23	28	26.50	29.00	1.0943
108.	57	34	55	58	51.00	390.00	7.6470
109.	35	44	44	36	39.75	72.75	1.8302
110.	47	42	49	48	46.50	29.00	.6237
111.	57	52	47	43	49.75	110.75	2.2261
112.	52	49	35	48	46.00	51.00	1.1100
113.	49	43	39	46	44.25	54.75	1.2373
114.	43	46	54	37	45.00	150.00	3.3333
115.	36	60	42	54	48.00	360.00	7.5000
116.	38	37	42	31	37.00	62.00	1.6757
117.	37	30	39	40	36.50	61.00	1.6712
118.	35	32	40	40	36.75	46.75	1.2721
119.	33	33	32	27	31.25	24.75	.7920
120.	37	28	38	32	33.50	57.00	1.7015
121.	37	34	41	22	33.50	201.00	6.0000
122.	23	23	23	15	21.00	48.00	2.2857
123.	32	28	34	32	31.50	19.00	.6032
124.	56	52	35	61	51.00	362.00	7.4902
125.	42	44	38	54	44.50	139.00	3.1236

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	X^2
126.	51	47	33	33	41.00	264.00	6.4390
127.	65	59	62	73	64.75	108.75	1.6795
128.	13	31	24	19	21.75	174.75	8.0345
129.	28	29	31	30	29.50	5.00	.1695
130.	39	28	27	24	29.50	129.00	4.3729
131.	30	34	28	35	31.75	32.75	1.0315
132.	36	26	19	17	24.50	221.00	9.0204
133.	43	37	37	42	39.75	30.75	.7736
134.	44	40	43	42	42.25	8.75	.2071
135.	32	31	23	49	33.75	359.25	10.6444
136.	41	33	28	35	34.25	86.75	2.5328
137.	31	27	34	35	31.75	38.750	1.2205
138.	39	33	46	34	38.00	106.00	2.7895
139.	44	49	31	33	39.25	225.25	5.7388
140.	42	44	44	58	47.00	164.00	3.4894
141.	32	34	46	42	38.50	131.25	3.4091
142.	48	53	48	57	51.50	57.00	1.1068
143.	44	46	44	55	47.25	82.75	1.7513
144.	60	55	59	62	59.00	26.00	.4407
145.	35	32	46	55	42.00	334.00	7.9524
146.	49	48	42	30	42.25	228.75	5.4142
147.	34	36	43	53	41.50	221.00	5.3253
148.	53	56	54	58	55.25	14.75	.2670
149.	48	57	50	61	54.00	110.00	2.0370
150.	85	79	78	82	81.00	30.00	.3704

	plate 1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	X^2
151.	48	41	50	48	46.75	46.75	1.0000
152.	23	30	20	16	22.25	104.75	4.7079
153.	25.	25	20	23	23.25	16.75	.7204
154.	13	05	12	17	11.75	74.75	6.3617
155.	22	17	16	24	19.75	44.75	2.2658
156.	16	13	05	12	11.50	65.00	5.6522
157.	18	23	22	21	21.00	14.00	.6667
158.	15	10	15	18	14.50	33.00	2.2759
159.	10	08	13	13	11.00	18.00	1.6364
160.	20	15	13	21	17.25	44.75	2.5942
161.	16	12	18	17	13.00	36.00	2.7692
162.	29	25	18	20	23.00	74.00	3.2174
163.	13	16	27	21	19.25	112.75	5.8572
164.	74	62	67	49	63.00	334.00	5.3016
165.	21	22	23	26	23.00	14.00	.6087
166.	54	54	63	56	56.75	54.75	.9648
167.	62	68	58	57	61.25	74.75	1.2204
168.	96	69	67	73	76.25	538.75	7.0557
169.	48	43	44	54	47.25	74.75	1.5820
170.	58	85	64	68	68.75	402.75	5.8582
171.	49	52	37	44	45.50	129.00	2.8352
172.	41	46	35	40	40.50	61.00	1.5062
173.	45	39	45	47	44.00	36.00	.8182
174.	41	42	38	38	39.75	12.75	.3208
175.	53	54	60	62	57.25	58.75	1.0262

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	X^2
176.	19	20	16	22	19.25	18.75	.9740
177.	22	15	20	19	19.00	26.00	1.3684
178.	28	27	22	18	23.75	64.75	2.7263
179.	29	30	34	27	30.00	26.00	.8667
180.	42	32	34	43	37.75	92.75	2.4570
181.	20	22	15	27	21.00	74.00	3.5238
182.	11	11	08	09	09.75	6.75	.6923
183.	15	19	12	18	16.00	30.00	1.8750
184.	29	27	29	27	28.00	4.00	.1429
185.	43	47	53	34	44.25	190.75	4.3107
186.	37	29	32	35	33.25	36.75	1.1053
187.	19	25	26	27	24.25	38.75	1.5979
188.	43	30	46	34	38.25	168.75	4.4118
189.	47	47	43	40	44.25	34.75	.7853
190.	36	46	51	42	43.75	120.75	2.7600
191.	38	65	51	50	51.00	366.00	7.1765
192.	53	38	49	45	46.25	122.75	2.6541
193.	45	48	35	53	45.25	172.75	3.8177
194.	48	46	44	57	48.75	98.75	2.0256
195.	41	45	52	55	48.25	122.75	2.5440
196.	50	47	41	43	45.25	48.75	1.0773
197.	44	56	43	44	46.75	114.75	2.4546
198.	51	56	45	64	54.00	194.00	3.5926
199.	40	48	66	71	56.25	644.75	11.4622
200.	42	48	61	49	50.00	190.00	3.8000

HISTOGRAM 2.


PSEUDOMONAS FLUORESCENS
1:10,000,000
EXPECTED FREQUENCY
OBSERVED FREQUENCY
NUMBER OF X^2 VALUES PER CLASS
45
40
35
30
25
20
15
10
5
EXPECTED NUMBER PER CLASS
2 2 6 10 20 20 40 40 20 20 10 6 2 2
OBSERVED NUMBER PER CLASS
2 3 7 5 24 23 39 39 18 18 11 7 4 5
CLASS BOUNDARIES
.12 .18 .35 .58 100 1.42 2.37 3.66 4.64 6.25 781 984 11.34
FINAL X^2 = 11.1334 n = 13 P = .60

EXPERIMENT 2

The second experiment deals with the effect of mixing Pseudomonas fluorescens with a pure culture of another common soil organism. Taylor and Lochhead (7), in following up work done by Conn and Darrow (2), reported that organisms of the type of Bacterium globiforme represent some ten percent of the organisms capable of being isolated by the plate method. This type of organism grows rapidly on nutrient agar. Dr. Lochhead very kindly provided a culture of Bacterium globiforme N. G.53.

A dilution of 1:10,000,000 was used, since the low counts have been shown to give equally satisfactory results and involve less work. In order to have results on the separate cultures used in the mixture, sets of plates of Pseudomonas fluorescens and of Bacterium globiforme were made at the same time and from the same dilutions as used in preparing the mixture. These might throw some light on the cause of the variation. One pipette was used for each dilution of each culture. One ml. of the final dilution of Pseudomonas fluorescens was placed in each of eight plates. One ml. aliquots of the final dilution of Bacterium globiforme were added to four of these and to four other plates. This resulted in a set of four plates each of Pseudomonas fluorescens, Bacterium globiforme and a mixture of the two. One hundred sets of twelve plates were prepared by this procedure.

RESULTS OF EXPERIMENT 2

The results of this experiment are shown in three tables. Table 3 presents the counts of Pseudomonas fluorescens, table 4 of Bacterium globiforme and table 5 of the mixture. The corresponding X^2 distributions are shown in histograms 3, 4, and 5, respectively.

These indicate that one hundred sets of parallel plates may be used to give an estimation of the distribution of the population. Obviously, the fit is rougher than that obtained when the two hundred sets are used. Again, the counts of *Pseudomonas fluorescens* and *Bacterium globiforme* and also those of the mixture give actual distributions of the X^2 values which indicate that the mean of four plates is reliable.

Table 3.
Counts of the 1:10,000,000 dilution of Pseudomonas fluorescens.

	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	X^2
1.	45	37	56	38	44.00	200.00	4.5454
2.	36	28	35	29	32.00	50.00	1.5625
3.	20	48	18	23	27.25	586.75	21.5321
4.	29	35	29	36	32.25	42.75	1.3256
5.	26	24	30	30	27.50	27.00	.9818
6.	29	26	17	33	26.25	128.75	4.9048
7.	23	26	38	31	29.50	129.00	4.3729
8.	14	18	09	11	13.00	46.00	3.3846
9.	16	10	18	15	14.75	34.75	2.3559
10.	23	17	14	20	18.50	45.00	2.4324
11.	21	23	15	22	20.25	38.75	1.9136
12.	09	12	12	07	10.00	18.00	1.8000
13.	16	16	16	11	14.75	18.75	1.2712
14.	21	34	39	37	32.75	196.75	6.0076
15.	38	40	34	41	38.25	28.75	.7516
16.	43	34	29	35	35.25	100.75	2.8582
17.	24	31	20	26	25.25	72.75	2.8812
18.	16	13	18	12	14.75	22.75	1.5424
19.	28	36	34	36	33.50	43.00	1.2836
20.	63	44	61	61	57.25	236.75	4.1354
21.	72	68	56	64	65.00	140.00	2.1538
22.	69	58	57	65	62.25	98.75	1.5863
23.	67	66	62	50	61.25	182.75	2.9837
24.	57	53	71	63	61.00	184.00	3.0164
25.	62	47	43	49	50.25	202.75	4.0348

	plate 1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	x^2
26.	70	67	73	84	73.50	165.00	2.2490
27.	68	87	84	64	75.75	392.75	5.1848
28.	77	82	86	86	82.75	54.75	.3616
29.	104	93	82	106	96.25	368.75	3.8312
30.	79	97	88	83	86.75	180.75	2.0836
31.	66	76	80	82	76.00	150.00	1.9727
32.	71	69	72	48	65.00	390.00	6.0000
33.	43	51	42	52	47.00	80.00	1.7021
34.	27	42	37	44	37.50	173.00	4.6133
35.	79	81	59	74	73.25	296.75	4.0512
36.	50	74	83	84	72.75	750.75	10.3196
37.	63	85	61	87	74.00	580.00	7.8378
38.	54	62	87	86	72.25	844.75	11.6920
39.	52	54	44	61	52.75	146.75	2.7820
40.	64	42	56	57	54.75	254.75	4.6530
41.	57	61	49	54	55.25	76.750	1.3891
42.	47	59	77	73	64.00	564.00	8.8125
43.	57	53	52	58	55.00	26.00	.4723
44.	42	55	36	43	44.00	190.00	4.3182
45.	29	44	33	34	35.00	122.00	3.4857
46.	49	54	67	69	59.75	286.75	4.7992
47.	56	57	58	54	56.25	8.75	.1556
48.	55	64	53	58	57.50	69.00	1.2000
49.	47	58	45	45	48.75	116.75	2.3949
50.	40	43	57	42	45.50	181.00	3.9780

	plate 1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	X^2
51.	51	45	50	49	48.75	20.75	.4256
52.	74	61	77	70	70.50	145.00	2.0567
53.	58	72	78	61	67.25	262.75	3.9071
54.	116	105	111	113	111.25	64.75	.5820
55.	58	71	65	74	67.00	150.00	2.2388
56.	90	81	85	96	88.00	126.00	1.4318
57.	66	64	56	67	63.25	74.25	1.1739
58.	67	40	58	60	56.25	396.75	7.0533
59.	42	44	47	49	45.50	29.00	.6374
60.	127	112	115	128	120.50	89.00	.7386
61.	126	108	107	91	108.00	614.00	5.6852
62.	49	43	38	41	42.75	64.75	1.5146
63.	40	41	46	38	34.75	41.25	.8424
64.	56	70	61	69	64.00	134.00	2.0938
65.	78	83	61	88	77.50	413.00	5.3290
66.	57	48	64	51	55.00	150.00	2.7273
67.	66	65	53	50	58.50	201.00	3.4359
68.	64	59	71	47	60.25	306.75	5.0913
69.	56	59	60	57	58.00	10.00	.1724
70.	52	43	51	54	50.00	70.00	1.4000
71.	73	68	71	64	69.00	46.00	.6667
72.	68	71	57	59	63.75	138.75	2.1765
73.	85	62	64	76	71.75	348.75	4.8606
74.	72	84	81	71	77.00	126.00	1.6364
75.	40	48	54	61	50.75	238.75	4.7044

	plate						
	1	2	3	4	$\bar{\bar{x}}$	$S(x - \bar{\bar{x}})^2$	X^2
76.	15	18	20	23	19.00	34.00	1.7895
77.	24	17	21	27	22.25	54.75	2.4607
78.	54	47	43	53	49.25	80.75	1.6396
79.	64	61	46	52	55.75	204.75	3.6726
80.	56	64	60	55	58.75	50.75	.8638
81.	35	38	46	42	40.25	68.75	1.7081
82.	37	52	42	36	41.75	160.75	3.8503
83.	71	52	55	67	61.25	252.75	4.1265
84.	63	56	68	63	62.50	73.00	1.1680
85.	46	63	54	57	55.00	150.00	2.7273
86.	47	51	49	57	51.00	56.00	1.0980
87.	47	64	58	56	56.25	148.75	2.6444
88.	46	45	27	36	38.50	237.00	6.1558
89.	56	63	58	59	59.00	26.00	.4407
90.	51	61	67	60	59.75	130.75	2.1883
91.	67	73	78	68	71.50	77.00	1.0769
92.	66	60	66	70	65.50	51.00	.7786
93.	58	76	77	75	71.50	247.00	3.4545
94.	58	66	54	64	60.50	30.50	.5041
95.	40	37	40	43	40.00	18.00	.4500
96.	29	28	41	35	33.25	108.75	3.2707
97.	53	62	41	54	52.50	225.00	4.2857
98.	49	49	46	44	47.00	18.00	.3830
99.	68	60	71	69	67.00	70.00	1.0448
100.	54	58	62	56	57.50	35.00	.6087.

HISTOGRAM 3.

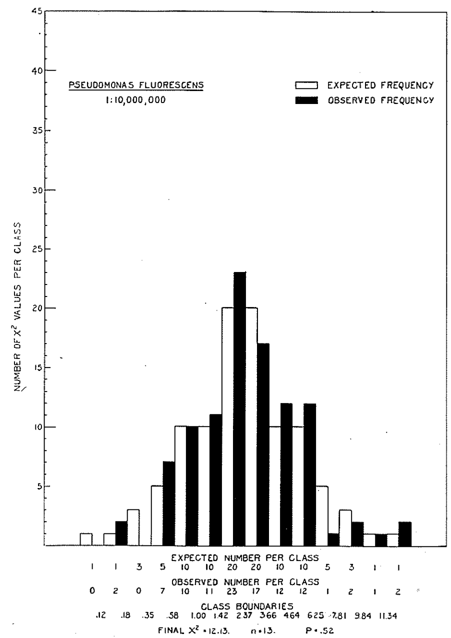

PSEUDOMONAS FLUORESCENS
1:10,000,000
EXPECTED FREQUENCY
OBSERVED FREQUENCY
NUMBER OF X² VALUES PER CLASS
45 40 35 30 25 20 15 10 5
EXPECTED NUMBER PER CLASS
1 1 3 5 10 10 20 20 10 10 5 3 1 1
OBSERVED NUMBER PER CLASS
0 2 0 7 10 11 23 17 12 12 1 2 1 2
CLASS BOUNDARIES
.12 .18 .35 .58 1.00 1.42 2.37 3.66 4.64 6.25 7.81 9.84 11.34
FINAL X² = 12.13. n = 13. P = .52

Table 4.

Counts on the 1:10,000,000 dilution of Bacterium globiforme.

	plate 1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	X^2
1.	73	76	93	85	81.75	246.75	3.0183
2.	63	53	52	44	53.00	182.00	3.4340
3.	11	20	18	17	16.50	45.00	2.7273
4.	38	28	25	36	31.75	116.75	3.6772
5.	13	16	11	17	14.25	22.75	1.5965
6.	21	14	11	16	15.50	53.00	3.4194
7.	23	25	19	20	21.75	22.75	1.0460
8.	21	29	21	28	24.75	56.75	2.2929
9.	19	21	20	19	19.75	2.75	.1392
10.	38	27	25	33	30.75	104.75	3.4065
11.	42	47	42	44	43.75	16.75	.3828
12.	35	21	32	30	29.50	109.00	3.6949
13.	25	29	29	21	26.00	44.00	1.6923
14.	06	05	05	08	06.00	.25	.0417
15.	19	16	11	13	14.75	36.75	2.4942
16.	03	11	09	04	06.75	44.75	6.6296
17.	25	22	23	20	22.50	13.00	.5778
18.	11	07	08	08	08.50	9.00	1.0588
19.	14	15	14	15	14.50	1.00	.0690
20.	60	57	54	55	56.50	21.00	.3717
21.	63	60	54	58	58.75	42.75	.7276
22.	57	68	65	71	65.25	108.76	1.6667
23.	55	48	54	51	52.00	30.00	.5769
24.	60	59	64	69	63.00	62.00	.9841
25.	52	63	58	53	56.50	133.50	2.3628

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	X^2
26.	71	62	72	68	68.25	60.75	.8901
27.	77	96	87	82	85.50	197.00	2.3041
28.	85	68	68	64	71.25	262.75	3.6877
29.	79	88	81	86	83.50	53.00	.6347
30.	84	96	88	77	86.25	188.75	2.1884
31.	79	78	83	81	80.25	14.75	.1838
32.	65	58	51	51	56.25	134.75	2.3956
33.	34	27	25	25	27.75	54.75	1.9730
34.	62	63	71	55	62.75	128.75	2.0518
35.	38	35	42	30	36.25	76.75	2.1172
36.	41	36	45	53	43.75	154.75	3.5375
37.	25	22	14	18	19.75	68.75	3.4810
38.	19	25	27	23	23.50	35.00	1.4894
39.	78	121	108	105	103.00	978.00	9.4951
40.	81	88	105	86	90.00	326.00	3.6222
41.	94	87	92	114	96.75	422.75	4.3695
42.	101	104	87	86	94.50	261.00	2.7619
43.	57	69	88	87	75.25	672.75	8.9402
44.	79	86	80	82	81.75	28.75	.3517
45.	88	72	74	89	80.75	242.75	3.0062
46.	85	64	72	73	73.50	225.00	3.0612
47.	84	71	78	82	79.00	106.00	1.3418
48.	78	97	75	86	84.00	290.00	3.4524
49.	105	81	89	75	87.50	507.00	5.7943
50.	82	76	77	79	78.50	21.000	.2675

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	X^2
51.	83	68	71	77	74.75	132.75	1.7759
52.	46	32	38	34	37.50	115.00	3.0667
53.	39	28	28	41	34.00	146.00	4.2941
54.	79	65	55	82	70.25	474.75	6.7580
55.	42	46	45	54	46.75	78.75	1.6845
56.	83	76	67	85	77.75	198.75	2.5563
57.	63	50	49	64	56.50	197.00	3.4867
58.	72	79	83	81	78.75	68.75	.8730
59.	81	87	85	78	82.75	48.75	.5891
60.	93	78	79	81	82.75	144.75	1.7492
61.	95	76	87	84	85.50	185.00	2.1637
62.	58	42	59	43	50.50	257.00	5.0811
63.	53	52	46	50	50.25	28.75	.5721
64.	133	114	134	105	121.50	617.50	5.0782
65.	121	118	140	132	127.75	308.75	2.4168
66.	220	164	191	202	194.25	1648.75	8.4878
67.	167	182	191	174	178.50	321.00	1.7983
68.	127	124	128	149	132.00	394.00	2.9848
69.	172	155	160	131	154.50	889.00	5.7540
70.	163	172	166	154	163.75	168.75	1.0305
71.	167	156	149	146	154.50	261.00	1.6893
72.	134	147	153	149	145.75	202.75	1.3911
73.	149	161	157	188	163.75	858.75	5.2443
74.	165	174	166	148	163.25	358.75	2.1975
75.	38	46	43	34	40.25	84.75	2.1056

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	χ^2
76.	23	15	19	19	19.00	32.00	1.6842
77.	22	25	30	26	25.75	32.75	1.2718
78.	48	43	38	37	41.50	77.00	1.8554
79.	40	45	36	41	40.50	41.00	1.0133
80.	12	16	10	11	12.25	20.75	1.6939
81.	21	27	26	25	25.00	22.00	.8800
82.	22	25	19	22	22.00	18.00	.8182
83.	38	33	33	33	34.25	18.75	.5474
84.	40	50	37	39	41.50	101.00	2.4337
85.	118	129	107	117	117.75	242.75	2.0616
86.	122	117	92	95	106.50	693.00	6.5070
87.	147	155	139	162	150.75	296.75	1.9685
88.	79	78	85	93	83.75	142.75	1.7045
89.	135	116	124	136	127.75	272.75	2.1350
90.	144	122	134	118	129.50	419.00	3.2355
91.	112	113	116	113	113.50	9.00	.0793
92.	103	121	127	105	114.00	420.00	3.6842
93.	101	119	92	121	108.25	594.75	5.4942
94.	136	137	129	129	132.75	56.75	.4275
95.	85	86	97	82	87.50	129.00	1.4743
96.	97	102	86	88	93.25	170.75	1.8311
97.	99	108	94	96	99.25	114.75	1.1562
98.	96	104	79	88	91.75	344.75	3.7575
99.	58	60	67	69	63.50	85.00	1.3386
100.	106	107	77	128	104.50	1317.00	12.6029

HISTOGRAM 4.

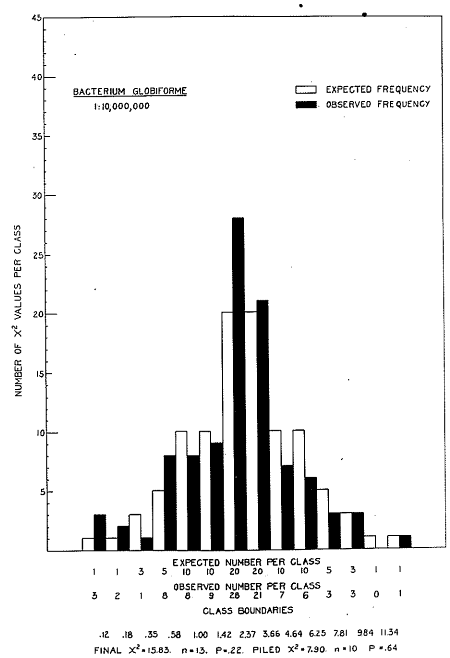

BACTERIUM GLOBIFORME
1:10,000,000
EXPECTED FREQUENCY
OBSERVED FREQUENCY
NUMBER OF X² VALUES PER CLASS
45
40
35
30
25
20
15
10
5
EXPECTED NUMBER PER CLASS
1 1 3 5 10 10 20 20 10 10 5 3 1 1
OBSERVED NUMBER PER CLASS
3 2 1 8 8 9 28 21 7 6 3 3 0 1
CLASS BOUNDARIES
.12 .18 .35 .58 1.00 1.42 2.37 3.66 4.64 6.25 7.81 9.84 11.34
FINAL X² = 15.83. n = 13. P = .22. PILED X² = 7.90. n = 10 P = .64

Table 5
Counts on 1:10,000,000 Dilution of the Mixture.

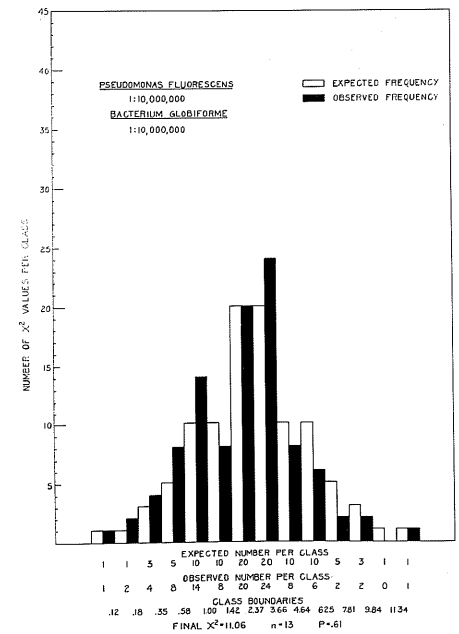
	plate 1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	X^2
1.	114	141	107	126	122.00	666.00	5.4590
2.	83	76	86	98	85.75	252.75	2.9475
3.	49	31	32	30	35.50	245.00	6.9014
4.	55	56	66	59	59.00	74.00	1.2542
5.	50	35	30	41	39.00	222.00	5.6923
6.	39	34	34	43	37.50	57.00	1.5200
7.	48	51	47	56	50.50	49.00	.9703
8.	37	48	35	39	39.75	98.75	2.4842
9.	37	26	29	41	33.25	144.75	4.3534
10.	47	39	34	49	42.25	146.75	3.4734
11.	76	71	73	67	71.75	42.75	.5958
12.	37	35	51	43	41.50	155.00	3.7349
13.	38	56	39	42	43.75	208.75	4.7714
14.	41	36	45	37	39.75	50.75	1.2767
15.	47	53	52	50	50.50	21.00	.4158
16.	40	42	39	47	42.00	38.00	.9048
17.	36	36	51	52	43.75	240.75	5.5028
18.	22	25	14	18	19.75	68.75	3.4810
19.	61	49	59	69	59.50	203.00	3.4118
20.	124	116	121	119	120.00	34.00	.2833
21.	132	124	125	147	132.00	338.00	2.5606
22.	141	145	137	142	141.25	128.25	.9080
23.	113	114	107	108	110.50	37.00	.3348
24.	135	139	94	92	115.00	1946.00	16.9217
25.	115	128	120	116	119.75	104.75	.8747

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	x^2
26.	149	143	146	144	145.50	21.00	.1443
27.	162	159	193	171	171.25	708.75	4.1387
28.	161	169	167	177	168.50	131.00	.7774
29.	172	154	155	167	162.00	238.00	1.4691
30.	181	185	165	184	178.75	260.75	1.4587
31.	177	156	155	164	163.00	310.00	1.9018
32.	113	131	143	141	132.00	564.00	4.2727
33.	88	77	89	90	86.00	110.00	1.2791
34.	135	123	118	117	123.25	205.75	1.6694
35.	106	113	96	111	106.50	173.00	1.6244
36.	147	131	148	146	143.00	194.00	1.3566
37.	96	98	93	77	91.00	274.00	3.0110
38.	97	96	101	108	100.50	89.00	.8856
39.	140	116	135	117	127.00	454.00	3.5748
40.	141	152	145	144	145.50	65.00	.4467
41.	153	144	141	123	140.25	475.75	3.3922
42.	136	157	140	159	148.00	410.00	2.7703
43.	135	159	137	161	148.00	580.00	3.9189
44.	132	121	129	132	128.50	81.00	.6304
45.	135	159	137	161	148.00	580.00	3.9189
46.	121	132	111	135	124.75	360.75	2.8918
47.	134	133	156	139	140.50	341.00	2.4270
48.	152	136	143	131	140.50	249.00	1.4875
49.	144	119	147	142	138.00	494.00	3.5797
50.	125	131	120	134	127.50	117.00	.9176

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	χ^2
51.	121	102	118	124	116.25	288.75	2.4839
52.	86	85	94	99	91.00	134.00	1.4725
53.	100	95	98	93	96.50	29.00	.3005
54.	158	181	162	167	167.00	302.00	1.8084
55.	112	110	101	114	109.25	98.75	.9059
56.	169	164	157	146	159.00	298.00	1.8742
57.	115	112	113	115	113.75	6.75	.0593
58.	161	142	142	155	150.00	274.00	1.8267
59.	164	169	188	181	175.50	361.00	2.0570
60.	185	199	195	196	193.75	110.75	.5716
61.	202	214	187	215	204.50	513.00	2.5086
62.	114	108	92	105	104.75	258.75	2.4702
63.	93	81	92	99	91.25	168.75	1.8493
64.	182	164	193	197	184.00	654.00	3.5543
65.	172	167	180	206	181.25	902.75	4.9807
66.	220	221	249	227	229.25	548.75	2.3937
67.	209	226	223	202	215.00	390.00	1.8140
68.	189	179	172	176	179.00	158.00	.8827
69.	234	209	209	223	218.75	440.75	2.0148
70.	237	221	212	215	221.25	372.75	1.6847
71.	224	236	243	247	237.50	305.00	1.2842
72.	221	246	222	208	224.25	752.75	3.3567
73.	231	240	229	238	234.50	85.00	.3625
74.	237	241	230	237	236.25	62.75	.2656
75.	87	89	88	80	86.00	50.00	.5814

	plate						
	1	2	3	4	$\bar{x}$	$S(x - \bar{x})^2$	χ^2
76.	42	44	38	43	41.75	20.75	.4970
77.	39	39	50	43	42.75	80.75	1.8889
78.	93	82	83	93	87.75	110.75	1.2621
79.	82	101	95	92	92.50	189.00	2.0432
80.	50	68	57	62	59.25	174.75	2.9494
81.	77	69	66	89	75.25	316.75	4.2093
82.	99	66	71	74	77.50	649.00	8.3742
83.	91	112	98	109	102.50	285.00	2.7805
84.	96	93	107	98	98.50	109.00	1.1066
85.	178	153	155	171	164.25	446.75	2.7199
86.	161	165	171	183	170.00	276.00	1.6235
87.	198	208	194	198	197.25	194.75	.9873
88.	127	148	138	137	137.50	221.00	1.6073
89.	183	206	210	174	193.25	918.75	4.7542
90.	192	209	186	212	199.75	484.75	2.4268
91.	191	194	204	201	197.50	109.00	.5519
92.	189	186	186	192	188.25	24.75	.1315
93.	173	171	167	159	167.50	115.00	.6866
94.	197	206	200	186	197.25	210.75	1.0684
95.	117	147	127	134	131.25	476.75	3.6324
96.	95	134	123	122	118.50	825.00	6.9620
97.	140	141	145	152	144.50	89.00	.6159
98.	143	133	141	135	138.00	68.00	.4928
99.	114	144	150	112	130.00	1176.00	9.0461
100.	178	159	158	143	159.50	617.00	3.8683

HISTOGRAM 5.


PSEUDOMONAS FLUORESCENS
1:10,000,000
BACTERIUM GLOBIFORME
1:10,000,000
EXPECTED FREQUENCY
OBSERVED FREQUENCY
NUMBER OF X² VALUES PER CLASS
45
40
35
30
25
20
15
10
5
EXPECTED NUMBER PER CLASS
1 1 3 5 10 10 20 20 10 10 5 3 1 1
OBSERVED NUMBER PER CLASS
1 2 4 8 14 8 20 24 8 6 2 2 0 1
CLASS BOUNDARIES
.12 .18 .35 .58 1.00 1.42 2.37 3.66 4.64 6.25 7.81 9.84 11.34
FINAL X²=11.06 n=13 P=.61

DISCUSSION OF RESULTS

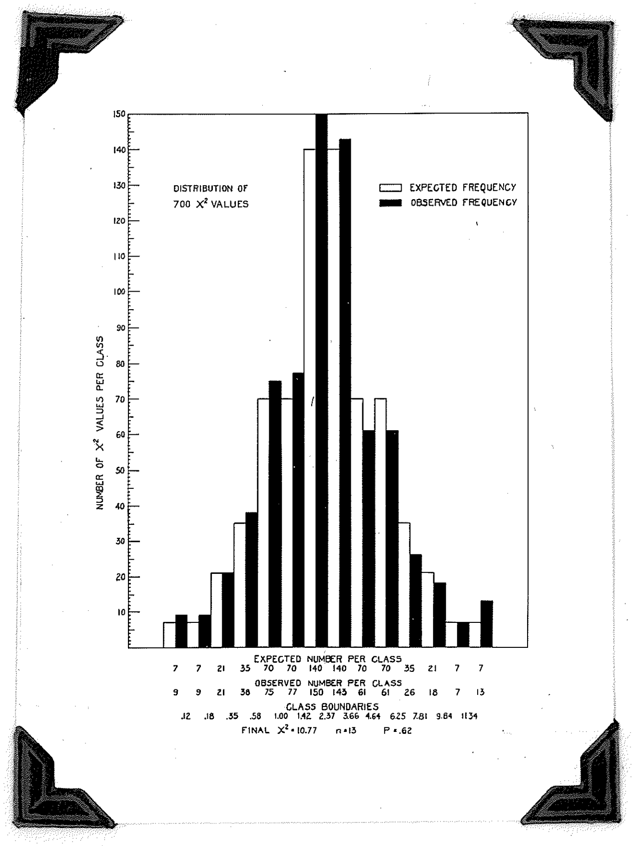
In fitting the observed to the theoretical distribution, one degree of freedom was taken arbitrarily as absorbed in the fitting. (11) This resulted in a P value of between .70 and .50 in all except the one hundred platings with Bacterium globiforme. In the goodness of fit test small theoretical and observed values make too large a contribution to the final X^2 value in proportion to their real significance. This is the reason one may group values of less than five in the theoretical distribution. Piling the values at the ends of the histogram to give theoretical frequencies of five in the case of Bacterium globiforme brought the final X^2 value within the .70 to .50 range.

The X^2 distributions in all the histograms show a reasonably good fit as determined by the goodness of fit test. This involves the acceptance of a P value within certain definite limits. Fisher (3) states that a range of values from .90 to .10 may be expected without giving reason to question the hypothesis being tested. A P value outside these limits may be taken as indicating that the hypothesis does not account for all the factors involved.

To test the hypothesis that all the samples were drawn from a normally distributed population, a distribution of the seven hundred X^2 values obtained in these two experiments was made and compared with the theoretical. These are presented in histogram number 6. The goodness of fit test as applied to this set shows a closer agreement with the theoretical than any of the separate sets of data.

These results indicate that the samples were taken from a normally distributed population. Any variations are the result of random sampling rather than serious error in the technique of plating and counting.

HISTOGRAM 6.


DISTRIBUTION OF
700 X² VALUES
EXPECTED FREQUENCY
OBSERVED FREQUENCY
NUMBER OF X² VALUES PER CLASS
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
EXPECTED NUMBER PER CLASS
7 7 21 35 70 70 140 140 70 70 35 21 7 7
OBSERVED NUMBER PER CLASS
9 9 21 38 75 77 150 143 61 61 26 18 7 13
CLASS BOUNDARIES
.12 .18 .35 .58 1.00 1.42 2.37 3.66 4.64 6.25 7.81 9.84 11.34
FINAL X² = 10.77 n = 13 P = .62

Consequently, the mean of four replicate plates may be accepted as giving a reasonable estimate of the populations sampled. This confirms the opinion expressed by Fisher, Thornton and MacKenzie (4) referred to in the introduction.

Further, the data presented under Experiment 1. show that the final X^2 value in the goodness of fit test on the 1:10,000,000 dilution was 11.1334, which gives a P value of .60. These counts ranged from twenty-five to seventy-five colonies per plate. The 1:2,000,000 dilutions from the same sources gave a final X^2 value of 11.3833 and a P of .58. In this case the counts were three to five times as high as in the former test, involving proportionately more work. As these results are so much alike there appears to be no sound reason for using a dilution yielding higher counts.

The two cultures used in the mixture give no indication of a disturbing factor as shown by the X^2 test. The average of the one hundred sets of the mixture is 126.54 colonies per plate while the sum of the separate platings averages 125.06. Since there is no apparent associative effect in the results when the two cultures were plated together, the discrepancies observed in soil platings are due to other factors than technique. Antagonism and mutual stimulation are known phenomena capable of causing considerable variation in the counts. There are a great many types of organisms in the soil. It is possible that one plate may have a single strongly antagonistic organism which reduces the number of colonies near it. Similarly, in the same set there may be evidences of mutual benefit, possibly from products diffusing into the medium and forming additional food supplies that enable other organisms to develop. This would increase the plate count. One or both of these factors may cause unusual variations in a set of replicates and give high X^2 values. Waksman (9) in 1937 published a very full resume of the literature on Associations of Microorganisms. The effects of specific microorganisms on others have been determined in a rough way. Nevertheless, much work remains to be done.

Various investigators interpret the pronounced deviations from the X^2 distribution as indicating that the mean may be wholly unreliable. There is general agreement among workers in this field that sets of replicate bacterial counts direct from soil yield too many high X^2 values. This would seem to indicate the need of accounting for these deviations from the expected rather than discarding the method as unreliable. A considerable part of this variation may be removed by using a dilution giving counts of about fifty colonies per plate as shown by data collected in this laboratory in 1937. In this way the organisms are spaced far enough apart to minimize the associative effects, with no loss of accuracy as shown by the results of Experiment 1. There still remains a great deal of variation to be accounted for.

In the light of the better X^2 distribution of counts of bacteria and actinomycetes direct from soil as a result of lower numbers on a plate, it is evident that the effect of specific microorganisms on the metabolism of others is an important factor in producing excessive variation in sets of replicates. Changed variation in replicates may result from associative effects. The normal variation may be determined for each species of bacteria by plating pure cultures. A difference found when plating mixtures of two pure cultures of known behaviour may be a measure of the effect of their association.

Variations in series of parallel plates may be caused by the composition of the medium used. Each medium is selective for definite organisms and populations of these organisms may not be distributed normally in the soil. Fisher, Thornton and MacKenzie presented data which suggests that subnormal variance in sets of replicates may be due to the use of a faulty medium. Harmsen and Verweel attempted to use the distribution of X^2 values as an indication of the defects in the medium in trials with yeast and soil extract agar. Their results are promising. It is desirable to develop a test by means of which the relative merits of different media may be tested.

The results presented in this paper show clearly that reasonably careful and standardized technique with a pure culture produces a mean of four plates that is reliable. Accordingly, one may begin to look for factors which cause variation, rather than attempt to refine the technique to a point where its application on a large scale is definitely impracticable.

Standard methods are needed in Soil Microbiology. There is no object in choosing the methods by preference alone. Some sound method of determining the relative value of each practice should be available.

CONCLUSIONS.

1. The technique used in plating the normal soil flora on a large scale produces a mean of four replicate counts that appears reliable as an index of the population sampled.

2. Plates yielding counts varying between twenty-five and seventy-five are as reliable, as judged by the X^2 test, as counts of three to five times as large, and result from much less labor.

3. Discrepancies in the X^2 distributions as obtained by other observers are due probably to other causes than technique. It is suggested that antagonisms or stimulations among organisms, or some inherent character peculiar to certain types of organisms, may make proper separation and distribution difficult under practical conditions.

4. When a pure culture of easily separated bacteria in a soil and water dilution is used, or a mixture of two non-antagonistic species, there is no question about practical plating technique producing an actual distribution of X^2 values that agrees reasonably well with the theoretical.

SUMMARY.

1. A pure culture of *Pseudomonas fluorescens* was mixed with sterile soil and plated in dilutions 1:2,000,000 and 1;10,000,000.

2. This was repeated two hundred times and the data were submitted to the X^2 test.

3. The distribution of the X^2 values in each set of two hundred samples agrees very well with the theoretical distribution.

4. There appeared to be little difference in the reliability of the means of the dilutions yielding high and low counts. On this assumption the following experiment was carried out using the higher dilution only, resulting in more readily counted plates.

5. One hundred series of *Bacterium globiforme* were plated along with one hundred of *Pseudomonas fluorescens* and one hundred of a mixture of the two.

6. The distribution of the X^2 values in each set is such that they might be considered to have been derived from a normally distributed population.

7. By distributing the X^2 values for the seven hundred samples a better fit results than when each set is considered alone.

8. This may be taken as additional proof that the samples were drawn from a normally distributed population.

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