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The Effect of Partial Sterilization by Steam and Formalin on the Numbers of Amoebae in Field Soil

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SUMMARY: In Sitka spruce nursery plots the numbers of amoebae in steam-treated soil rose with the increase in the bacterial population. The population of amoebae over a period of seven months was much higher in this soil than in the untreated or the formalin-treated soils. The formalin-treated soil had significantly lower numbers of amoebae compared with the untreated soil over a period of one year, although the bacterial numbers were often higher in the former than in the latter. It is suggested that the unsuitable quality of bacterial food supply might be responsible for keeping the numbers of amoebae in check in the formalin-treated soil. Double formalin treatment seemed to suppress further the numbers of amoebae.

The occurrence of Protozoa in soils was known to the biologists of the nineteenth century. Russell & Hutchinson's (1909) theory to explain the effects of partial sterilization stimulated the development of research on soil Protozoa. This theory drew attention to the fact that many soil Protozoa are primarily devourers of bacteria. It attempted to account for the development of 'soil sickness' by supposing this to be due to an excessive number of active Protozoa which diminished the bacterial population and for the remedial effect of partial sterilization by the suppression of these Protozoa by the sterilizing agents. The theory put forward by Russell & Hutchinson has never been fully accepted. Laboratory experiments with pure cultures of bacteria with and without the additions of Protozoa have shown that the latter do not always depress the biochemical activity of the bacteria, but may in fact stimulate it under certain conditions (Nasir, 1923; Cutler & Bal, 1926; Cutler & Crump, 1929; Harvey & Greaves, 1941; Meiklejohn, 1930, 1932). Further critical work is needed to explain this most interesting phenomenon.

Amoebae are numerically the most important group of soil Protozoa which feed on bacteria. Their importance is perhaps increased by the fact that they feed differentially (Singh, 1941, 1942, 1945, 1947*a*, *b*, 1948) and are known to diminish bacterial numbers in sterilized soil. It seems, therefore, that this group is of prime importance to the examination of the Protozoa theory of partial sterilization. The earlier work on amoebae in partially sterilized soils has been carried out under laboratory conditions. The programme of field trials carried out at Ampthill on Sitka spruce nursery plots, some of which were treated with steam and formalin, provided an opportunity to investigate the action of these agents on numbers of amoebae and bacteria under field conditions, while modern technique for estimating numbers of amoebae

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(Singh, 1946) has greatly improved the validity of results now obtainable. The present paper records the results of such estimates made from samples taken at intervals over a period of twelve months from the time of applying the sterilizing agents to the plots.

MATERIALS AND METHODS

The plots from which the samples were taken were part of an experiment conducted by the Chemistry Department of Rothamsted Experimental Station at the Ampthill Forest Nursery, Bedfordshire, on a light sandy soil having a pH value of about 6·0, which was not appreciably changed by the treatments. The main results of this experiment will be published elsewhere.

Small plots were steamed for about 20 min., using four grids at a time, each grid having five pipes, 4 ft. long and 9 in. apart buried at a depth of about 9 in. A solution of 10% commercial formalin in water at the rate of 1 gallon/sq.yd. was added to the formalin-treated plots. A more detailed description of the plots is given by Mollison (1953). The dates on which steam and formalin were applied to the soil and the intervals at which the amoebae were counted are given below.

Twelve 6 in. borings from one plot of each treatment were taken and thoroughly mixed to form a composite sample. The samples were brought to the laboratory and passed through a 3 mm. sieve. The numbers of amoebae were estimated by the dilution technique of Singh (1946), eight replicate subsamples being tested from each dilution. The cultures in which amoebae could be seen by microscopical examination were considered positive. The analysis of the data is based on the theory developed by Fisher (cf. Introduction to Fisher & Yates, 1947; Appendix, Singh, 1946). With the technique used a difference of approximately 100% in populations estimated from two individual samples is significant at 5% level.

No counts of amoebae from duplicate soil samples were made; the differences between a number of duplicate field soil samples in earlier work (Singh, 1946, 1949) were in no case found to be significant.

The bacterial numbers were estimated by plate counts on the following medium: $Ca(NO_3)_2$, 0·5 g.; $MgSO_47H_2O$, 0·1 g.; Ca gluconate, 0·2 g.; K_2HPO_4, 0·1 g.; KH_2PO_4, 0·1 g.; $NaHCO_3$, 0·2 g.; H_2O, 1 l.; $FeCl_3$, 0·2% (w/v.), 1 ml.; agar, 15 g.; filter and add mannitol, 0·1 g.; asparagine, 0·1 g.; 'Difco' yeast extract, 0·1 g., adjusted to pH 7·4.

The results given below were derived from three different field trials carried out at Ampthill in 1950, 1951 and 1952.

RESULTS

Experiment 1, 1950

Table 1 gives the counts of amoebae in untreated, formalin-treated and steam-treated soils. In the tables the numbers of active amoebae given are obtained by subtracting cystic from total numbers. Thus where these do not differ significantly the number of active forms may be nil. The soils were

Table 1. *The numbers of amoebae and bacteria in untreated, formalin-treated and steam-treated soils under natural conditions*

Date	Amoebae count (no./g. wet soil) Total	Cystic	Active	Bacterial count (10^6/g. wet soil)	Soil water content (%)
		Untreated soil			
23 Mar. 1950	12,200	979	11,221	3·3	12·4
20 Apr. 1950	8,570	3,300	5,270	10·75	12·6
4 May 1950	10,200	4,670	5,530	13·0	11·3
25 May 1950	15,800	6,040	9,760	—	—
8 June 1950	17,300	4,670	12,630	11·9	8·5
6 July 1950	14,500	3,600	10,900	10·4	11·2
31 July 1950	13,300	4,280	9,020	5·25	11·0
24 Oct. 1950	17,300	4,670	12,330	8·3	8·4
		Formalin-treated soil			
23 Mar. 1950	635	412	—*	2·5	12·6
20 Apr. 1950	1,650	1,390	—*	27·55	12·1
4 May 1950	2,770	1,270	1,500	13·75	11·7
25 May 1950	11,100	5,540	5,560	—	—
8 June 1950	8,570	2,770	5,800	28·35	8·5
6 July 1950	7,210	3,020	4,190	14·15	8·6
31 July 1950	11,100	5,540	5,560	7·0	10·9
24 Oct. 1950	10,200	5,540	4,660	20·1	7·5
		Steam-treated soil			
23 Mar. 1950	693	126	567	5·0	13·9
20 Apr. 1950	12,200	2,540	9,660	27·85	12·8
4 May 1950	20,500	5,540	14,960	38·25	13·9
25 May 1950	54,000	12,200	41,800	—	—
8 June 1950	37,900	1,960	35,940	42·55	8·8
6 July 1950	59,000	5,540	53,460	18·0	10·0
31 July 1950	45,200	5,540	39,660	8·3	13·1
24 Oct. 1950	45,200	13,300	31,900	15·65	8·6

* Total and cystic not significantly different.

treated with steam on 10 March and with formalin on 16 March. In the first count taken on 23 March the total numbers of amoebae found in steam- and formalin-treated plots (column 2) did not differ significantly from the number of encysted forms found in the untreated plot (column 3). This suggests that both treatments killed the active forms. At this date the amoebae in the formalin-treated soil were still nearly all in the cystic condition, since the numbers of encysted amoebae (column 3) did not differ significantly from the total numbers (column 2). In the case of steam-treated soil the majority of the amoebae were beginning to excyst, most of them being in the active condition (column 4). The next count taken on 20 April showed that the population of amoebae had greatly increased in both the formalin- and steam-treated soils. In subsequent samples the amoebae in the steam-treated soil were several times higher than the control soil, and a very large proportion of them were in the active condition. This clearly demonstrates that the beneficial effect of partial sterilization by steam is not due to the killing of the amoebae.

The bacterial population in the steamed soil was very much higher than the control. This treatment probably stimulates the growth and the reproduction of amoebae owing to the increase in the amount of bacterial food. The numbers of amoebae were very much lower on the whole in the formalin-treated than in the untreated plot, although the bacterial numbers were, after the first 4 weeks, higher in the former soil. Thus it seems that some factor or factors other than bacterial numbers were responsible for keeping the numbers of amoebae in check in the formalin-treated soil.

Experiment 2, 1951

Table 2 gives the results of samplings from a second experiment. The soil was treated with formalin on 13 March and the first count was taken on 8 May. In this and in all later counts taken over a period of 1 year the total

Table 2. *The numbers of amoebae and bacteria in untreated and formalin-treated soils under natural conditions*

Date	Amoebae count (no./g. wet soil)			Bacterial count (10^6/g. wet soil)	Soil water content (%)
	Total	Cystic	Active		
		Untreated soil			
8 May 1951	10,200	2,330	7,870	12·4	12·7
5 Feb. 1952	13,200	5,540	7,660	9·2	—
3 Apr. 1952	11,100	5,540	5,560	19·65	—
13 May 1952	15,800	3,330	12,470	7·8	13·4
17 June 1952	10,200	3,600	6,600	9·75	10·0
		Formalin-treated soil			
8 May 1951	534	377	—*	42·85	11·4
5 Feb. 1952	2,540	490	2,050	8·05	—
3 Apr. 1952	1,960	639	1,321	32·2	—
13 May 1952	3,960	534	3,426	7·45	12·9
17 June 1952	3,600	1,270	2,330	8·75	10·0

* Total and cystic not significantly different.

numbers of amoebae were significantly lower in the formalin-treated than the untreated soil. The amoebae were present in active and cystic condition in both soils. This observation confirms the findings of the previous year.

Experiment 3, 1952

Table 3 shows the results of a few counts of amoebae and bacteria in untreated, formalin-treated and twice formalin-treated soils. The formalin was applied to the soil on 29 February. The soil termed 'twice formalin-treated' was from the plot (Exp. 2) that received formalin in 1951 and was given a second dose in 1952. Although the counts of amoebae were not continued for a long period, the results confirm those of previous years and also suggest that the effect of two treatments with formalin further decreased the population of amoebae.

Table 3. *The numbers of amoebae and bacteria in untreated, formalin-treated and twice formalin-treated soils under natural conditions*

Date	Amoebae count (no./g. wet soil) Total	Cystic	Active	Bacterial count (10^6/g. wet soil)	Soil water content (%)
3 Apr. 1952	11,100	5,540	5,560	19·65	—
13 May 1952	15,800	3,300	12,470	7·8	13·4
17 June 1952	10,200	3,600	6,600	9·75	10·0
		Formalin-treated soil			
3 Apr. 1952	582	979	—*	41·45	—
13 May 1952	1,800	582	1,218	21·5	13·3
17 June 1952	5,080	2,140	2,940	9·05	10·4
		Twice formalin-treated soil			
3 Apr. 1952	265	154	—*	34·9	—
13 May 1952	1,270	265	1,005	22·85	13·8
17 June 1952	1,800	635	1,165	8·0	10·7

* Total and cystic not significantly different.

DISCUSSION

According to Russell & Hutchinson's (1909) theory of partial sterilization the treatment of soil by steam or various volatile chemical antiseptics has a detrimental effect on the Protozoa which were thought to be the agents in causing 'soil sickness'. The counts of amoebae in this paper clearly demonstrate that no generalizations can be made as to the effects of partial sterilizing agents on the subsequent growth and multiplication of amoebae in field soils. In the case of steam treatment the population of amoebae is ultimately much higher than in the untreated soil. Formalin treatment considerably lowered the numbers of amoebae, but the bacterial numbers were often higher than in the untreated soil in all the experiments. It may be that the factor responsible for keeping the amoebae in check is the unsuitable type of food bacteria which develop in formalin-treated soil. One of the authors (L.M.C.) has found that the bacterial flora which develop in steam- and formalin-treated soils are to some extent qualitatively different, and that this difference persists over a long period. Further work on the bacterial relationships in soils partially sterilized by volatile antiseptics is needed to investigate the influence of partial sterilization on the feeding qualities of the different bacterial populations. The importance of the quality of the bacterial flora for the growth of amoebae in field soils was also suggested by the different effects of artificial fertilizers and dung on the numbers of amoebae in Rothamsted soils (Singh, 1949). The results obtained from these Rothamsted plots as well as those here recorded from partially sterilized soils suggest that the quality of the bacterial flora in the field is of more importance than its quantity in determining the numbers of amoebae, a result to be expected from laboratory experiments on the differential feeding of these organisms (Singh, 1941, 1942, 1945, 1947*a*, *b*, 1948).

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