

CAN SOIL BE STERILIZED WITHOUT RADICAL ALTERATION?¹

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The widespread adoption of soil as a culture medium in preference to solutions in the study of soil biology problems involves a fundamental difficulty, which merits more attention than is at present being bestowed upon it. In a word, the sterilization of soil, whether by heat or antiseptics, induces certain profound changes in the chemical and physical constitution of the soil. Since the guiding motive in the use of soil as a culture medium is to obtain results which may be correlated more closely with actual field conditions, that purpose is in a measure defeated, so long as the methods of sterilization, as commonly practiced, are drastic in their effects. A review of the literature (4) failing to offer any adequate solution, a preliminary investigation was undertaken in an effort to devise some method whereby the soil might be rendered sterile with a minimum amount of alteration. Experimentation was conducted along the following lines: 1. The intermittent sterilization of soil by dry heat; 2. The relative sterilizing efficiency of various chemical substances used as soil antiseptics; 3. Volatile antiseptics applied in partial vacuum; and 4. Volatile antiseptics applied under pressure at 80° C.

THE INTERMITTENT STERILIZATION OF SOIL BY DRY HEAT.

It has been pointed out by Russell and Hutchinson (11, 12), Pickering (8, 9), Schreiner (13), Seaver and Clark (14), and others, that heating the soil causes a distinct change in its chemical composition. Various temperatures from 60° to 170° C. have been employed with the inevitable result of an alteration in the constitution of the soil. Pickering (9) states, however, that heating at 82° C. does not cause a much greater production of toxins than heating at 50° or 60° C., consequently it was deemed advisable to use this temperature in an attempt to sterilize the soil completely. From the work of Russell and Hutchinson (11, 12), as well as that of Cunningham and Löhnis (2), it seemed valid to conclude that this degree of heat would be sufficient to kill all the living protozoa and most of the bacteria. Likewise, the cysts of protozoa were killed at 72° C. in solutions (which would represent about 60° to 65° C. in the soil). Moist heat is known to be more efficient in its destructive action than dry heat, but the laboratory facilities did not permit the use of the former.

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In view of the fact that the spores of bacteria or fungi might resist 82° C., it seemed desirable to follow a well-known bacteriological procedure and sterilize intermittently for a number of days at the same temperature. In this way the spores present would have ample opportunity to germinate, and the living forms would immediately succumb to the temperature which the spores might have been able to resist. Likewise, by this method the production of toxins, and the alteration of the chemical composition of the soil was reduced to a minimum, while the biological factors suffered decimation.

A point of considerable importance which had been attacked by Richter (10) and Koch (3), namely, the difference in the effect of sterilization upon air-dry and moist soil, was included in this investigation.

The method of procedure was as follows, using Penn Clay Loam soil, a chemical analysis of which is given in Table I. Because of the fact that it is more difficult to sterilize a heavier than a lighter soil, the results obtained by using Penn Clay Loam would thus be more exacting, than if a sandier soil were employed.

TABLE I.
CHEMICAL ANALYSIS OF PENN CLAY LOAM.
(HCL sp. gr. 1.115)

	Per Cent		Per Cent
Insoluble Matter	78.2820	P ₂ O ₅1282
K ₂ O5496	SO ₃0497
Na ₂ O1790	Volatile Matter	8.9200
CaO3962	Total Nitrogen1722
MgO9041	Total Phosphoric Acid1340
Fe ₂ O ₃	4.1900	Total Potash	2.1700
Al ₂ O ₃	6.3168	Total Carbon	2.4500

Fifty-gram portions of soil were placed in cotton-plugged 200 c.c. Erlenmeyer flasks. One series of ten flasks containing air-dry soil (having 4.5 per cent water), the other series of ten flasks containing 25 per cent of water (calculated on the water-free basis), which was equivalent to 60 per cent of the moisture-holding capacity of the soil.

Both series of flasks were incubated at 22° C. for 24 hours to allow excystation of protozoa in moist soil and to ensure the presence of vegetative forms of bacteria. (It might be noted, however, that a three-day incubation would have been preferable.) The flasks were then placed in a hot-air oven and heated until the constant temperature of the soil registered 82° C. for one hour. After this treatment all the flasks were again placed in the incubator at 22° C. for 24 hours, and two flasks of each series were taken for bacterial counts on Lipman and Brown's (5) "synthetic" agar. The counts were made in triplicate. The plates were counted after 3 days had elapsed.

The process outlined above was repeated for 5 successive days, two flasks of each series being removed each day and bacterial counts made on the results of each day's heating. On the last day, the soil was incubated for 48 hours instead of 24 to make doubly certain of having all the biological forms in the soil in the active living state. Also the plates were incubated for 7 instead of 3 days.

TABLE II.
INTERMITTENT STERILIZATION BY DRY HEAT AT 82° C.

MOIST SOIL (PENN CLAY LOAM) 25% H₂O.

Treatment	Bacterial Count Millions per gm. Soil		Total Water-Solu- ble Solids in gm.		Organic Solids in gm.		Inorganic Solids in gm.	
	Dup.	Av.	Dup.	Av.	Dup.	Av.	Dup.	Av.
Check	48.000		.0216		.0108		.0108	
	47.500	47.750	.0224	.0220	.0132	.0120	.0092	.0100
	12.300		.0304		.0164		.0140	
After 1st Day's Heating	12.400	12.350	.0316	.0310	.0180	.0172	.0136	.0138
	0.711		.0320		.0176		.0144	
After 2nd Day's Heating	0.632	0.672	.0316	.0318	.0172	.0174	.0144	.0144
	0.075		.0282		.0118		.0164	
After 3rd Day's Heating	0.067	0.071	.0296	.0289	.0184	.0151	.0112	.0138
	0.037		.0310		.0170		.0140	
After 4th Day's Heating	0.046	0.042	.0325	.0318	.0170	.0176	.0145	.0143
	0.001		.0322		.0173		.0149	
After 5th Day's Heating	0.002	0.005	.0320	.0321	.0167	.0170	.0153	.0151

AIR-DRY SOIL (PENN CLAY LOAM) 4.5% H₂O.

Check	45.000		.0216		.0108		.0108	
	42.000	43.050	.0224	.0220	.0132	.0120	.0092	.0100
	52.500		.0200		.0164		.0036	
After 1st Day's Heating	50.200	51.350	.0212	.0206	.0164	.0164	— .0048	— .0042
	11.300		.0332		.0176		.0166	
After 2nd Day's Heating	11.800	11.500	.0320	.0326	.0164	.0170	— .0156	— .0161
	9.250		.0260		.0172		.0084	
After 3rd Day's Heating	9.660	9.400	.0270	.0265	.0180	.0176	— .0092	— .0088
	3.525		.0237		.0121		.0116	
After 4th Day's Heating	4.400	3.962	— .0220	— .0229	.0112	.0116	— .0108	— .0112
	3.424		.0228		.0075		.0153	
After 5th Day's Heating	3.500	3.462	.0271	.0256	.0122	.0093	.0149	.0151
			.1810		.0900		.0795	
Moist heat at 120° C. for 15 min. at 15 lbs. pres're		Sterile	.1800	.1805	.1005	.0953	.0910	.0853

Treatment in Moist Soil (25 % H ₂ O)	Ammonia Release Average in grams
Check0014
After 1st Day's Heating.....	.0021
After 2nd Day's Heating.....	.0019
After 3rd Day's Heating.....	.0019
After 4th Day's Heating.....	.0019
After 5th Day's Heating.....	.0019

The results are recorded in Table II. All the protozoa were killed after the initial treatment by heating to 82° C. for one hour. Löhns' (2) soil extract was employed as a medium for determining the presence of protozoa, 100 c.c. being inoculated with 50 gm. of soil, and a microscopical examination made for 5 successive days. Fungi, however, persisted throughout the experiment; species of *Penicillium* and *Mucor* being recognized.

An examination of the results of the bacterial counts represented in figure 1 reveals the fact that in moist soil the numbers of bacteria decrease successively, in a remarkable manner from 47,750,000 per gram on the first day to 1,500 on the last day. On the other hand, however, there is an initial depression of bacterial numbers exhibited in the air-dry soil, followed by a gradual decrease in the numbers of bacteria on the subsequent days, which is substantially less effective than the decrease operating in the moist soil. A variation is noted in the bacterial counts on air-dry soil on the second day, for instead of a decrease there is an increase which may be ascribed to the immediate utilization of some nutrients becoming more available by the first day's heating.

It may be observed, parenthetically, that the soil was incubated for 48 hours instead of 24 on the last day, thus enabling the bacteria to multiply to a considerable extent after removal from the sterilizer and magnifying the bacterial count accordingly. Consequently, the number recorded is not a fair index of the actual number of bacteria present in the soil immediately after heating, but represents an addition over and above that amount.

Further, the plates were incubated for 7 days instead of 3 days for the purpose of ascertaining whether the number of colonies increased to any appreciable extent after 3 days. A negligible increase was noted.

Therefore, it is evident from the above results, that the intermittent sterilization of moist soil by dry heat is decidedly more efficacious in reducing the bacterial numbers than the same treatment with air-dry soil.

For the purpose of determining the amount of increase of the water-soluble constituents of the soil as a result of intermittent sterilization by dry heat, the following method was employed.

Fifty grams of the same kind of soil were heated under the same conditions as previously and then vigorously shaken with distilled water until the leachings made up a volume of 200 c.c. An aliquot portion of 50 c.c. was evaporated to dryness on a water-bath, dried in a hot-air oven at 108° C. for one hour (according to the method described by Seaver and Clark) (5), weighed, ignited, and then weighed again. The first weight represents the total solids, the final weight represents the inorganic constituents, and the difference between the two weights represents the organic constituents.

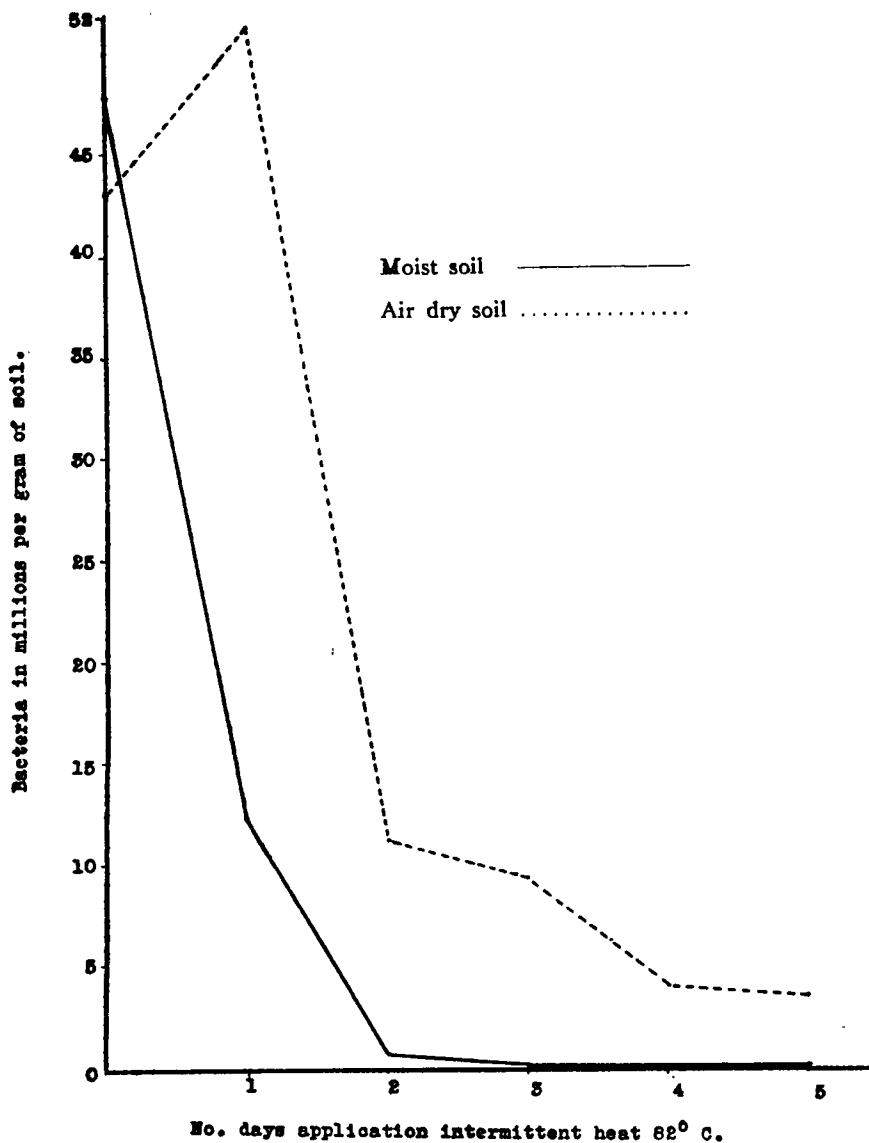


Fig. 1. Diagram showing the effect of intermittent heat for 5 days at 82° C. upon the numbers of bacteria in moist soil and in air-dry soil.

It is seen from Table II that sterilization under these conditions increases the amount of total solids 45.91 per cent; and that after the initial heating the amount of total solids does not increase within experimental error.

It is interesting to note the comparison between the increase in the amount of water-soluble solids as a result of intermittently dry heat at 82° C. and the increase due to subjecting the soil to steam at 120° C. under 15 pounds pressure for 15 minutes, which is the usual laboratory method employed in soil biology investigations. The latter method is responsible for almost sixteen times the amount liberated by subjection to intermittent dry heat. The ammonia released by the sterilizing treatment at 82° C. is greatest after the first day and remains practically constant thereafter.

Considerable variation is noted in the amount of total solids in the air-dry soil, and this discrepancy may be accounted for by the fact that the heat was not able to permeate uniformly throughout the soil in a dry state.

In summarizing the foregoing results, the following points are to be noted:

1. Intermittent partial sterilization at 82° C. kills the greater portion of the bacterial population, as indicated by growth on Lipman and Brown's synthetic agar.
2. The treatment kills all the protozoa in the soil, as indicated by their non-appearance in Löhnis' soil extract medium.
3. Fungi persist throughout the experiment, as indicated by their presence on the culture media.
4. The sterilization treatment increases the total solids in the soil about 46 per cent, thereby altering the chemical composition of the soil and changing it as a medium for biological activity, but only one-sixteenth as much as by the common method of steam sterilization.
5. Where the time-element is of considerable importance the above method is undesirable.

THE RELATIVE STERILIZING EFFICIENCY OF VARIOUS CHEMICAL SUBSTANCES USED AS SOIL ANTISEPTICS.

The use of various chemical substances for the purpose of sterilizing soil has long been practiced not only in the laboratory, but in the greenhouse and under field conditions as well. Russell and Hutchinson (12), and recently Buddin (1), have made an extensive survey of substances which would prove adequate in presenting the so-called "partial sterilization" phenomena. The results obtained by the former investigators indicate that volatile antiseptics in quantities of one per cent are as efficient as when used in greater amounts. For this reason one per cent (on the

basis of 100 gm. of air-dry soil) of the following volatile antiseptics were employed: ethyl alcohol (C_2H_5OH), ethyl ether [$(C_2H_5)_2O$], hydrogen peroxide (H_2O_2), toluene ($C_6H_5CH_3$), carbon bisulfid (CS_2), and chloroform ($CHCl_3$).

The procedure of this experiment was as follows: 100-gm. portions of Penn Clay Loam (passing a 20-mesh sieve) were placed in cotton-plugged 200 c.c. Erlenmeyer flasks. The soil was then brought to optimum moisture content by the addition of 25 c.c. of sterile tap water. One per cent quantities of the above-mentioned volatile antiseptics were then added to the soil. Each treatment was carried out in duplicate. In the case of the addition of hydrogen peroxide, 5 c.c. of concentrated solution were previously added to 20 c.c. of sterile tap water and the mixture used to bring the soil to the optimum moisture content. Following the addition of the antiseptics the flasks were sealed with corks which had previously been steeped in paraffine. The antiseptics were allowed to remain in contact with the soil for 3 days at room temperature. At the expiration of this time, the paraffine corks were removed from the flasks, and sterile cotton plugs substituted. In their work on partial sterilization, Russell and Hutchinson (11)* removed the volatile antiseptics from the soil after treatment, by exposing the latter to the air for a time. It would be practically impossible to accomplish this under sterile conditions, consequently the following apparatus, illustrated in figure 2, was devised.

The vacuum chamber (A) is a strong metal tank (in this case a fire extinguisher emptied of its contents was employed), which is connected with a water-pump (F) for exhausting the air from the chamber. Another connection is made with the barometer (I), which consists of a graduated glass tube inverted in a bottle of mercury. In a subsequent portion of this paper mention will be made of the volatilization of antiseptics by immersion of a bottle of antiseptic (D) in boiling water (E). This connection is likewise indicated in the diagram. Stop-cocks were placed at the points marked C. It might be added parenthetically that the capacity of the vacuum chamber (A) in which the flasks (B) were placed is 11,500 c.c.

In the experiment under discussion the flasks (B) were placed in the vacuum chamber (A) and the latter exhausted (G) to one-half inch mercury pressure (F). After remaining in the chamber for one-half hour, the flasks were removed and the soils plated out on Lipman and Brown's (5) synthetic agar for bacterial counts in the usual manner. The total water-soluble solids were determined by the method previously described under the sterilization of soil by intermittent dry heat. An examination of the soils for the presence of any antiseptics, revealed the fact that toluene was the only substance remaining in any appreciable quantity in the soil after such treatment.

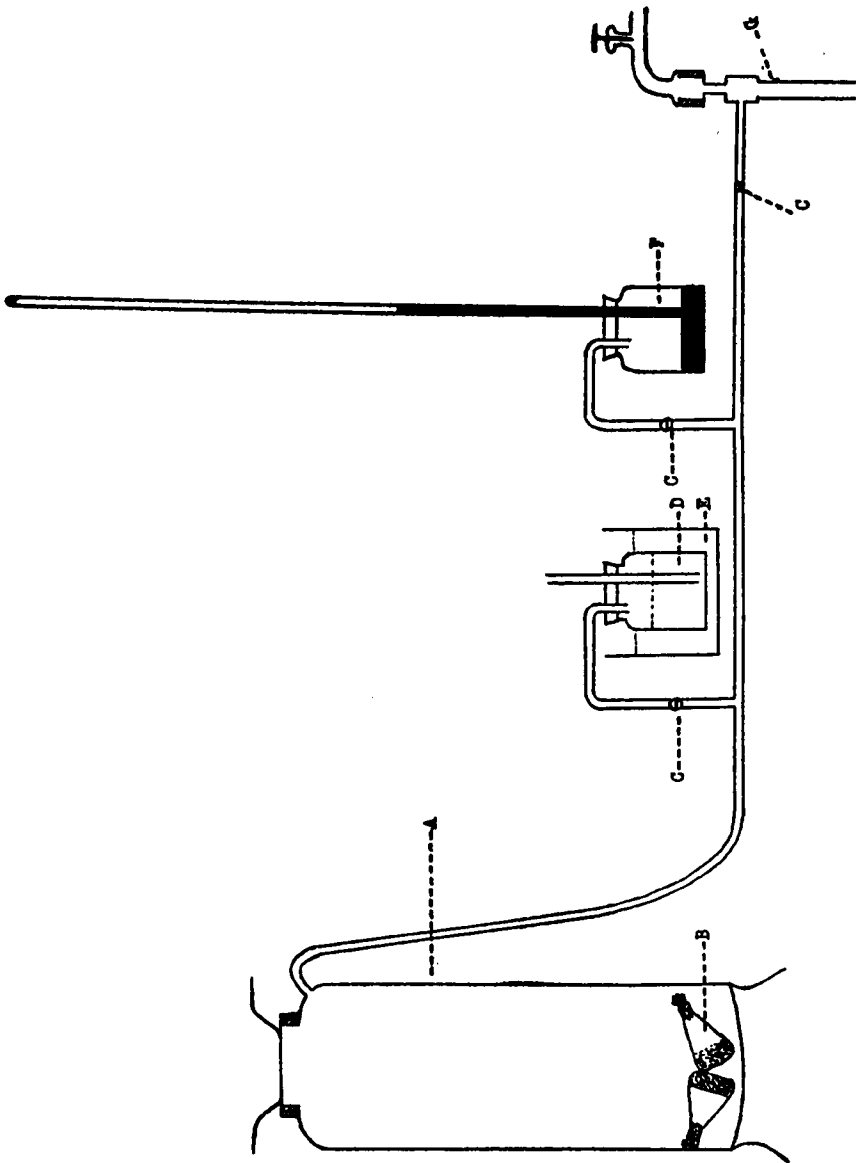


Fig. 2. Diagram of apparatus used in experiment on sterilizing soil with various antiseptics.

It will be seen from the results in Table III that this method of applying antiseptics is not sufficiently efficient to justify its use as a practical means of soil sterilization. Of the substances employed, chloroform,

TABLE III.
THE RELATIVE EFFICIENCY OF VOLATILE ANTISEPTICS (1%)
IN SOIL STERILIZATION.

MOIST SOIL.				
Lab. No.	Antiseptic	Bacteria in millions per gm. of soil	Average	Total Water-soluble Solids, Av. in gm.
210	Original Soil		47.75	.0220
298	Check	44.50		
299	Check	51.00	47.75	.0902
334	Alcohol (Ethyl)	44.50		
335	Alcohol (Ethyl)	42.50	43.50	.1045
300	Ether (Ethyl)	41.50		
301	Ether (Ethyl)	37.00	39.25	.1090
322	Hydrogen Peroxide	41.00		
323	Hydrogen Peroxide	26.50	33.25	.0990
310	Toluene	11.50		
311	Toluene	27.00	19.25	.0947
328	Carbon Bisulfid	13.50		
329	Carbon Bisulfid	5.50	9.50	.1097
316	Chloroform	9.00		
317	Chloroform	4.50	6.75	.0915

carbon bisulfid, and toluene, in the order named, were effective in decimating the bacterial flora; chloroform being responsible for a decrease of 86 per cent of the original bacterial content. Furthermore, chloroform caused the least alteration in the chemical constitution of the soil, as indicated by the total water-soluble solids, which in this case amounted to something more than three times the original quantity present.

Under the conditions of the experiment, volatile antiseptics when applied to moist soil in amounts of 1 per cent were not efficient as sterilizing agents. Acting upon the assumption that dry soil is more difficult to sterilize than moist soil, the plan of repeating this experiment with air-dry soil was abandoned.

THE RELATIVE EFFICIENCY OF VOLATILE ANTISEPTICS APPLIED AS VAPOR IN PARTIAL VACUUM.

The problem of equal distribution throughout the soil mass is a serious one in the application of volatile antiseptics in such small amounts as 1 per cent. Obviously, moist soil is superior to dry soil in facilitating uniform distribution; nevertheless, it would be of advantage to increase the efficiency even of the former, in this direction. With this in view, the vacuum chamber (figure 2) previously described was adapted to the needs of the following experiment.

The principle involved is, in effect, the application of volatile antiseptics *in vacuo*, thus obtaining an intimate and uniform mixture of soil and chemical. A further modification has been introduced which is based upon the fundamental physical law that gases diffuse more rapidly than liquids; namely, the volatile antiseptics are applied in the form of vapor rather than in the usual liquid state. This combination of the two principal factors of vapor and vacuum is effected by means of the apparatus devised; ergo, a more uniform distribution of the antiseptics in the soil is achieved.

As in former cases, 100-gm. portions of soil were placed in Erlenmeyer flasks (plugged with cotton). In the first series of treatments air-dry soil was employed, whereas in the second series moist soil was used. The volatile antiseptics tested were: osmic acid, ethyl ether, carbon bisulfid, toluene, and ethyl alcohol.

The method of procedure was as follows: The flasks containing soil were placed in the vacuum chamber (A—see figure 2), and the latter exhausted to one-half inch of mercury pressure (F). A bottle containing the antiseptic (D) was immersed in boiling water (E), in order that the application might be made in the form of vapor. By means of a direct connection the vapor entered the vacuum chamber. The flasks were allowed to remain in this antiseptic atmosphere for one and one-half hours, thereby allowing the soil to take up as much vapor as it could. The vacuum chamber (A) was kept in close proximity to a source of heat in order that the antiseptic might remain in a volatile state. During the course of the various treatments pressure was developed within the vacuum chamber as a result of the antiseptics passing from the liquid to the gaseous state. Indirectly, this development of pressure may be considered as being indicative of the fact that the antiseptic vapor has saturated the pore spaces of the soil, which had previously been exhausted.

After the antiseptic vapor had been allowed to remain in intimate contact with the soil for one and one-half hours, the vacuum chamber was again exhausted to one-half inch of mercury pressure in order that the antiseptics might be removed from the soil. Air, which had been rendered sterile by filtering through cotton, was then admitted to the vacuum chamber, and the soils plated immediately for the bacterial count. The process outlined above was repeated on each of three successive days and the total water-soluble solids determined at the conclusion of that period.

It will be observed from the results recorded in Table IV that carbon bisulfid, toluene, and ethyl alcohol, in the order named, were quite effective in decreasing the bacterial content of the air-dry soil, as is evidenced by the fact that the number of bacteria fell from 43,000,000 to 160,000 in the case of the carbon bisulfid and to 250,000 and 330,000 as a result

of treatment with toluene and ethyl alcohol, respectively. In effect, this is a decrease of more than 99 per cent of the original soil flora.

TABLE IV.
THE RELATIVE EFFICIENCY OF VOLATILE ANTISEPTICS APPLIED AS VAPOR
IN PARTIAL VACUUM.

AIR-DRY SOIL.

Antiseptic	Bacteria in millions per gram of soil						Total Water-Soluble Solids Av. in gm.	Pressure developed during treatment
	1st Day	Av.	2d Day	Av.	3d Day	Av.		
Original Soil		43.05		43.05		43.05	.0220	
Check	10.00		8.80		4.80			
Check	9.00	9.50	8.00	8.40	4.20	4.50	.0890	29 in. Hg.
Osmic Acid	7.85		6.50		5.00			
Osmic Acid	7.95	7.90	10.00	8.75	2.70	3.85	.0895	28 " "
Ethyl Ether	3.60		3.40		2.75			
Ethyl Ether	3.50	3.55	2.70	3.05	2.00	2.37	.0975	20 " "
Carbon Bisulfid	3.50		0.80		0.12			
Carbon Bisulfid	lost	3.50	0.80	0.80	0.20	0.16	.1190	20 " "
Toluene	6.90		5.10		0.29			
Toluene	8.00	7.45	lost	5.10	0.21	0.25	.1190	12 " "
Ethyl Alcohol	2.20		2.50		0.30			
Ethyl Alcohol	2.10	2.15	2.70	2.60	0.36	0.33	.1195	4½" "

In considering the decrease in bacterial numbers from day to day as a result of treatment, in the case of carbon bisulfid there is a decrease on the second day 75 per cent of the number of bacteria present on the first day, followed by a decrease on the third day of 80 per cent of the number present on the second day. There is, then, good reason to believe that if the treatment were prolonged over a greater period of time, the number of bacteria might be still further reduced. The operation of the time-factor, however, must be regarded as a distinct limitation upon any method of protracted duration. With toluene and ethyl alcohol there is likewise a striking reduction in numbers from the second to the third day. It will be noted in this experiment that osmic acid proved to be unsuccessful as a sterilizing agent for soil. Ethyl ether, as well, was found to be inefficient.

With regard to the total water-soluble solids it will be observed that there is, generally speaking, a fourfold increase. No correlation can be established between the sterilizing efficiency of the antiseptics in question, and the pressure developed by them in the gaseous state during treatment, although the data may not be altogether without interest.

It may be seen from Table V that the final results for moist soil are similar to those obtained where dry soil was employed, with the exception of ethyl ether which yields the largest reduction of bacterial num-

bers. Carbon bisulfid, toluene and ethyl alcohol again manifest a fair degree of efficiency, although their effectiveness is not as great in moist soil as in dry soil. This may possibly be explained on the grounds that in the case of the moist soil the bacteria had ample opportunity to multiply in the 24-hour interval between sterilizations; or, on the other hand, it is not impossible to suppose that the moist soil offers greater resistance to the penetration of the antiseptic vapors than the dry soil. The reduction in bacterial numbers on successive days is noteworthy, although not quite so marked as in the treatment of dry soil.

TABLE V.
THE RELATIVE EFFICIENCY OF VOLATILE ANTISEPTICS APPLIED AS VAPOR
IN PARTIAL VACUUM.

Antiseptic	Bacteria in millions per gram of soil						Total Water-Soluble Solids Av. in gm.	Pressure developed during treatment
	1st Day	Av.	2d Day	Av.	3d Day	Av.		
Original Soil		47.75		47.75		47.75	.0220	
Check	8.30		5.30		3.90			
Check	8.00	8.15	4.80	5.05	3.60	3.75	.0895	29 in. Hg.
Osmic Acid	3.50		4.00		4.70			
Osmic Acid	2.70	3.10	4.90	4.45	2.00	3.35	.0860	28 " "
Ethyl Ether	3.00		1.10		0.15			
Ethyl Ether	4.50	3.75	0.92	1.01	0.20	0.17	.1215	20 " "
Carbon Bisulfid	10.00		0.70		0.50			
Carbon Bisulfid	13.00	11.50	0.70	0.70	0.46	0.48	.1145	20 " "
Toluene	4.30		1.80		1.60			
Toluene	4.40	4.35	0.70	1.25	0.63	1.11	.0950	12 " "
Ethyl Alcohol	6.70		2.20		1.00			
Ethyl Alcohol	6.80	6.75	2.70	2.45	1.20	1.10	.1190	4 $\frac{3}{8}$ " "

The superiority of osmic acid over the check treatment is virtually insignificant. The treatments employed were responsible for an increase in total water-soluble solids, as formerly, of approximately four times the original amount. From the data presented in Tables IV and V it may be inferred that volatile antiseptics applied as vapor in partial vacuum are relatively efficient in the sterilization of soil. This method, if sufficiently prolonged, might render the soil totally sterile, yet the increase in water-soluble constituents must also be considered, and with this point in view it is evident that the soil is compelled to undergo some alteration.

THE RELATIVE EFFICIENCY OF VOLATILE ANTISEPTICS WHEN APPLIED
UNDER HEAT AND PRESSURE.

Having obtained results of a somewhat encouraging nature from the use of intermittent heat at 82° C., it was deemed advisable to combine this method with a still further modification of the application of volatile antiseptics, namely, employing the latter under pressure at 80° C. for three successive days. It was observed in the preceding experiment that when antiseptics were vaporized in an air-tight chamber they automatically developed pressure.

The method of procedure was as follows: A vertical steam pressure autoclave (American Standard), commonly employed in the bacteriological laboratory for sterilization, was filled to within 5 inches of the top with water and the temperature raised to 80° C. An agate pan was floated on the surface of the water and the Erlenmeyer flasks (plugged with cotton) containing moist soil were placed therein. One hundred cubic centimeters of the volatile antiseptics under investigation were poured into the agate pan. The lid of the autoclave was then quickly clamped down and the check valve closed upon the appearance of the vapor. As a result of the vaporization of the antiseptic, pressure was developed. (It is assumed throughout this work with volatile antiseptics that the usual precautionary methods are observed, and all flames in the vicinity of the vapors extinguished.) The flasks were allowed to remain in the autoclave for one hour,¹ after which they were removed to the vacuum chamber, which was exhausted to one-half inch mercury pressure. After remaining in the chamber for one-half hour to allow sufficient time for the removal of the antiseptic, sterile air was admitted and the soils plated immediately for bacterial counts. The entire 100 gm. of soil were taken up with one liter of sterile water and the ordinary dilutions employed. It was impracticable to use alcohol in this experiment for the reason that it would remain in solution in the water.

From the results in Table VI it will be noted that this method of treatment was fairly effective in depopulating the bacterial flora of the soil. The decrease, in general, approximated 98 per cent. Carbon bisulfid was the only antiseptic employed that proved superior to the check treatment. In this case a possible correlation might be obtained between the pressure developed during treatment and the effectiveness of the sterilizing agent. Thus carbon bisulfid developed a pressure of 20 pounds, whereas ethyl ether, which proved least efficient in sterilization, developed only 6 pounds of pressure. Carbon tetrachloride, with only 5 pounds pressure, is an exception to such a generalization.

¹ During the period of treatment the temperature fell, on the average, ten degrees.

TABLE VI.
THE RELATIVE EFFICIENCY OF VOLATILE ANTISEPTICS APPLIED UNDER
HEAT AND PRESSURE.

MOIST SOIL.

Antiseptic	Bacteria in millions per gram of soil						Total Water-Soluble Solids Av. in gm.	Pressure developed during treatment
	1st Day	Av.	2d Day	Av.	3d Day	Av.		
Original Soil		47.75		47.75		47.75	.0220	
Check	10.00		1.27		0.55			
Check	9.00	9.50	1.17	1.22	0.65	0.60	.0885	0
Carbon Tetrachloride	11.50		1.17		0.45			
Carbon Tetrachloride	12.50	12.00	2.60	1.88	0.80	0.62	.1097	5 lbs.
Carbon Bisulfid	10.50		0.41		0.30			
Carbon Bisulfid	6.50	8.50	0.34	0.37	0.15	0.22	.1170	20 "
Ethyl Ether	9.50		0.96		1.55			
Ethyl Ether	11.50	10.50	0.75	0.85	1.30	1.42	.1102	6 "
Chloroform	8.50		1.50		0.95			
Chloroform	4.50	6.50	1.50	1.50	0.80	0.87	.1055	10 "

In the matter of total water-soluble solids, the increase is again approximately fourfold. This experiment was repeated, substituting air-dry for moist soil, with little or no increase in effectiveness of volatile antiseptics over the check treatment.

In a general comparison of the volatile antiseptics employed under the various treatments as outlined, shown in Table VII, the total decrease (in per cent) of the bacterial numbers from the original bacterial content of the soil is recorded, as well as the increase in total water-soluble solids (in per cent) over the amount originally present in the soil.

Under the conditions of this experiment it will be readily observed from the calculations represented in Table VII that intermittent dry heat at 82° C. for 5 successive days in moist soil was responsible for the greatest bacterial decrease, or 99.996 per cent. Likewise, this method caused the minimum alteration in the chemical constitution of the soil as indicated by the amount of total water-soluble solids. It is of interest to note that, whereas the above method caused a 46 per cent increase of total water-soluble solids, the ordinary moist heat sterilization at 120° C. for 15 minutes at 15 pounds pressure, which is widely used in soil biology investigations, caused an increase of 720.45 per cent. Thus it is evident that the latter method induces a radical alteration in the composition of the soil compared with the former.

Of the three other methods devised, that of applying volatile antiseptics in partial vacuum for three successive days proved somewhat superior to the application of these chemicals under heat (80° C.) and pressure for a similar period. Volatile antiseptics applied in 1 per cent. quantities in the liquid state are inefficient. Carbon bisulfid, in general,

proved to be the most efficient volatile antiseptic tested with regard to its practical value as a sterilizing agent. The three methods just mentioned caused approximately a fourfold increase in total water-soluble solids.

TABLE VII.

COMPARISON OF THE VOLATILE ANTISEPTICS EMPLOYED UNDER VARIOUS TREATMENTS SHOWING TOTAL DECREASE (IN PER CENT) OF BACTERIAL NUMBERS FROM ORIGINAL BACTERIAL CONTENT OF THE SOIL; AND INCREASE OF TOTAL SOLIDS (IN PER CENT).

Treatment	Total decrease of bacterial numbers resulting from treatment (av.)							
	1% applied as liquid 3 days in moist soil		Partial Vacuum 3 Days				Pressure and heat (80° C.) for 3 days	
			Moist Soil		Air-Dry Soil			
	Bact. Dec. %	Total Solids Inc. %	Bact. Dec. %	Total Solids Inc. %	Bact. Dec. %	Total Solids Inc. %	Bact. Dec. %	Total Solids Inc. %
Original Soil—Ck. Treatment.		310.00	92.15	306.81	89.55	304.54	98.75	302.27
Carbon Tetrachloride							98.71	398.63
Carbon Bisulfid	80.11	394.08	99.00	420.45	99.63	440.90	99.54	431.81
Ethyl Ether	17.80	395.45	99.65	452.27	94.50	343.18	97.03	400.91
Chloroform	85.87	315.89					98.18	379.54
Osmic Acid			92.98	290.91	91.06	306.81		
Toluene	59.69	330.45	97.68	331.81	99.43	440.90		
Ethyl Alcohol	8.91	375.00	97.70	440.91	99.24	443.18		
Hydrogen Peroxide	30.37	350.00						
	Moist Soil		Air-Dry Soil					
Intermittent Dry Heat at 82° C. for 5 days	99.996	45.91	91.96	13.63				
Moist Heat at 120° C. for 15 min. at 15 lbs. pressure.....	Sterile	720.45						

The data presented above are preliminary in character, and although no definite conclusions can be established, several lines of investigation are indicated which might prove adequate in solving the problem of soil sterilization without radical alteration.

SUMMARY.

1. Under the conditions of the experiment, with Penn Clay Loam, intermittent sterilization by means of dry heat at 82° C. for 5 successive days in moist soil, almost completely decimated the bacterial flora of the soil. This was accomplished with but a slight change in the chemical constitution of the soil, as indicated by the amount of water-soluble solids. Ordinary steam sterilization under pressure causes a change sixteen times as great.

2. There is a strong indication that the application of volatile antiseptics either in partial vacuum or under a combination of heat and pressure, if repeated for more than three successive days, would achieve complete soil sterilization without involving any radical alteration in the chemical constitution of the soil.

In conclusion, it is a privilege to express an appreciation of Dr. J. G. Lipman's suggestions, ever at our disposal.

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