ON THE BACTERICIDAL ACTION OF COPPER.

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EARLY in 1904, Moore and Kellerman[1] published the results of their studies of the effect of copper salts on the growth of algæ and bacteria, claiming that minute quantities of copper were sufficient to prevent the development of many troublesome algæ in water supplies, and that a large percentage of the bacteria, including all of the pathogenic bacteria, were destroyed as the result of such treatment. In addition, these observers claimed that storage of a few hours in a copper vessel would effectually free any water from pathogenic bacteria.

Appreciating that a method, apparently so simple, would soon be brought into practical application, and realizing that many factors concerning its safety and efficiency yet remained to be worked out, experiments were begun by the Massachusetts State Board of Health to investigate thoroughly all phases of the subject as applied to the treatment of Massachusetts waters. In the course of this work, much information has been acquired as to the action of copper on the bacterial contents of waters of different kinds, and it is with this phase of the problem that the present paper treats.

The experimental data fall naturally under two heads: (1) experiments in which waters were treated with definite amounts of copper as copper sulphate; and (2) experiments in which the water was placed in contact with metallic copper and allowed to absorb an unknown amount of copper. Each of these two lines of investigation may be subdivided as follows:

a) Effect on the total numbers of bacteria in the water.

b) Effect on the numbers of B. coli naturally present in polluted waters.

c) Effect on the numbers of B. coli added to water in the form of laboratory cultures.

d) Effect on the numbers of B. typhosus added to water in the form of laboratory cultures.

[1] *U. S. Dept. Agri. Bureau Plant Industry, Bull. No. 64*, 1904.

In addition, experiments have been made to compare the action of other salts frequently used in water purification, such as ferrous sulphate and aluminum sulphate, with copper sulphate, and also to compare the effect of other metals with that of metallic copper.

To the sanitarian the question arises: Does copper in dilute solution destroy the bacteria completely, and do the same laws which apply to the common water bacteria apply equally when dealing with the bacillus of typhoid fever? As we have frequently pointed out, tests made with laboratory cultures of B. typhosus, while they have a certain value, are not conclusive evidence as to what would be the behavior of typhoid organisms which have come from fecal matter and have become accustomed to life in water.

The difficulty of isolating typhoid from mixed cultures in water, and the preponderance of other germs in sewage-polluted water, rendered it necessary to attack our problem in a roundabout manner. The method which has given the most reliable information at Lawrence, in investigations of this character, has been to study the relative viability of different laboratory cultures of both B. typhosus and B. coli, to study the viability of the colon bacillus in a natural state under similar conditions, and from the two sets of experiments to draw inferences as to the viability of the typhoid bacillus in nature under similar conditions.

The weak point in the conclusions of Moore and Kellerman with regard to the destruction of typhoid by copper is that they were drawn from analyses in which the largest amount of water tested was 1 c.c., and the usual amount tested was less than .01 c.c. It is generally conceded, especially when dealing with laboratory cultures, that the great majority of the typhoid bacilli are quickly destroyed by conditions unfavorable to their growth. It has also been repeatedly shown that a few germs are much more resistant than the majority, and may survive even under the most unfavorable conditions for many days. All epidemiological evidence points to the conclusion that the germs which are able to live under unfavorable conditions are also extremely pathogenic, and that, while it may help to destroy the majority of the bacilli, no method of sterilizing water is thoroughly effective unless it will accomplish the destruction of the especially resistant individuals.

It is unsafe to conclude that because a certain species of bacteria, especially a pathogen like B. typhosus, is not found in a loopful of the water, or even in 1 c.c., that there is no danger from the use of that water. The average drinking-glass holds about 300 c.c., and until repeated tests of volumes as large as 100 c.c. have been made and the germ proved to be absent, the water under observation cannot safely be said to be free from the test forms.

EXPERIMENTAL METHODS AND EXPRESSION OF RESULTS.

In experiments where the water was treated with metallic salts, the water was first drawn in bulk, carefully mixed and sampled, then divided into portions of uniform size, one of which, the control, was carried through untreated, the others being treated with varying amounts of copper sulphate. The containers in all cases were of glass, and the samples were in every case kept in the dark at room temperature. Daily bacterial analyses were made of the contents of the various bottles in each experiment, and in a few experiments a number of analyses were made during the first 24 hours. In every case the contents of the bottles were shaken thoroughly before samples were removed for analysis, thus insuring a fair sample. The volume of water used in the different experiments varied considerably; in some experiments only 100 c.c. of water were placed in each bottle, in others 1,000 c.c. were used, and in a few experiments 3,000 c.c. was the volume treated.

In the experiments in which the waters have been exposed to metallic copper, about 15 liters of water were used in every case, except in the experiments in which a number of metals were under comparison, in which case the volume of water used was about 1,000 c.c.

The containers in the metal experiments have in some cases been of copper, with the control in enameled ware or in glazed stoneware, and in others the waters have been placed in glass and the metals inserted as thin sheets.

In the experiments where the metal was in the form of the container, or was inserted as sheets, it was impossible to shake the contents, and stirring was inadvisable, since it was likely to cause abrasions in the metal and affect the normal rate of solution of the

metal by the water. In these experiments we have resorted to such mixing as could be produced by blowing strongly through the pipettes at the time of sampling. Samples collected under such circumstances are not as representative as would have been the case had a thorough shaking occurred, and some of the variations in such experiments may be attributed to this imperfect sampling.

The determination of the numbers of bacteria and of B. coli in raw waters has been made by the regular "Lawrence" methods. In dealing with sterile waters seeded with laboratory cultures of B. coli and B. typhosus, counts were made on agar plates incubated 18 hours at body temperature, and tests of larger volumes than 1 c.c. were made by mixing broth with the water, incubating at body temperature, and, in case a growth was obtained, by identifying the test organisms by the usual cultural tests. In the following tables the sign + indicates that there was no growth on plates, but that the organism was proved to be present by qualitative tests.

A number of different methods of expressing the copper content of waters have been used in recent publications, the most common being the expression of the ratio of copper sulphate to water by weight. While this method of expression is satisfactory in speaking of treatment with copper sulphate, it is rather out of place in speaking of the amount of copper absorbed in the metallic or colloidal state. As copper sulphate is dissociated in dilute solutions, and as it is the copper *ion* which is apparently the germicide, it is more convenient to express our results in parts of copper per 100,000 parts of water, especially when we wish to compare copper sulphate treatment with the metallic copper treatment. This method of expression has been used throughout in the tables, although in some cases both methods of expression have been used in the text. The following equivalent weights of the metals and of the metallic salts are given for comparison:

Copper sulphate ($CuSo_45H_2O$): Copper (Cu)=1:0.253
Ferrous sulphate ($FeSO_47H_2O$): Iron (Fe)=1:0.201
Aluminum sulphate ($Al_2(SO_4)_5$): Aluminum (Al)=1:0.158

EXPERIMENTS WITH COPPER SULPHATE.

In the following digest of experiments the various bottles in each experiment are designated by the amount of copper added:

Experiment 159.—Merrimac River water. Copper range 0.000253 to 253. Duration 10 days. The bacteria in 0.000253 and 0.00253 act like the control. In 0.0253 and 0.253 the bacteria increased. In 2.53 and 25.3 all but a few of the bacteria were killed, these few remaining throughout the experiment. In 253 the bacteria were all destroyed. The bacterial results are shown in Table 1.

TABLE 1.

MERRIMAC RIVER WATER.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.000253	0.00253	0.0253	0.253	2.53	25.3	253
Start	3,800	.....	.....	.....	.....	..	..	..
1 day	....	10,000	22,400	340,800	30	9	3	0
2 days	....	4,800	16,000	357,000	88,600	9	0	0
3 "	....	2,500	11,800	300,000	103,000	10	6	1
4 "	....	3,600	9,700	315,000	157,500	12	2	0
6 "	....	1,100	2,400	10,300	79,200	27	0	0
8 "	....	1,800	3,500	7,500	97,500	11	2	0
10 "	....	410	870	4,700	118,800	9	8	0

Experiment 163.—Merrimac River water containing 1 per cent of sewage Range 0.000253 to 253. Duration 20 days. The numbers of bacteria in the control were higher in 24 hours than they were at the start, but decreased slowly during the experiment. The bacteria in 0.000253, 0.00253, and 0.253 were nearly all destroyed in 24 hours, but the few remaining increased to large numbers in the course of three or four days. In 0.0253 the bacteria increased immediately and then decreased slowly. In 2.53 and 25.3 nearly all of the bacteria were destroyed, but the few remaining were alive during 20 days. In 253 all of the bacteria were destroyed in two days. The bacterial results are shown in Table 2.

TABLE 2.

MERRIMAC RIVER WATER TO WHICH WAS ADDED 1 PER CENT OF LAWRENCE SEWAGE.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.000253	0.00253	0.0253	0.253	2.53	25.3	253
Start	46 000	.....	.....	.....	.....	..	..	..
1 day	69,000	37	8	333,000	80	20	8	3
2 days	41,500	19	13	404,500	4,200	6	9	0
3 "	12,500	65	34	610,000	3,900	26	23	0
4 "	13,700	447,300	125,700	138,500	4,400	28	35	1
6 "	7,400	590,000	55	83,100	130,800	14	3	0
8 "	8,000	71,000	18,500	220,000	85,200	20	16	0
10 "	4,200	37,350	21	56,200	55,500	15	8	0
13 "	4,400	25,600	23	33,000	15,600	14	12	0
20 "	1,400	100	15	1,700	3,300	7	8	1

Experiment 210.—Merrimac River water containing $\frac{1}{10}$ of 1 per cent of sewage. Duration 131 days. Range 0.000253 to 2.53. The bacteria in the control increased at first and then decreased slowly until the 116th day, when a slight secondary increase was noted. 0.000253, 0.00253, and 0.0253 all increased largely during the first two or

TABLE 3.

MERRIMAC RIVER WATER TO WHICH WAS ADDED 0.1 PER CENT OF LAWRENCE SEWAGE.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000					
	Control	0.000253	0.00253	0.0253	0.253	2.53
Start	17,000	.....	.....	.....	.....	....
1 day	72,500	18,500	115,000	132,000	800	150
2 days	64,800	68,400	87,800	87,800	7,000	18
4 "	35,000	5,500	24,300	175,000	372,800	65
5 "	10,700	12,300	32,600	223,200	458,900	65
6 "	17,500	56,500	57,500	162,000	581,400	100
7 "	12,000	15,200	50,500	90,000	151,200	100
8 "	2,200	4,800	7,300	19,500	75,600	50
11 "	11,000	13,300	7,300	35,500	266,300	24
13 "	8,900	4,700	4,600	14,000	532,500	25
15 "	8,000	7,400	3,000	7,500	450,000	2,600
18 "	6,200	5,800	5,100	30,000	770,000	2,400
21 "	3,300	2,400	2,650	9,100	320,000	550
25 "	600	800	900	5,500	18,400	33
32 "	390	450	1,600	4,500	194,400	120
39 "	190	135	180	1,700	210,000	30
46 "	110	210	205	830	11,400	475
53 "	95	475	250	700	10,500	240
60 "	65	90	65	300	8,850	75
67 "	75	90	160	140	4,500	9
74 "	170	85	210	100	1,425	4
81 "	21	275	275	24	480	4
88 "	140	1,100	725	190	175	35
95 "	120	2,700	1,100	530	500	9
103 "	38	75	210	55	230	1
109 "	55	160	350	300	275	5
116 "	1,100	120	5,000	150	325	7
123 "	1,100	750	2,350	90	180	27
131 "	500	400	1,100	400	550	1

TABLE 4.

MERRIMAC RIVER WATER TO WHICH WAS ADDED 0.1 PER CENT OF LAWRENCE SEWAGE.

(B. coli per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000					
	Control	0.000253	0.00253	0.0253	0.253	2.53
Start	70	..	..	..	..	..
1 day	31	45	32	28	17	10
2 days	2	3	2	0	0	0
4 "	20	7	10	101	13	23
5 "	15	2	17	6	0	0
6 "	43	56	48	47	34	42
7 "	12	11	0	9	2	0
8 "	7	7	8	0	0	50
11 "	12	30	5	3	18	0
13 "	+	3	0	2	4	3
15 "	10	+	0	30	7	6
18 "	0	17	27	41	10	16
21 "	0	10	11	5	13	13
25 "	8	18	25	26	8	11
32 "	0	12	16	3	16	6
39 "	3	0	10	4	0	1
46 "	3	0	3	14	20	14
53 "	0	1	0	5	2	1
60 "	4	1	0	5	0	8
67 "	0	0	7	0	5	3
74 "	2	1	0	0	0	1
81 "	0	0	0	0	0	0
88 "	4	3	2	0	18	5
95 "	1	0	0	0	0	1
103 "	0	0	0	0	0	1

three days and then decreased slowly. In 0.000253 and 0.00253 a secondary increase in the numbers of bacteria was noted on the 88th and 95th days respectively, but no secondary increase of any importance occurred in 0.0253. In 0.0253 the numbers were considerably reduced in 24 hours, but immediately increased to large numbers on the fifth day, and then slowly declined. In 2.53 over 90 per cent of the bacteria were destroyed in 24 hours, and these numbers remained small and gradually decreased throughout the experiment, with the exception of one small secondary increase which occurred from the 15th to the 18th day.

The B. coli in all of the bottles fluctuated considerably, but decreased gradually throughout the experiment, and were found in small numbers up to the 95th day and 103d day, with the exception of 0.253, from which they disappeared on the 67th day. Tests in volumes larger than 1 c.c. were not made. The bacterial results are shown in Table 3, and the B. coli results in Table 4.

Experiment 193.—Tap water containing 3 per cent of sewage. Duration 187 days. Range 0.0000253 to 25.3. The bacteria in the control increased until the second day and then slowly decreased during 96 days, when a large secondary increase occurred, lasting through the 145th day, when a decrease again started. In 0.0000253, 0.000253, and 0.00253 the bacteria all follow much the same laws as the

TABLE 5.

LAWRENCE CITY WATER TO WHICH WAS ADDED 3 PER CENT OF LAWRENCE SEWAGE.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.0000253	0.000253	0.00253	0.0253	0.253	2.53	25.3
Start	96,400	.....	.....	.....	.....	.....	.....	....
½ hour	93,000	76,400	92,600	44,400	51,000	4,800	24,200	4,600
1 "	76,000	123,000	92,600	73,200	50,000	2,800	7,700	3,100
2 hours	106,500	84,200	110,800	60,200	31,000	1,420	6,200	530
4 "	92,300	84,000	132,100	73,000	13,200	350	730	130
6 "	84,400	95,000	140,600	84,400	11,000	210	300	80
8 "	86,400	75,600	114,500	72,500	8,800	150	175	70
24 "	1,000,000	1,120,000	1,150,000	1,150,000	1,500,000	20	2	1
2 days	1,100,000	1,400,000	795,200	284,000	1,930,000	220,000	2	0
3 "	108,000	370,000	6,400	324,000	1,420,000	990,000	2	3
5 "	13,400	44,700	32,200	22,600	1,020,000	1,500,000	5	0
7 "	7,500	11,200	16,500	270,000	475,200	276,500	43	5
13 "	2,300	4,300	1,000	4,600	4,100	2,360,000	2,400	4
16 "	2,400	5,500	5,800	5,400	19,500	73,400	6,600	5
21 "	800	4,600	4,800	4,000	11,500	33,800	13,200	1
26 "	3,100	4,800	7,200	6,200	12,800	47,000	32,000	5
33 "	800	6,500	2,000	3,300	8,500	26,500	15	6
40 "	500	6,000	1,250	1,700	5,350	15,500	6	0
47 "	3,400	7,700	4,600	2,500	7,600	11,800	1,080	4
55 "	480	3,700	2,600	1,200	2,400	162,000	6,800	3
61 "	230	185	200	370	440	210,000	4	7
68 "	190	650	390	110	850	.....	4	4
75 "	225	325	510	160	1,850	150,000	3	3
82 "	300	310	550	350	600	88,600	2	0
89 "	750	4,700	500	225	275	.....	0	0
96 "	300	2,200	250	1,150	510	6,500	0	0
103 "	29,100	20,000	740	1,700	660	41,000	0	0
110 "	75,000	3,200	1,500	650	270	37,500	0	0
117 "	60,000	3,800	475	3,400	300	40,000	0	0
124 "	28,600	10,400	590	1,200	240	25,000	0	0
131 "	22,600	1,300	880	600	300	7,900	0	0
145 "	504,000	5,600	660	725	650	33,500	0	0
160 "	650	1,800	1,100	650	57	8,000	0	0
173 "	6,600	30,900	3,500	5,300	2,000	47,200	0	0
187 "	1,600	2,750	700	300	70	5,900	0	0

control both as to increase, gradual decrease, and a secondary increase after about 100 days. In 0.0253 a primary increase and decrease of bacteria followed the normal curve, only a slight secondary increase being noted. In 0.253 over 99 per cent of the bacteria had disappeared in 24 hours, but these increased to large numbers during the succeeding week and then slowly increased throughout the experiment. In 2.53 the bacteria decreased over 99 per cent in 24 hours, and the numbers remained practically constant until the seventh day, when an increase started which lasted about two weeks, the numbers fluctuating and gradually falling off until the water became sterile on the 89th day. In 25.3 practically all of the bacteria were destroyed in 24 hours, small numbers being occasionally found until the 82d day, when the water became sterile. The bacterial results are shown in Table 5.

Experiment 176.—Water from a stagnant reservoir. Duration three days. Range 0.000253 to a.0253. The bacteria in the control and in 0.000253 decreased, while in 0.00253 and 0.0253 they increased, the greatest increase being noted in the bottle containing the most copper. The bacterial results are shown in Table 6.

TABLE 6.

WATER FROM STAGNANT RESERVOIR.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000			
	Control	0.000253	0.00253	0.0253
Start	120	...	...	...
1 day	110	210	400	700
2 days	34	55	1,900	19,000
3 "	13	31	1,800	12,400

Experiment 177.—Water from a stagnant reservoir. Duration 12 days. Range 0.00253 to 0.0253. The bacteria in all of the bottles followed the same law, increasing rapidly during the first two or three days and then decreasing slowly. The bacterial results are shown in Table 7.

TABLE 7.

WATER FROM STAGNANT RESERVOIR.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000			
	Control	0.00253	0.00506	0.0253
Start	3,900	.....	.....	.....
1 day	29,400	22.000	24,500	24,500
2 days	16,900	22,900	90,200	144,000
3 "	10,100	16,400	40,000	165,600
5 "	6,800	4,100	21,000	10,500
7 "	5,000	4,700	4,000	6,900
9 "	4,200	10,500	32,500	90,000
12 "	270	420	3,600	2,500

Experiment 179.—Water from a stagnant reservoir. Duration eight days. Range 0.00253 to 2.53. The bacteria in the control fluctuated somewhat, but decreased throughout the experiment. In 0.00253 the numbers decreased during the first six

days and then showed an increase on the eighth day. In 0.0253 the numbers decreased during the first two days and then increased largely, the same being true with 0.253. In 2.53 over 90 per cent of the bacteria were destroyed in 24 hours, and continued to decrease to the sixth day, a large secondary increase being noted on the eighth day. The bacterial results are shown in Table 8.

TABLE 8.

WATER FROM STAGNANT RESERVOIR.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000				
	Control	0.00253	0.0253	0.253	2.53
Start	300	....	.....	.....	.....
1 day	35	255	100	135	21
2 days	45	400	36	28	9
4 "	700	300	113,600	97,200	1
6 "	80	75	10,600	1,800	2
8 "	245	4,100	52,000	1,900	2,300

Experiment 187.—Water from a stagnant reservoir. Duration 12 days. Range 0.00253 to 2.53. The bacteria in the control increased slowly throughout the experiment. In 0.00253 they increased during the first three days, and then fluctuated, but decreased throughout the remainder of the experiment. In 0.0253 over 90 per cent of the bacteria were killed in 24 hours, but the remainder increased rapidly throughout the experiment. In 0.253 the bacteria decreased during the first two days and then increased largely until the fifth day, a decrease following throughout the experiment. In 2.53 over 95 per cent of the bacteria were killed in 24 hours, the rest remaining practically constant, with the usual fluctuations. The bacterial results are shown in Table 9.

TABLE 9.

WATER FROM STAGNANT RESERVOIR.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000				
	Control	0.00253	0.0253	0.253	2.53
Start	265	.....	.....	.....	..
1 day	230	210	16	95	9
2 days	1,500	2,500	1,300	65	0
3 "	425	11,000	10,400	23,200	2
4 "	1,260	720	38,800	442,800	0
5 "	1,118	7,200	48,400	630,000	..
7 "	4,500	700	1,900	241,900	3
9 "	8,900	865	51,000	75,600	5
12 "	15,500	3,200	24,000	240,000	8

Experiment 185.—Driven well water from Methuen town supply. Duration 133 days. Range 0.000253 to 0.253. There were only 31 bacteria in this water at the start. In all the samples a large increase was noted in 24 to 48 hours. The numbers in the control, 0.000253, and 0.00253 remained practically constant after 48 hours during 45 days, after which they began to decrease slowly. In 0.0253 a considerable decrease

occurred about the 84th day, the numbers remaining low after that time. In 0.253 the numbers were below 100 on the 31st day and continued low, with the exception of one count throughout the experiment. The bacterial results are shown in Table 10.

TABLE 10.

DRIVEN WELL WATER FROM METHUEN TOWN SUPPLY.

(Bacteria per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000				
	Control	0.000253	0.00253	0.0253	0.253
Start	31	.....	.....	.....	.....
1 day	15,700	6,700	6,300	3,700	300
2 days	3,240	275	19,500	27,000	53,500
3 "	1,050	440	17,000	30,000	10,300
4 "	1,300	315	3,500	66,000	33,600
5 "	11,800	14,500	4,700	181,100	55,000
7 "	14,000	675	13,500	97,200	21,000
8 "	920	9,500	20,000	66,000	41,600
10 "	600	440	13,800	9,100	1,400
12 "	500	5,600	16,600	46,000	1,200
15 "	900	13,500	11,000	80,600	500
17 "	7,500	3,600	17,500	33,000	100
19 "	1,500	7,200	13,500	10,800	3,100
21 "	5,200	4,700	10,600	20,600	260
23 "	6,400	1,600	4,700	11,600	125
25 "	8,000	9,600	13,200	17,600	230
28 "	430	2,900	5,600	14,800	140
31 "	4,100	6,500	5,900	13,600	15
35 "	4,300	3,100	3,000	14,800	38
38 "	2,500	4,600	4,300	12,000	95
42 "	335	85	3,600	11,000	65
45 "	1,100	1,800	2,400	9,200	4
49 "	290	100	600	5,100	1
57 "	200	40	1,600	4,000	5
65 "	3,200	75	2,000	800	9
70 "	2,400	800	700	250	18
77 "	1,600	12	55	1,700	340
84 "	900	65	550	10	0
91 "	1,000	475	2,900	165	10
99 "	200	200	350	0	6
105 "	95	25	625	33	8
112 "	35	24	110	2	2
119 "	140	37	375	28	10
126 "	133	15	250	8	7
133 "	143	330	265	24	10

Experiment 164.—48 hour broth culture of B. coli diluted 1:10,000 with sterile tap water. Duration six days. Range 0.000253 to 253. The B. coli in the control decreased during the first two days and then began to increase. In 0.000253 about

TABLE 11.

48 HOUR BROTH CULTURE B. COLI DILUTED 1:10,000 WITH STERILE TAP WATER.

(B. coli per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.000253	0.00253	0.0253	0.253	2.53	25.3	253
Start	15,400	...	..	..	..	..	..	..
1 day	2,500	800	2	1	0	0	0	0
2 days	1,200	67	3	0	0	0	0	0
3 "	1,600	7	0	0	0	0	0	0
4 "	25,600	5	0	0	0	0	0	0
6 "	580,000	0	0	0	0	0	0	0

50 per cent were killed in 24 hours, the numbers decreasing rapidly until the water became sterile on the sixth day. In 0.00253 and 0.0253 all but a few of the germs were killed in 24 hours, and the water became sterile on the third and second days, respectively. In 0.253 to 253 inclusive all of the test organisms were killed in 24 hours. The detailed results are shown in Table 11.

Experiment 161.—48 hour broth culture of B. typhosus diluted 1:10,000 with sterile tap water. Duration three days. Range 0.000253 to 253 inclusive. The test organisms disappeared from the control in 24 hours. In 0.000253 a large increase was noted in 24 hours. In 0.00253 about 90 per cent of the organisms were destroyed in 24 hours, and the water became sterile on the third day. In 0.0253 to 253 inclusive, like the control, the waters were sterile in 24 hours. The detailed results are shown in Table 12.

TABLE 12.

48 HOUR BROTH CULTURE OF B. TYPHOSUS DILUTED 1:10,000 WITH STERILE TAP WATER.

(B. typhosus per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000							
	Control	0.000253	0.00253	0.0253	0.253	2.53	25.3	253
Start	3,100	.....	...	..	..	..	..	..
1 day	0	23,300	48	0	0	0	0	0
2 days	0	8,400	2	0	0	0	0	0
3 "	0	21,000	0	0	0	0	0	0

Experiment 191.—48 hour broth culture of B. typhosus diluted 1:10,000 with sterile tap water. Duration 28 days. Range 0.000253 to 0.253. The test organisms in the control dropped off gradually from 115,000 at the start to only four organisms

TABLE 13.

24 HOUR BROTH CULTURE OF B. TYPHOSUS DILUTED 1:10,000 WITH STERILE TAP WATER.

(B. typhosus per c.c.)

ELAPSED TIME	COPPER—PARTS PER 100,000				
	Control	0.000253	0.00253	0.0253	0.253
Start	115,500	.....	.....	.....	...
½ hour	71,000	234,000	14,500	6,500	520
1 "	100,800	90,000	195	1,000	24
2 hours	47,000	30,000	85	65	26
4 "	25,000	4,800	2	18	1
6 "	3,000	900	+*	2	+*
8 "	2,800	770	1	+*	+*
24 "	770	5	+*	1	+*
2 days	12	6,300	6,525	+*	+*
3 "	4	240	.....	+*	+*
5 "	52,000	1,100	8	+*	+*
6 "	420,000	22,000	8	+†	+†
7 "	260,000	54,000	8	0‡	0‡
8 "	230,000	47,000	5	0‡	0‡
9 "	220,000	35,000	4	..	..
10 "	80,000	30,000	2	..	..
12 "	260,000	29,000	7	..	..
14 "	118,800	42,000	+*	..	..
16 "	86,400	36,000	+*	..	..
20 "	61,200	12,300	+*	..	..
22 "	72,100	24,200	+*	..	..
28 "	237,000	16,000	+*	..	..

*1 c.c.=0, 10 c.c.=+. †1–10 c.c.=0, 100 c.c.=+. ‡1, 10 and 100 c.c.=0.

on the third day, and then increased rapidly to over 400,000 on the sixth day, after which they decreased slowly throughout the experiment. The same phenomenon was noted in 0.000253, the bacteria dropping off to five individuals in 24 hours, after which they increased to over 54,000 on the seventh day, and then gradually decreased. In 0.00253 the organisms were destroyed rapidly, about 90 per cent being killed during the first hour, and in 24 hours it required 10 c.c. of the water to detect the test organism. On the second day, however, the organisms had increased, and small numbers were present until the 12th day. From the 14th to 28th days B. typhosus was not found in 1 c.c., but was present in 10 c.c. In 0.0253 over 90 per cent of the organisms were destroyed in 30 minutes, and nearly all had disappeared in six hours. From the second to fifth days the organism was found present in 10 c.c., and on the sixth day it was found present in 100 c.c., the water becoming sterile on the seventh day. In 0.253 over 95 per cent of the organisms disappeared in the first 30 minutes and the decrease was so rapid that after six hours it required 10 c.c. of the water to obtain a positive test. The organism was found continuously in 10 c.c. through the fifth day, and was found in 100 c.c. on the sixth day, the water becoming sterile on the seventh day. The detailed results are shown in Table 13.

Experiment 194.—24 hour broth culture of B. typhosus diluted 1:500 with sterile tap water. Duration 10 days. Range 0.0000253 to 2.53. In this experiment the typhoid bacilli were added to the water as a broth culture, and the water in the various bottles was allowed to stand four days before adding copper, the idea being that the organisms would have become in a measure inured to their new environment before they were subjected to the action of the copper sulphate. The numbers of B. typhosus

TABLE 14.

24 HOUR BROTH CULTURE OF B. TYPHOSUS DILUTED 1:500 WITH STERILE TAP WATER AND ALLOWED TO STAND FOUR DAYS BEFORE ADDING COPPER SULPHATE.

(B. typhosus per c.c.)

ELAPSED TIME	BEFORE ADDING COPPER							
	A	B	C	D	E	F	G	H
Start	1,280,000	1,200,000	1,200,000	1,200,000	1,200,000	1,280,000	1,280,000	1,280,000
1 day	450,000	200,000	120,000	104,400	200,000	420,000	480,000	300,000
2 days	1,080,000	79,200	28,500	59,000	93,600	1,090,000	950,000	940,000
3 "	2,200,000	210,000	75,600	75,600	165,600	1,850,000	2,430,000	2,200,000
4 "	3,000,000	313,200	118,800	85,200	777,600	2,500,000	2,430,000	73,000

Elapsed Time	Control	Duplicate Control	COPPER ADDED—PARTS PER 100,000. 0.0000253	0.000253	0.00253	0.0253	0.253	2.53
30 minutes			149,100	70,000	399,600	2,099,500	1,887,600	34,000
1 hour			183,600	239,000	529,100	1,120,000	1,358,500	27,500
2 hours			159,800	72,000	626,400	1,657,500	640,000	2,225
4 "			175,000	149,000	748,400	4,406,400	323,800	142
6 "			205,200	109,400	1,101,600	2,814,000	48,800	15
24 "	3,400,000	902,900	604,800	576,000	3,700,000	4,200,000	4	1
2 days	2,750,000	2,000,000	1,040,000	1,980,000	2,520,000	2,750,000	0	0
3 "		3,000,000	1,120,000	1,200,000	2,200,000	2,450,000	0	0
4 "	265,000	4,400,000	330,000	14,400,000	4,500,000	3,000,000	0	0
8 "	2,200,000	4,600,000	10,300,000	9,360,000	3,100,000	2,060,000		
10 "	1,300,000	2,230,000		250,000	1,650,000	1,300,000		

during these four days increased in four of the bottles and decreased more or less in the other four. In the two bottles which were retained as controls the B. typhosus continued to increase during the 10 days they were under observation, the same being true of the waters containing copper 0.0000253, 0.000253, and 0.00253. In the water containing copper 0.0253 the numbers of B. typhosus remained practically constant during the 10 days. In 0.253 and 2.53 the numbers fell off rapidly, being practically all eliminated in 24 hours, and the waters becoming sterile in 48 hours. Tests were not made in volumes larger than 1 c.c. in this experiment. The detailed results are shown in Table 14.

EXPERIMENTS WITH METALLIC COPPER.

Experiment 175.—18.5 liters of Merrimac River water in copper dish. Control in a stone crock. Exposed copper area 3,200 square c.c. Duration 20 days. The bacteria in the control increased. The bacteria in the copper dish, with the exception of one slight increase, fell off gradually.

The B. coli in the control were found in 1 c.c. on the eighth day and in 100 c.c. on the 15th day. In the copper dish the B. coli disappeared from 100 c.c. on the third day. The bacterial results are shown in Table 15 and the B. coli results in Table 16.

Experiment 178.—18.5 liters of tap water containing 10 per cent of sewage in a copper dish. Control in a stone crock. Exposed copper area 3,200 square c.c. Duration 76 days. Copper absorbed 0.46 parts per 100,000 (87 days). The bacteria

TABLE 15.

(Bacteria per c.c.)

Elapsed Time	Experiment 175 Merrimac River Water		Experiment 178 Tap Water Containing 10 per Cent of Sewage	
	Control in Stone Crock	Copper Dish	Control in Stone Crock	Copper Dish
Start	1,400	1,400	320,000	390,000
1 day	9,300	1,500	1,950,000	1,440,000
2 days	11,000	17,300	2,980,000	3,430,000
3 "	488,500	337	470,000	2,690,000
4 "	1,114,000	1,600	350,000	250,000
6 "	704,900	3,100	12,000	420,000
8 "	16,200	95	12,000	69,000
10 "	107,000	180	1,230,000	210,000
13 "	125,000	85	110,000	30,000
15 "	307,000	85	73,400	26,500
17 "	414,000	30	77,800	39,000
20 "	1,490,000	37	300,000	39,000
22 "	.....	.....	73,000	20,000
24 "	.....	.....	240,000	4,000
28 "	.....	.....	180,000	12,300
30 "	.....	.....	230,000	5,000
32 "	.....	.....	180,000	4,500
34 "	.....	.....	118,800	2,800
36 "	.....	.....	29,000	3,000
38 "	.....	.....	168,500	2,700
41 "	.....	.....	55,000	2,500
44 "	.....	.....	86,400	2,400
48 "	.....	.....	101,500	3,000
55 "	.....	.....	174,200	1,100
62 "	.....	.....	93,600	200
70 "	.....	.....	172,800	100
76 "	.....	.....	350,000	3

TABLE 16.

(B. coli per c.c.)

Elapsed Time	Experiment 175 Merrimac River Water		Experiment 178 Tap Water Containing 10 per Cent of Sewage	
	Control in Stone Crock	Copper Dish	Control in Stone Crock	Copper Dish
Start	11	11	4,600	5,000
1 day	11	..	750	550
2 days	11	0†	700	100
3 "	+	0†	100	+
4 "	+	0†	+	+
6 "	+	0†	6	+
8 "	5	0†	+	3
10 "	+*	0†	1	6
13 "	+*	..	2	+
15 "	+*	..	0†	4
17 "	0†	..	0†	3
20 "	0†	..	0†	4
22 "	..	..	0†	0†
24 "	..	..	0†	0†

*1 c.c.=0, 100 c.c.=+. †100 c.c.=0.

in the control were practically constant throughout. The bacteria in the copper dish increased rapidly and then slowly decreased until the water was practically sterile on the 76th day.

The B. coli decreased rapidly in both cultures. In the control it was found in 1 c.c. on the 13th day, but disappeared from 100 c.c. on the 15th day. In the copper, B. coli were found in small numbers on the 20th day, five days after they had completely disappeared from the control. The bacterial results are shown in Table 15 and the B. coli results in Table 16.

Experiment 183.—18.5 liters of Merrimac River water in a copper dish, with control in an enameled dish. Exposed copper area 2,900 square c.c. Duration 71 days. Copper absorbed 0.30 parts per 100,000. Bacteria drop off gradually in both copper and control, both becoming practically sterile at end of experiment.

B. coli were found in 1 c.c. of the control on the 17th day and in 100 c.c. on the 28th day, disappearing on the 30th day. In the copper, B. coli were found in small numbers up to the sixth day, but disappeared from 100 c.c. on the eighth day. The bacterial results are shown in Table 17 and the B. coli results in Table 18.

Experiment 200.—17.3 liters of Merrimac River water in a copper dish with control in an enameled dish. Exposed copper surface 2,900 square c.c. Duration 64 days. Copper absorbed 1.0 part per 100,000 (78 days). Bacteria in both control and copper dish decrease gradually, the decline in the copper dish being more rapid. The contents of the copper dish were sterile on the 55th day, and in the control only a few bacteria remained alive.

B. coli were found in the control in 1 c.c. as late as the 20th day, but disappeared from 100 c.c. on the 28th day. In the copper, B. coli disappeared from 1 c.c. on the fourth day, but were found in 100 c.c. as late as the 34th day, six days after they had disappeared from the control. The bacterial results are shown in Table 17 and the B. coli results in Table 18.

TABLE 17.
(Bacteria per c.c.)

Elapsed Time	Experiment 183 Merrimac River Water		Experiment 200 Merrimac River Water	
	Control in Enameled Dish	Copper Dish	Control in Enameled Dish	Copper Dish
Start	600	1,200	11,000	10,500
½ hour	....	....	12,300	16,500
1 "	....	....	11,200	11,500
2 hours	800	500	10,800	7,300
4 "	700	490	11,000	7,200
6 "	400	135	12,000	6,000
8 "	800	200	20,000	3,800
24 "	1,300	65	16,000	104
2 days	1,044	120	6,500	400
3 "	7,700	....	12,000	200
4 "	103	763	1,200	42
6 "	164	133	5,500	133
8 "	245	156	5,400	40
10 "	250	110	12,800	15
13 "	275	25	300	7
15 "	180	12	....	....
17 "	792	13	....	....
20 "	20	10	1,200	15
23 "	2,590	9	....	....
28 "	100	13	46	9
30 "	260	13	....	....
34 "	82	10	28	2
42 "	90	12	11	0
48 "	10	6	65	3
55 "	3	1	6	0
64 "	3	4	2	0
71 "	5	6	....	....

TABLE 18.
(B. coli per c.c.)

Elapsed Time	Experiment 183 Merrimac River Water		Experiment 200 Merrimac River Water	
	Control in Enameled Dish	Copper Dish	Control in Enameled Dish	Copper Dish
Start	32	44	...	...
½ hour	..	..	91	146
1 "	..	..	118	114
2 hours	48	10	118	97
4 "	23	12	120	96
6 "	48	+	74	63
8 "	44	12	105	15
24 "	30	+	52	+*
2 days	0	3	8	+*
3 "	25	4	8	11
4 "	7	+	+	+*
6 "	1	10	11	+*
8 "	11	0†	+*	+*
10 "	7	0†	+*	+*
13 "	1	0†	+*	+*
15 "	+	0†	..	..
17 "	+	..	..	..
20 "	+*	..	6	+*
23 "	+*	..	..	..
28 "	+*	..	0†	+*
30 "	0†	..	..	..
34 "	0†	..	0†	+*
42 "	..	..	0†	0†
48 "	..	..	0	0†

*1 c.c.=0, 100 c.c.=+. †100 c.c.=0.

Experiment 182.—Three glass vessels containing respectively 18.9 liters of Merrimac River water, effluent from a sewage filter, and driven well water. 2,200 square c.c. of copper surface were inserted in each in the form of thin sheets. Duration seven days. Copper absorbed by river water 0.062, by sewage effluent 1.822, by driven well water 0.035. The bacteria in all samples increased. The bacterial results are shown in Table 19.

Experiment 186.—Duplicate of Experiment 182. Duration 14 days. Copper absorbed by river water 0.082, by sewage effluent 2.400, by well water 0.047. The numbers of bacteria in the river water showed only small fluctuations. In the sewage effluent the bacteria decreased about 85 per cent until the fifth day, and then increased until the 13th day. The bacteria in the well water increased to a maximum on the 10th day and then slowly decreased. The bacterial results are shown in Table 19.

TABLE 19.

WATER IN GLASS VESSELS CONTAINING SHEETS OF METALLIC COPPER.

(Bacteria per c.c.)

ELAPSED TIME	EXPERIMENT 182			EXPERIMENT 186		
	Merrimac River Water	Effluent Sewage Filter	Driven Well Water	Merrimac River Water	Effluent Sewage Filter	Driven Well Water
Start	1,600	14,500	900	2,400	2,000	195
1 day	1,500	972	443	525	245	375
2 days	1,600	4,300	3,400	545	345	560
3 "	2,000	1,100	12,300	340	240	46,000
4 "	7,500	32,500	6,175	3,500	370	87,300
5 "	21,000	142,000	30,000	655	1,800	78,100
6 "	4,400	22,000	5,110	240	1,420	78,100
7 "	5,000	10,800	21,600	720	1,800	60,000
8 "	.....	.....	.....	900	11,200	43,000
9 "	.....	.....	.....	700	6,875	66,600
10 "	.....	.....	.....	6,000	12,000	100,000
11 "	.....	.....	.....	2,400	8,000	73,400
12 "	.....	.....	.....	585	63,000	70,000
13 "	.....	.....	.....	575	60,000	72,000
14 "	.....	.....	.....	3,200	4,750	69,000

Experiment 184.—48 hour broth culture of B. coli diluted 1:10,000 with sterile tap water in a copper dish, with control in an enameled dish. 18.5 liters of water. Copper surface 2,900 square c.c. Duration six days. Copper absorbed 0.50 parts per 100,000 (sterilized in dish and stood 12 days). B. coli in the control show a normal increase. In the copper over 99 per cent died out in 24 hours, but a few were alive on the sixth day. The detailed results are shown in Table 20.

Experiment 189.—48 hour broth culture of B. coli diluted 1:10,000 with sterile tap water in a copper dish, with a control in an enameled dish. 18.5 liters of water. Copper surface 2,900 square c.c. Duration 12 days. Copper absorbed 0.68 parts per 100,000 (21 days). B. coli in control show a normal increase. In copper the numbers remained practically constant during five days, the test organisms being found in one c.c. on the eighth day, but having disappeared from that volume on the 10th day. The detailed results are shown in Table 20.

TABLE 20.

48 HOUR BROTH CULTURE OF B. COLI DILUTED 1:10,000 WITH STERILE TAP WATER.

(B. coli per c.c.)

ELAPSED TIME	EXPERIMENT 184		EXPERIMENT 189	
	Control in Enameled Dish	Copper Dish	Control in Enameled Dish	Copper Dish
Start	370,000	350,000	430,000	360,000
2 hours	750,000	550,000	700,000	420,000
4 "	320,000	450,000	118,800	98,000
6 "	340,000	500,000	275,300	110,200
8 "	440,000	159,800	260,000	350,000
24 "	750,000	735	21,000	9,500
2 days	390,000	2	77,800	190,800
3 "	4,300,000	1	479,300	2,100,000
4 "	.....	.....	2,851,200	180,000
5 "	1,570,000	+	3,800,000	539,600
6 "	4,700,000	+	1,430,000	400
8 "	.....	.....	392,100	1
10 "	.....	.....	2,750,000	0
12 "	.....	.....	450,000	0

Experiment 199.—48 hour broth culture of B. coli diluted 1:10,000 with sterile tap water in a copper dish, with control in an enameled dish. 19 liters of water. Copper surface 3,050 square c.c. Duration 62 days. Copper absorbed 0.54 parts per 100,000 (72 days). The numbers of B. coli in the control remained practically unchanged during 62 days. In the copper over 99 per cent of the organisms were killed in 24 hours, and it required 10 c.c. to give a positive for the organisms on the second and third days. On the fourth day a secondary increase commenced, and

TABLE 21.

48 HOUR BROTH CULTURE OF B. COLI DILUTED 1:10,000 WITH STERILE TAP WATER.

(B. coli per c.c.)

Elapsed Time	Control in Enameled Dish	Copper Dish
Start	280,000	440,000
½ hour	280,000	380,000
1 "	380,000	290,000
2 hours	360,000	300,000
4 "	240,000	78,400
6 "	291,600	68,400
8 "	147,600	10,500
24 "	111,600	15
2 days	146,900	+*
3 "	1,026,000	+*
4 "	852,000	22
6 "	639,000	1
8 "	410,000	4,900
10 "	1,560,000	750
13 "	1,000,000	+*
20 "	170,000	+*
27 "	46,100	+*
33 "	229,000	+†
40 "	990,000	+†
47 "	400,000	0‡
54 "	520,000	0‡
62 "	100,000	0‡

*1 c.c.=0, 10 c.c.=+. †1 and 10 c.c.=0, 100 c.c.=+. ‡1, 10, and 100 c.c.=0.

on the eighth day the number of B. coli had increased to 4,900. On the 10th day a decline was noted. From the 13th to the 27th day the organism was found in 10 c.c., but disappeared from 100 c.c. on the 33d day. The detailed results are shown in Table 21.

Experiment 190.—48 hour broth culture of B. typhosus diluted 1:10,000 with sterile tap water in a copper dish, with control in an enameled dish. 18.5 liters of water. Copper surface, 2,900 square c.c. Duration three days. The numbers of B. typhosus in the control decreased rapidly, over 99 per cent having disappeared in three days. In the copper the same rapid decrease was noted, over 99 per cent of the organisms having disappeared in six hours, and practically all in eight hours. After 24 hours the organism was not found in 1 c.c., but was detected in 100 c.c., and on the second day it had disappeared from 100 c.c. The detailed results are shown in Table 22.

TABLE 22.

48 HOUR BROTH CULTURE OF B. TYPHOSUS DILUTED 1:10,000 WITH STERILE TAP WATER

(B. typhosus per c.c.)

Elapsed Time	Control in Enameled Dish	Copper Dish
Start	180,000	270,000
½ hour	200,000	93,600
1 "	47,000	39,000
2 hours	26,500	14,500
4 "	300	700
6 "	2,000	200
8 "	1,800	+
24 "	2,000	+*
2 days	330	0†
3 "	110	0†

*1-10-100 c.c.=0. †1-10 c.c.=0, 100 c.c.=+.

COMPARISON OF COPPER AND COPPER SULPHATE WITH OTHER METALS AND SALTS.

In the following digest of experiments, the various bottles of experiments with ferrous sulphate and aluminum sulphate, are designated by the amount of iron or aluminum present, in parts per 100,000.

Experiment 210.—Merrimac River water treated with ferrous sulphate. Range 0.002 to 2.01. Duration 131 days. The bacteria in the control increased during the first 24 hours and then gradually decreased until the 116th day, when a small secondary increase occurred. In .002 the increase in 24 hours was larger than in the control, after which the numbers decreased steadily throughout the period of observation. In 2.01 over 95 per cent of the bacteria were destroyed in 24 hours, but those remaining were able to increase steadily until the sixth day, after which a decline set in, lasting till the 109th day. A slight secondary increase began about the 116th day.

B. coli were found in gradually decreasing numbers in the control until the 95th day, and in .002 until the 88th day. In 2.01 the B. coli were nearly all destroyed in

TABLE 23.

MERRIMAC RIVER WATER TO WHICH WAS ADDED 0.1 PER CENT OF LAWRENCE SEWAGE.

(Bacteria per c.c.)

ELAPSED TIME	IRON—PARTS PER 100,000		
	Control	0.002	2.01
Start	17,000	.....	.....
1 day	72,500	123,500	500
2 days	64,800	86,400	1,300
4 "	35,000	13,300	8,500
5 "	10,700	10,500	7,300
6 "	17,500	36,000	83,000
7 "	12,000	12,500	6,550
8 "	2,200	61,400	.....
11 "	11,000	10,800	26,500
13 "	8,900	1,200	9,500
15 "	8,000	3,700	9,500
18 "	6,200	5,000	7,400
21 "	3,300	900	2,200
25 "	600	1,600	1,440
32 "	390	110	350
39 "	190	205	410
46 "	110	245	305
53 "	95	90	260
60 "	65	90	95
67 "	75	110	46
74 "	170	75	250
81 "	21	57	28
88 "	140	110	85
95 "	120	12	67
103 "	38	8	18
109 "	55	100	80
116 "	1,100	180	700
123 "	1,100	375	275
131 "	500	170	1,200

TABBE 24.

MERRIMAC RIVER WATER TO WHICH WAS ADDED 0.1 PER CENT OF LAWRENCE SEWAGE.

(B. coli per c.c.)

ELAPSED TIME	IRON—PARTS PER 100,000		
	Control	0.002	2.01
Start	70	..	..
1 day	31	20	+
2 days	2	1	+
4 "	20	+	+
5 "	15	+	1
6 "	43	58	+*
7 "	12	+	+*
8 "	7	+	+*
11 "	12	3	+*
13 "	+	+*	+*
15 "	10	5	0
18 "	0	28	0
21 "	0	20	0
25 "	8	10	0
32 "	0	5	..
39 "	3	1	..
46 "	3	5	..
53 "	0	3	..
60 "	4	2	..
67 "	0	+*	..
74 "	2	1	..
81 "	0	+*	..
88 "	4	5	..
95 "	1	0	..
103 "	0	0	..

*1 c.c.=0, 10 c.c.=+ .

24 hours, the organism being detected in 1 c.c. up to the fifth day, and in 10 c.c. up to the 13th day. The bacterial results are shown in Table 23, and the B. coli results in Table 24.

Experiment 218.—Merrimac River water treated with ferrous sulphate. Range 0.020 to 20.1. The experiment was not complete when this was written.

TABLE 25.

MERRIMAC RIVER WATER.

(Bacteria per c.c.)

ELAPSED TIME	IRON—PARTS PER 100,000				
	Control	0.020	0.201	2.01	20.1
Start	600	.....	.....	.....	.....
1 day	1,300	425	700	300	250
2 days	600	500	44	85	130
3 "	4,900	2,400	900	1,500	1,700
4 "	.....	.....	.....	.....	.....
5 "	20,000	12,900	8,800	2,200	9,100
7 "	7,700	2,000	9,700	1,400	400
9 "	7,500	2,000	1,600	2,000	50
11 "	2,100	300	1,400	15,400	0
15 "	3,800	375	275	2,000	18
18 "	2,200	1,400	800	2,800	600
21 "	300	230	70	1,100	14
28 "	140	250	120	600	5
35 "	230	75	47	325	23
42 "	16	50	130	190	11
49 "	38	70	35	65	29
56 "	21	51	47	31	16
63 "	11	10	50	22	37
70 "	35	12	36	17	52
78 "	46	12	32	11	30
84 "	108	65	37	72	30
91 "	15	26	19	49	28

TABLE 26.

MERRIMAC RIVER WATER.

(B. coli per c.c.)

ELAPSED TIME	IRON—PARTS PER 100,000				
	Control	0.020	0.201	2.01	20.1
Start	20	..	..	..	..
1 day	26	15	6	2	3
2 days	8	23	9	6	3
3 "	19	9	15	20	4
4 "	7	4	6	11	4
5 "	3	4	0	2	3
7 "	0	7	0	0	8
9 "	3	0	2	14	0
11 "	4	0	5	..	0
15 "	11	16	6	6	7
18 "	0	1	2	10	0
21 "	0	2	0	0	0
28 "	0	0	0	0	0
35 "	15	6	3	0	0
42 "	12	0	4	11	3
49 "	3	3	1	2	5
56 "	7	9	0	1	1
63 "	1	4	0	0	0
70 "	10	5	16	16	10
78 "	8	11	4	6	1
84 "	0	0	0	1	0
91 "	0	15	14	17	3

The bacteria in all the bottles increased steadily for a few days, then decreased slowly. The maximum was reached in the control, 0.020, and 20.1 on the fifth day, in 0.201 on the ninth day and in 2.01 on the 11th day. B. coli were found in small numbers as late as the 78th day in the control, on the 91st day in the waters which had been treated. The bacterial results are shown in Table 25 and the B. coli results in Table 26.

Experiment 158.—Merrimac River water treated with sulphate of alumina. Range 0.134 to 0.537. Duration four days. The bacteria in all samples were reduced during the first 24 hours, and then began to increase about the third or fourth day. The results are shown in Table 27.

TABLE 27.

MERRIMAC RIVER WATER.

(Bacteria per c.c.)

ELAPSED TIME	ALUMINA—PARTS PER 100,000				
	Control	0.134	0.269	0.403	0.537
Start	950	.....	.....	.....	.....
6 hours	800	20	255	110	145
1 day	205	143	120	55	95
2 days	.....	248	176	155	70
3 "	1,200	400	790	10	2,800
4 "	.....	2,800	3,800	20,000	4,700

Experiment 165.—Merrimac River water treated with sulphate of alumina. Range 0.134 to 0.537. Duration 11 days. The bacteria in the control increased to a maximum on the third day and then declined. In all of the treated samples the bacteria were much reduced on the second day, but all showed a material increase from the third to the seventh days.

The B. coli results in this experiment are interesting, showing the occasional appearance of considerable numbers in all treated samples at intervals, with intermediate periods when the test organism was not detected. This phenomenon was probably due to errors in sampling caused by the precipitated aluminum hydrate, and would indicate that the B. coli were able to live in the precipitate and perhaps to increase. The bacterial results are shown in Table 28 and the B. coli results in Table 29.

TABLE 28.

MERRIMAC RIVER WATER.

(Bacteria per c.c.)

ELAPSED TIME	ALUMINA—PARTS PER 100,000				
	Control	0.134	0.269	0.403	0.537
Start	1,450	.....	.....	.....	.....
1 day	176,400	4,900	3,200	800	1,900
2 days	321,900	550	290	110	125
3 "	430,000	11,200	11,400	1,050	155
5 "	129,000	25,800	4,600	1,700	260
7 "	7,700	3,200	40,000	5,900	75,600
9 "	15,500	1,600	26,300	3,000	25,500
11 "	16,500	18,500	30,500	41,400	28,200

TABLE 29.

MERRIMAC RIVER WATER.

(B. coli per c.c.)

ELAPSED TIME	ALUMINA—PARTS PER 100,000				
	Control	0.134	0.269	0.403	0.537
Start	36	..	..	..	..
1 day	6	0	0	0	0
2 days	95	11	20	0	25
3 "	65	1	16	0	0
5 "	20	0	0	22	0
7 "	25	0	0	0	0
9 "	11	35	63	40	114
11 "	0	0	0	21	0

NOTE.—The occasional appearance of B. coli was probably caused by imperfect sampling due to the precipitated hydrate of alumina.

Experiment 209.—1,000 c.c. of Merrimac River water were placed in each of six jars, and thin sheets of copper, aluminum, lead, zinc, tin, and iron were inserted in each of six jars respectively, the seventh being retained as a control. Exposed metal surface 625 square c.c. Duration 132 days. The bacteria in the control showed a steady decrease after the first 24 hours. With the copper, the bacteria decreased during the first 24 hours, and then increased to a maximum on the eighth day, dropping

TABLE 30.

MERRIMAC RIVER WATER.

(Bacteria per c.c.)

Elapsed Time	Control	Copper	Aluminum	Lead	Zinc	Block Tin	Iron
Start	10,000	75,600	14,600	18,000	9,000	75,600	7,700
4 hours	11,000	700	11,000	12,700	4,100	15,200	18,000
8 "	19,200	180	15,500	9,300	6,200	24,000	14,700
24 "	9,700	430	8,500	2,900	5,200	14,000	5,500
2 days	2,500	75,600	7,000	4,500	11,000	6,700	7,300
3 "	800	850	5,000	24,000	14,500	4,300	1,400
4 "	800	.....	1,200	33,000	5,500	1,750	600
6 "	1,000	360,000	600	30,800	28,500	1,700	65
8 "	1,450	673,000	900	38,300	4,050	900	62
10 "	250	97,200	150	2,000	650	700	29
13 "	110	181,500	120	2,000	950	50	20
15 "	125	108,000	65	21,400	450	55	80
17 "	23	51,000	100	21,250	65	105	300
20 "	250	59,000	85	22,500	7	45	110
23 "	180	31,500	9	22,100	10	90	230
27 "	43	0	8	800	0	43	10
34 "	....	310	55	1,100	0	12	360
41 "	180	10	10	1,200	....	43	175
48 "	290	4	100	490	....	720	430
55 "	225	2	35	400	....	770	60
62 "	260	4	5	130	....	60	60
69 "	200	0	0	55	....	70	80
76 "	160	2	3	20	....	550	42
83 "	350	10	0	10	....	375	47
90 "	150	9	26	6	....	120	110
97 "	125	4	12	14	....	210	220
105 "	35	4	55	180	....	350	130
111 "	75	30	65	500	....	350	75
118 "	23	0	40	7	....	75	36
125 "	38	13	60	19	....	260	100
132 "	5	7	3	22	....	52	85

TABLE 31.

MERRIMAC RIVER WATER.

(B. coli c.c.)

Elapsed Time	Control	Copper	Aluminum	Lead	Zinc	Block Tin	Iron
Start	32	52	18	19	5	4	25
4 hours	18	47	38	14	35	32	15
8 "	1	2	+	1	7	6	+
24 "	12	3	+	+	+	10	6
2 days	10	2	10	3	3	3	+
3 "	+	+	10	+	5	+	+
4 "	1	+	+	1	+	+	1
5 "	5	4	6	12	+	4	+
8 "	26	10	10	19	6	25	0
10 "	9	3	+	+	0	9	0
13 "	6	+	+	2	0	3	6
15 "	1	+	2	3	0	3	0
17 "	7	+	2	+	0	6	0
20 "	8	+	3	10	0	16	0
23 "	+	+	21	4	0	10	0
27 "	3	4	9	2	0	13	0
34 "	2	9	6	5	..	2	0
41 "	5	3	0	3	..	0	0
48 "	+	0	0	+	..	0	0
55 "	2	0	0	12	..	0	0
62 "	4	0	0	7	..	0	0
69 "	+	0	0	+	..	0	0
76 "	1	..	..	1	..	..	..
83 "	0	..	..	+	..	..	..
90 "	0	..	..	3	..	..	..
97 "	..	..	..	0	..	..	..
105 "	..	..	..	0	..	..	..

off slowly till the 23d day. With the aluminum there was a steady decrease in bacteria throughout the experiment. With the lead, the bacteria increased slightly to a maximum on the eighth day, remaining practically constant till the 23d day, after which they decreased steadily, with a slight secondary increase on the 105th day. With the zinc the bacteria increased slightly to a maximum on the sixth day and then declined rapidly, the water becoming sterile on the 27th day. The bacteria decreased steadily in the tin until the 41st day, when a small secondary increase began, lasting until the 125th day. With the iron, the bacteria decreased steadily, with small secondary increases from the 17th to the 48th days, and from the 90th to the 105th days.

The B. coli in all the samples decreased steadily, with the usual fluctuations, and disappeared from the zinc on the 10th day, from the iron on the 15th day, from the tin and aluminum on the 41st day, and from the copper on the 48th day, from the control on the 83d day, and from the lead on the 97th day. The bacterial analyses are given in Table 30 and the B. coli determinations in Table 31.

Experiment 237.—1,100 c.c. of Merrimac River water containing 1 per cent of sewage was placed in each of seven jars, and thin sheets of copper, aluminum, lead, zinc, tin, and iron were inserted in each of six of the jars respectively, the seventh being retained as a control. The exposed metal surface in each case was 625 square c.c. The experiment had only run 31 days at the time this was written. In the control, lead, and tin, the bacteria decreased steadily throughout the period of observation. In the copper a large increase occurred during the first four days, after which the bacteria declined steadily. In the aluminum the bacteria decreased steadily until the 15th day, when a secondary increase started lasting till the 23d day. With the zinc a considerable reduction occurred during the first 24 hours, after which the num-

bers increased till the third day, when a decline set in lasting throughout the period of observation. In the iron an increase occurred during the first 24 hours, after which a steady decrease was noted until the 15th day, when a secondary increase commenced which lasted until the 20th day.

The numbers of B. coli in the control increased largely during the first 24 hours, after which they decreased steadily until the water was practically sterile after 31 days. In all of the jars containing metals the B. coli decreased steadily, a slight secondary increase being noted on the 17th day in the aluminum. The fluctuations in the numbers of B. coli were quite noticeable, in a number of instances no organisms being found in one c.c. for a number of days, and then appearing in small numbers. The B. coli disappeared from the copper on the sixth day, from the zinc on the 10th day, from the tin on the 15th day, from the iron and lead on the 23d day, and probably from the aluminum on the 31st day. The bacterial analyses are given in Table 32 and the B. coli determinations in Table 33.

TABLE 32.

MERRIMAC RIVER WATER CONTAINING 1 PER CENT OF SEWAGE.

(Bacteria per c.c.)

Elapsed Time	Control	Copper	Aluminum	Lead	Zinc	Tin	Iron
Start	44,500	.....	.....	.....	.....	.....	.....
1 day	21,000	127,800	44,000	56,000	7,000	35,000	86,600
2 days	150,000	362,100	16,500	26,000	13,400	12,000	16,500
3 "	11,000	180,000	2,800	8,500	16,500	1,400	35,000
4 "	4,600	305,000	11,500	8,500	10,500	4,800	18,000
6 "	415	35,000	310	5,500	11,200	200	205
8 "	2,800	96,300	220	3,500	3,700	138	93
10 "	52	3,600	95	3,000	19	94	63
13 "	69	6,700	55	155	115	18	8
15 "	17	245	1,000	80	21	27	105
17 "	23	790	7,600	3	100	105	760
20 "	31	115	3,000	9	9	235	1,500
23 "	5	100	3,850	18	40	275	525
28 "	10	65	480	20	5	210	30
31 "	25	275	325	135	116	160	125

TABLE 33.

MERRIMAC RIVER WATER CONTAINING 1 PER CENT OF SEWAGE.

(B. coli per c.c.)

Elapsed Time	Control	Copper	Aluminum	Lead	Zinc	Tin	Iron
Start	3,500	3,500	3,500	3,500	3,500	3,500	3,500
1 day	38,900	2	1,370	149	22	3,100	650
2 days	250	....	200	0	0	100	50
3 "	108	12	75	3	3	45	230
4 "	25	25	21	12	2	20	125
6 "	20	0	9	1	0	8	20
8 "	13	2	8	0	4	7	5
10 "	15	0	0	0	0	0	0
13 "	3	0	5	5	0	6	1
15 "	1	0	0	2	0	0	1
17 "	1	0	125	7	0	1	25
20 "	0	0	45	1	0	1	6
23 "	4	0	7	0	0	0	0
28 "	1	0	3	0	0	0	0
31 "	0	0	0	0	0	0	0

RÉSUMÉ.

In the following résumé, the results of the copper sulphate experiments are brought together according to the amount of copper added to the water. This copper is expressed as the ratio of copper sulphate by weight to the weight of water, with the equivalent of metallic copper in parts per 100,000 in parentheses.

Controls.—In three experiments with polluted water, one showed a gradual decrease in bacterial contents, and the other two showed an increase, then a decrease which was followed by a secondary increase after some time. In four experiments with stagnant water, run for a short time only, one showed a decrease, one increased, one remained practically constant, and one increased and then decreased. In one experiment with driven well water, the bacteria increased, then decreased, and continued to fluctuate up and down during the 133 days the experiment continued.

In the one experiment made, B. coli under natural conditions showed a gradual decrease, but continued alive in 1 c.c. during 95 days. B. coli added to water as broth culture decreased, then increased. In three experiments using laboratory cultures of B. typhosus, the control was sterile after 24 hours in one case; in another a sharp decrease was noted till the third day, when an enormous increase commenced which lasted through 28 days; and in a third case the typhoid bacilli, which were present in large numbers, remained practically unchanged during 14 days.

1: billion (*0.0000253*).—In the one experiment made the bacteria followed the same laws as did the control.

1:100 million (*0.000253*).—The behavior of the normal water bacteria was observed in five experiments, four with polluted water and one with stagnant water. In four of the experiments the bacteria followed the same curve as in the control, and in the fifth they were nearly all destroyed at first, but the few remaining were able to multiply to large numbers.

In one experiment, B. coli, naturally present, acted much like the control, decreasing slowly but remaining alive some 88 days in 1 c.c. In one experiment with a laboratory culture a rapid decrease occurred, and the B. coli had disappeared at the end of six days.

Three experiments were made with laboratory cultures of B. typhosus. In one experiment large numbers survived three days, although the control died out in 24 hours; in another nearly all the B. typhosus were killed in 24 hours, but the very few remaining were able to increase to large numbers. In the third experiment the B. typhosus had become somewhat accustomed to life in water before the copper was added, and increased steadily during the 10 days they were under observation.

1:10 million (*0.00253*).—The normal bacteria were observed in eight experiments, four with polluted water, three with stagnant water, and one with deep well water. Three of the polluted waters acted like the control, but in the other nearly all the bacteria were destroyed at first, the few remaining, however, being able to increase rapidly. In the three stagnant waters the bacteria increased, although they failed to do so in one of the stagnant water controls.

Natural B. coli in one experiment decreased slowly, as they did in the control, but the numbers were usually larger than in the control. In one experiment with a laboratory culture the B. coli were killed in three days.

Two experiments were made with laboratory cultures of B. typhosus; in one the test organisms disappeared from 1 c.c. in three days, and in another nearly all were killed in four hours, but some remained alive in 1 c.c. till the 12th day, and tests of 10 c.c. showed them to be alive up to the 28th day. In one experiment with a culture of B. typhosus which had been grown four days in water, a steady increase occurred during the 10 days it was under observation.

1:million (*0.0253*).—The behavior of the water bacteria was observed in eight experiments, four with polluted water, three with stagnant water, and one with deep well water. In two of the polluted waters the bacteria followed the control, while in the other two they increased. In the three stagnant waters the bacteria increased, although they decreased in one of the controls. The bacteria in the well water followed the control.

Natural B. coli in one experiment decreased slowly, as did the control, but showed higher numbers than in the control. In one

experiment with a laboratory culture, the B. coli were killed in two days, testing 1 c.c.

In one experiment with a laboratory culture of B. typhosus the organism disappeared from 1 c.c. in 24 hours. In another experiment the B. typhosus were nearly all destroyed in six hours, a few were alive on the fifth day, but none were found in 100 c.c. on the seventh day. In the experiment where the typhoid bacilli were inured to the water, the numbers increased steadily during the 10 days they were under observation.

1:100,000 (*0.253*).—The bacteria were observed in seven experiments, four with polluted water, two with stagnant water, and one with deep well water. In three of the polluted waters nearly all of the bacteria were killed out at first, but the few remaining were able to multiply largely. In the other polluted water a large increase occurred at once. The numbers of bacteria in the two stagnant waters remained low for two days and then increased rapidly. In the well water the bacteria followed the same curve as in the control. Natural B. coli followed the same curve as the control. With a laboratory culture of B. coli the organism disappeared from 1 c.c. in 24 hours. In one experiment with B. typhosus the organism disappeared from 1 c.c. in 24 hours. In another experiment all but a few were killed in six hours, but 10 c.c. tests showed some to be alive on the fifth day, and 100 c.c. tests showed some alive on the sixth day. In the experiment with water-grown typhoid, the organisms disappeared from 1 c.c. in 24 hours.

1:10,000 (*2.53*).—The total bacteria were observed in six experiments, four with polluted water and two with stagnant water. In two of the polluted waters the bacteria decreased gradually, and the waters became practically sterile after 89 and 131 days respectively. In one experiment nearly all of the bacteria were killed immediately, and in another the bacteria were practically all destroyed at first, but a few remained during 20 days. The bacteria in the two stagnant water experiments were practically all destroyed in 24 hours.

Natural B. coli acted like the control and remained alive in 1 c.c. after 103 days. With a laboratory culture of B. coli the organism disappeared from 1 c.c. in 24 hours. A laboratory culture of B.

typhosus and the special water culture of the same organism both disappeared from 1 c.c. in 24 hours.

1:1,000 (25.3).—The behavior of the normal bacteria was observed in three experiments with polluted water. Nearly all of the bacteria were destroyed in a short time in all of these experiments, but a few remained alive for 10 days, 20 days, and 68 days, respectively.

With a laboratory culture of B. coli and one of B. typhosus the organisms disappeared from 1 c.c. in 24 hours.

1:100 (253).—Two experiments were made with polluted water, in which all of the bacteria were killed in 24 hours.

The complete sterilization of water by allowing it to stand in a clean copper dish does not seem to be an accomplished fact; in only one of six experiments was the water completely sterilized, and that only after standing 55 days. The bacterial curves, taking both controls and copper dish cultures, seem to follow the same laws hitherto noted for standing water experiments. In two experiments the copper dish cultures consistently decreased in bacterial contents throughout, while in another experiment the numbers increased sharply and then declined slowly, both of which phenomena have been frequently observed in the various control cultures. In two experiments three entirely different waters were under comparative observation. The bacterial contents of each of these waters increased constantly, although one of them absorbed relatively large amounts of copper.

In four of the copper dish experiments, determinations of B. coli and of bacteria were made simultaneously. In these experiments the B. coli were present naturally, i. e., they came from fecal matter directly polluting the water, as opposed to experiments in which B. coli were added to the water in the form of a laboratory culture. Naturally we should expect to find the organisms under such conditions more resistant than in the case where we are dealing with cultures, and this proves to be the case. In one experiment the test organism disappeared from the water, testing 100 c.c. on the third day. In two other experiments the B. coli died out in the control dishes before they disappeared from the waters contained in copper, being found in 1 c.c. in one experiment on the 20th day, or five days after they had disappeared from 100 c.c. of the control; and in

the other experiment they were found in 100 c.c. on the 34th day, or six days after they had disappeared from a like volume of the control.

Three experiments were made with laboratory cultures of B. coli in sterile water standing in copper, and one experiment was made with B. typhosus under the same conditions. In two experiments B. coli were found in 1 c.c. on the sixth and eighth days, respectively, tests not being made in larger volumes in these experiments. In another experiment B. coli appeared in some numbers as late as the 10th day, and were found in 100 c.c. as late as the 40th day. In the experiment with a typhoid the bacilli were practically all destroyed after eight hours, and were not found in 100 c.c. after 24 hours.

From the two experiments made with sulphate of alumina and ferrous sulphate, these two salts appear to have about the same action on the bacteria and B. coli as have equal strengths of copper sulphate.

Judging from the results of experiments comparing metallic copper with other metals, all of the metals tested seem to be about equal in their effect on the numbers of bacteria in waters with which they are in contact. In one experiment the water in contact with zinc became sterile after about three weeks, while the numbers of bacteria increased in the water which was in contact with copper, and with all the metals, excepting zinc, a few bacteria were alive after 132 days.

Based on the disappearance of B. coli in 1 c.c. the metals in one experiment ranked:—zinc 10 days, iron 15 days, tin 41 days, aluminum 41 days, copper 43 days, lead 97 days; and in the other experiment:—zinc 10 days, copper 10 days, tin 23 days, iron 23 days, lead 23 days, aluminum 31 days.

CONCLUSIONS.

In conclusion, the writers believe that the treatment of water with copper sulphate or by storing it in copper vessels has little practical value, for the following reasons:

I. The use of any method of sterilization which is not absolutely effective is dangerous in the hands of the general user, tending to

induce a feeling of false security, and leading to the neglect of ordinary precautions which would otherwise be employed.

II. The removal of bacteria, B. coli and B. typhosus, by allowing a water to stand in copper vessels for short periods, while occasionally effective, is not sure, and the time necessary to accomplish complete sterilization is so long that the method would be of no practical value to the ordinary user. Furthermore, metallic copper seems to have little more germicidal power than iron, tin, zinc, or aluminum.

III. Although the removal of B. coli and B. typhosus is occasionally accomplished by dilute solutions of copper sulphate, these organisms may both live for many weeks in water containing copper sulphate in greater dilutions than 1:100,000; and in order to be safe dilutions of 1:1,000 must be used, in which case the water becomes repugnant to the user because of its strongly astringent taste.

IV. In some instances very dilute solutions of copper sulphate or colloidal copper absorbed from contact with clean metallic copper, appear to have a decidedly invigorating effect on bacterial activity, causing rapid multiplication, when the reverse would have been true had the water been allowed to stand the same length of time without any treatment.