Cutler, D. W., Crump, L. M. and Dixon, A. 1932. Some factors influencing the distribution of certain protozoa in biological filters. *Journal of Animal Ecology.* 1 (2), pp. 143-151.

Some Factors Influencing the Distribution of Certain Protozoa in Biological Filters
Author(s): D. Ward Cutler, L. M. Crump and A. Dixon
Source: *Journal of Animal Ecology*, Vol. 1, No. 2 (Nov., 1932), pp. 143-151
Published by: British Ecological Society
Stable URL: https://www.jstor.org/stable/975

SOME FACTORS INFLUENCING THE DISTRIBUTION OF CERTAIN PROTOZOA IN BIOLOGICAL FILTERS

By D. WARD CUTLER, L. M. CRUMP and A. DIXON.

(*From the Department of General Microbiology, Rothamsted Experimental Station.*)

I. Introduction.

During the past four years investigations have been carried out to discover whether the principle of biological purification on percolating filters could be applied to the waste effluent from the beet sugar factories. At an early stage in the research it was found that large numbers of Protozoa, as well as bacteria and other organisms, were inhabitants of such filters. At first the work was done on a large scale at a factory, but when it was transferred to the laboratory with specially designed filters (**1**) it was thought of interest to make frequent observations of the protozoan species, since opportunity would thus be afforded for studying the effects of changes in hydrogen-ion concentration and in chemical composition of the medium on the fauna. Knowledge of the ecology of Protozoa has mostly been derived from the study of inland waters and soil, where the conditions could not be varied at will, or from culture solutions under laboratory conditions. In the filters, on the other hand, which depend for their population on chance contaminations and therefore approximate to natural conditions, the medium can be changed at will and therefore a good opportunity is afforded for work of this type.

Records have been kept of the following types of Protozoa occurring in the filters, and as, in some cases, the species have not been identified only the generic names are included in the list.

Sarcodina		Mastigophora		Ciliata	
Genus	No. of species	Genus	No. of species	Genus	No. of species
Amoeba	3	Bodo	2	Colpoda	2
Vahlkampfia	2	Heteromita	2	Colpidium	2
Naegleria	1	Cercomonas	1	Glaucoma	2
Hartmanella	1	Oicomonas	1	Chilodon	2
Sappinia	1	Trepomonas	1	Paramoecium	2
Pelomyxa	1	Proleptomonas	1	Lionotus	2
Arcella	1	Astasia	1	Vorticella	2
Hyalosphenia	1	Entosiphon	1	Pleuronema	1
Euglypha	1			Histrio	1
Cochliopodium	1			Spathidium	1
				Oxytricha	1
				Uroleptus	1
				Epistylis	1
				Podophrya	1
				Cinetochilum	1

In this paper only the following species have been considered as regards the effect of the varying conditions: *Bodo saltans* Ehrbg., *Trepomonas agilis* Dujardin, *Arcella vulgaris* Ehrbg., *Colpidium colpoda* Stein, *Paramoecium putrinum* Clap. and Lach., *Pleuronema chrysalis* Ehrbg., *Cinetochilum margaritaceum* Ehrbg., *Lionotus fasciola* Ehrbg.

II. Methods.

The object of the experimental filters was to convert a 0·2 per cent. solution of sucrose into carbon dioxide and water in the shortest possible space and time. Each filter consisted of six earthenware pipes each 12 in. long by 4 in. diameter; these were arranged in vertical series, each separated from the next by a space deep enough to allow of sampling the effluent from each section. The filters were filled with gravel ($\frac{1}{4}$–$\frac{3}{8}$ in.=0·6–1 cm.), and the sections were seeded with some of the film obtained from larger experimental filters which had been working at the Colwick factory of the Anglo-Scottish Beet Sugar Corporation for two years, and which therefore contained a representative fauna and flora. The filters were fed with about 5 litres of solution in 24 hours from an aspirator fitted with a Mariotte's constant head device. The solution unless otherwise stated had the following composition:

Sucrose	... ...	0·2 per cent.
NaCl	... ...	0·03 ,,
K_2HPO_4	... ...	0·03 ,,
KH_2PO_4	... ...	0·02 ,,
$MgSO_4$	... ...	0·01 ,,
$(NH_4)_2SO_4$	... ...	0·03 ,,
$CaCO_3$	... ...	0·03 ,,

A record was kept of the changes in acidity in each section and of the purification, as evidenced by the test for dissolved oxygen taken up in 5 days, which is a measure of the amount of reducing material present in the solution (**3**).

The decomposition of carbohydrates and ammonia in their passage through the filters is roughly as follows (**1**, **2**): in the first section the greater part of the sugar is converted into various organic acids, among which lactic, pyruvic, acetic and formic may occur; in the second and third sections these acids undergo oxidation, and in the fourth, fifth and sixth sections practically no carbohydrates are present; the ammonia disappears after the first section and amino acids are built up in the second section and to a lesser extent in the third; in the fourth section ammonia appears again from the amino acids and this is subsequently converted into nitrites and nitrates. Crudely therefore the sections may be classified as follows:

1. Organic acids, *p*H under 7·0.
2. Amino acids, *p*H under 7·0.
3. Amino acids decreasing, *p*H about 7·0.
4. Amino acids decreasing, appearance of nitrites and nitrates, *p*H over 7·0.
5. Very little organic matter, appearance of nitrites and nitrates, *p*H over 7·0.
6. Very little organic matter, appearance of nitrites and nitrates, *p*H over 7·0.

III. RESULTS.

The five principal factors which may be involved in determining the protozoan distribution are: hydrogen-ion concentration, purification, food supply, direct chemical action, and lastly the presence of large numbers of particular bacteria, developing in the solution in response to the presence of certain chemical substances.

Purification. As judged by the five-day tests the percentage of oxidisable material decreased from the top of the filter (section 1) down to the bottom (section 6), and to discover what effect this had on the Protozoa observations were made on the various sections. The percentage of occurrences of the different species of Protozoa varied with the different levels of the filter as is seen from Table I. In this table the percentage occurrences were calculated on the total number of observations made, that is the total number of times the organism might have occurred, in each section. The figures quoted in the tables bear no reference to the relative abundance of the animals on any one day, since presence or absence alone were recorded. It is difficult in such material to estimate actual numbers with any degree of accuracy, but under exactly similar conditions the numbers fluctuate to a very marked extent.

Table I. *Distribution of the species of Protozoa through the filters.*

	Section 1		Section 2		Section 3		Section 4		Section 5		Section 6	
	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Bodo	275	39·6	273	36·9	273	34·4	271	29·9	265	28·8	270	26·3
Trepomonas	244	36·9	250	40·0	247	28·7	245	21·2	249	17·6	245	14·3
Colpidium	274	64·6	274	74·4	274	59·1	274	58·0	274	39·0	274	27·0
Paramoecium	262	67·1	266	80·1	267	80·5	266	78·5	266	69·9	267	62·9
Pleuronema	271	56·1	270	61·5	270	69·6	270	54·1	270	45·2	270	41·1
*Arcella	162	11·7	162	22·8	162	40·7	162	67·6	162	71·2	134	72·4
*Cinetochilum	148	21·6	148	27·0	148	43·9	148	68·9	148	69·9	112	65·2

* These two species differ from the rest in that they were not present in all filters and the results for the periods when they were known not to occur have been omitted.

It will be noted that the protozoan species can be divided into three groups, firstly those like *Arcella* and *Cinetochilum*, which prefer the lower levels of the filter, secondly those like *Trepomonas*, *Colpidium* and *Bodo*, which are found mostly in the upper layers; and thirdly those which are comparatively indifferent, like *Paramoecium* and possibly *Pleuronema*.

This distribution is undoubtedly influenced by two main factors: the purity of the medium, and the food supply. *Cinetochilum* which feeds on organic débris, small organisms and bacteria (**4**) could undoubtedly obtain a more adequate food supply higher in the filter; it is therefore clear that it requires an environment relatively free from soluble organic compounds and so is limited in its range by the conditions of the effluent. On the other hand it is very problematical whether *Colpidium*, which feeds almost exclusively on bacteria (**4**, **5**), could obtain a living in the bottom sections, where the bacterial flora is greatly reduced, quite apart from any considerations of the purity of the medium, and so in this case the food supply is possibly the limiting factor. This was borne out by observation, for the animals in the lower sections were small and obviously starved.

Further information on these points was obtained when the filters were receiving only tap water. During this period *Colpidium* and *Bodo*, which commonly occur at the top, were reduced in numbers, while the percentage of occurrences of *Cinetochilum* changed from 10·7 in the sucrose solution to 65·5 in the tap water. The percentage occurrence of *Paramoecium* was comparatively unchanged (Table II).

Table II. *Percentage occurrences of species during* 14 *days with sucrose solution and* 14 *days with tap water, all sections added together.*

Species	Total No. of observations	0·2 % sucrose	Tap water
Paramoecium putrinum	84	77·4	82·1
Cinetochilum margaritaceum	84	10·7	65·5
Colpidium colpoda	84	77·4	19·1
Bodo saltans	84	78·6	17·8

Effect of p*H values.* The *p*H values in the various sections of the filters ranged from 4·3 to 8·0; the acid conditions being found more often in the first two sections. Tables III–IX have therefore been prepared from separate sections, so as to eliminate other variables, such as purification, as far as possible. The majority of the Protozoa found are able to live throughout the range of *p*H values, provided they are not adversely affected by other conditions, such as impurity, though in many cases the low or high *p*H values have a limiting effect. As the behaviour of the species is different, consideration of each one individually is necessary.

Trepomonas. As is seen in Table I this species is not an ubiquitous inhabitant of the filters; but when it does occur its distribution is largely affected by the purity of the solution. The *p*H values *per se* have no marked effect (Table III).

Colpidium. Here again the distribution is little affected by *p*H values though there is some evidence that neutrality or slight alkalinity are the most suitable conditions (Table IV).

Paramoecium. This species is practically indifferent to section distribution and occurs through a wide range of *p*H values, though less frequently in the more acid groups (Table V).

Pleuronema. In the case of this animal the distribution through the sections is similar to that of *Paramoecium* except that there is a more definite falling off in the first and last sections. This is of interest in view of the fact that there is a narrower *p*H range in which *Pleuronema* occurs. As is seen from the table the optimum range lies between 6·5–7·6, and as the *p*H values outside this range tend to occur in the top and bottom sections the lower numbers of occurrences in these two situations are explained (Table VI).

Arcella. *Arcella* shows a very marked preference for the lower sections of the filter. In these sections (5 and 6) it occurs freely at all pH values; nevertheless in these two sections and still more in sections 1 and 2 there is definite evidence that it prefers an acid medium (Table VII).

Cinetochilum. *Cinetochilum* also shows a preference for the three lower sections; but in this case, although it can occur freely at pH values down to 6·1 greater degrees of acidity definitely act as limiting factors (Table VIII).

Bodo. This particular species shows a slight falling off in numbers at the bottom of the filter; but like *Trepomonas* it is not one of the commoner forms found. As regards its pH distribution it exhibits curious irregularities, for reference to the table demonstrates that maximum development occurs at an acid value of about 5·3 and again at an alkaline one of about 7·6. It is possible that this peculiarity was due to the presence of different physiological strains, but the evidence is insufficient to establish this with certainty (Table IX).

Effect of food supply. The effects of three changes in the composition of the solution to be filtered were studied, these changes being the omission of phosphate, the substitution of urea for ammonium sulphate, and of lactic acid for sucrose. The period during which phosphate was omitted was only a short one, 11 days: and though only tentative conclusions can be drawn from such limited data, it was thought worth while to present the results. Table X gives the percentage of occurrences of the different species during the period when the filter was receiving no phosphate compared with the previous 11 days when the population was receiving a normal diet. In general the omission of phosphate causes a reduction in numbers, but *Colpidium* and *Cinetochilum* are exceptions to this rule. The slight falling off of the other species is what might be expected owing to the lowering of the bacterial numbers and therefore the food supply; but this would only be a gradual effect as the filter for the first few days had reserves of phosphate. Why this effected *Colpidium* and *Cinetochilum* in the reverse manner is inexplicable.

The effect of urea (Table XI) was a depressing one on most species though again *Colpidium* was increased as was *Pleuronema*. These results are interesting, though difficult of explanation, since the expectation would be that urea would have no effect, for it normally breaks down to give salts of ammonia.

Another set of conditions connected with a change in the food supply arose when lactic acid was given to one filter instead of sucrose, the object being to study the effect of extreme acidity on the protozoan population. The average pH value of the effluent from the top section during the period when lactic acid was given was 4·0, but in all the other sections the effluents showed no increase in acidity above the normal values that were found when the filters were receiving sucrose. As is seen from Tables III–IX a pH value of less than 4·9 limits the occurrence of all the protozoan species under

Table III. Trepomonas agilis. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	27	0·0	8	25·0	—	—
4·9–5·2	5	40·0	5	60·0	—	—
5·3–5·6	17	41·1	17	58·7	16	33·3
5·7–6·0	36	41·7	39	46·1	2	50·0
6·1–6·4	23	52·3	36	47·2	21	14·2
6·5–6·8	70	62·0	115	40·0	59	8·4
6·9–7·2	38	55·1	66	39·4	146	18·5
7·3–7·6	10	30·0	14	21·4	72	15·3
Over 7·6	5	40·0	8	37·5	22	27·1

Table IV. Colpidium colpoda. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	36	38·9	8	50·0	—	—
4·9–5·2	10	50·0	6	50·0	—	—
5·3–5·6	72	68·1	21	47·6	6	50·0
5·7–6·0	34	67·6	30	83·3	3	66·6
6·1–6·4	25	84·0	34	73·5	21	81·1
6·5–6·8	86	86·1	112	58·1	66	19·7
6·9–7·2	40	90·0	70	72·8	150	48·0
7·3–7·6	13	69·3	14	100·0	71	26·7
Over 7·6	5	100·0	8	100·0	22	68·2

Table V. Paramoecium putrinum. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	36	25·0	8	37·5	—	—
4·9–5·2	12	33·3	6	66·6	—	—
5·3–5·6	29	41·8	19	26·3	6	33·3
5·7–6·0	38	50·0	40	57·5	3	100·0
6·1–6·4	26	61·6	33	75·8	21	28·5
6·5–6·8	90	83·3	111	81·1	64	65·5
6·9–7·2	52	82·7	63	68·3	147	74·4
7·3–7·6	12	66·7	14	71·5	71	63·4
Over 7·6	5	80·0	8	75·0	22	63·1

Table VI. Pleuronema chrysalis. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	32	0	8	12·5	—	—
4·9–5·2	12	8·5	6	0	—	—
5·3–5·6	27	25·9	19	5·2	6	0
5·7–6·0	39	20·5	40	32·5	3	0
6·1–6·4	30	53·3	34	47·1	21	23·9
6·5–6·8	98	77·5	112	78·6	65	23·1
6·9–7·2	52	88·0	68	66·2	149	55·0
7·3–7·6	10	50·0	14	100·0	70	61·4
Over 7·6	5	60·0	8	25·0	22	18·1

Table VII. Arcella vulgaris. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	1	0	2	100·0	—	—
4·9–5·2	3	33·3	1	0	—	—
5·3–5·6	13	15·4	17	23·5	2	100·0
5·7–6·0	32	9·4	29	31·1	7	100·0
6·1–6·4	36	13·9	29	34·5	33	89·9
6·5–6·8	41	9·7	52	7·7	98	68·4
6·9–7·2	62	14·5	52	9·6	168	71·5
7·3–7·6	2	0	3	0	13	69·3
Over 7·6	0	0	0	0	0	0

Table VIII. Cinetochilum margaritaceum. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	2	0	4	0	—	—
4·9–5·2	2	0	3	0	—	—
5·3–5·6	15	0	17	5·9	2	0
5·7–6·0	32	9·4	32	21·8	7	28·5
6·1–6·4	34	23·5	31	35·5	34	55·8
6·5–6·8	43	27·9	45	22·2	98	70·5
6·9–7·2	34	29·4	42	28·6	136	65·5
7·3–7·6	0	0	3	33·3	4	50·0
Over 7·6	0	0	0	0	0	0

Table IX. Bodo saltans. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	36	5·5	8	25·0	—	—
4·9–5·2	11	54·5	5	20·0	—	—
5·3–5·6	25	56·0	20	60·0	16	33·3
5·7–6·0	29	27·6	38	65·8	3	33·3
6·1–6·4	23	43·5	39	43·5	21	28·5
6·5–6·8	72	52·8	121	30·6	66	27·2
6·9–7·2	43	74·4	73	28·8	149	24·8
7·3–7·6	9	55·5	14	21·4	62	32·2
Over 7·6	5	80·0	8	75·0	22	91·0

Table X. *Effect of the omission of phosphate.*

		Percentage occurrences	
Species	Total No. of observations. Sections 1–5	Normal diet	No phosphate
Colpidium	55	19·8	73·3
Paramoecium	55	61·2	48·6
Cinetochilum	55	45·0	59·4
Pleuronema	55	50·4	43·2
Bodo	55	52·2	14·4
Arcella	55	50·4	32·4
Trepomonas	55	41·4	28·8

Table XI. *Effect of urea.*

Species	Total No. of observations. Sections 1–6	Percentage occurrences: No urea	Percentage occurrences: Urea
Colpidium	148	19·4	25·5
Paramoecium	148	79·8	63·0
Cinetochilum	148	51·6	42·9
Pleuronema	148	54·9	74·4
Arcella	148	57·0	40·9

Table XII. *Effect of lactic acid.*

		Percentage occurrences					
	Total No. of observa-tions	Before lactic. Sections		Lactic. Sections		After lactic. Sections	
Species		1 2 3	4 5 6	1 2 3	4 5 6	1 2 3	4 5 6
Bodo	36	39·2	8·4	5·6	2·8	0	5·6
Trepomonas	36	58·3	2·8	25·2	2·8	14·0	0
Colpidium	36	81·2	25·2	0	0	2·8	0
Paramoecium	36	98·0	100·0	70·0	98·0	100·0	75·5
Pleuronema	36	95·2	42·0	33·6	22·4	92·4	11·2
Lionotus	36	30·8	84·0	2·8	5·6	47·5	2·8

consideration to some extent, therefore as might be expected the top section during the lactic acid period was almost devoid of living Protozoa, though bacteria and fungi were present and good purification was ultimately obtained. The fact that the percentage purification brought about by the top section changed from 17 on the second day of the experiment to 58 on the fourteenth day suggests that the bacterial population also underwent a marked change, strains presumably being selected out from the normal population which were able to oxidise the acid, while the development of the sucrose splitting strains was inhibited. Table XII shows the percentage occurrences of each species in the top and bottom parts of the filter during the lactic acid period, when observations were made during 12 days, and also during the 12 days immediately preceding and following this period when the food supply was normal. It will be seen that *Paramoecium* shows no change in numbers except in the top half of the filter during the acid period, the diminution being entirely due to the absence of this organism from the top section (average pH 4·0). *Pleuronema* was depressed throughout the acid period but recovered as soon as normal food was resumed; the other four species considered not only were very much reduced during the acid period, but showed little or no recovery in the succeeding period, except in the case of *Lionotus* when there was a definite rise in numbers. In the case of the other species no recovery took place, and they were not seen until the gravel of the filter had been autoclaved and reinoculated. Reinoculation of the sections before sterilisation had no effect. These results suggest that in these species it is the flora encouraged by the lactic acid that is inimical, while in the case of *Pleuronema* and still more in that of *Lionotus* it is possibly the oxidation products of the acid that inhibit development.

IV. ACKNOWLEDGMENT.

The investigation described in this paper was carried out at Rothamsted Experimental Station as part of the programme of the Water Pollution Research Board of the Department of Scientific and Industrial Research and is published by permission of the Department.

V. SUMMARY.

1. The purity of a medium, as measured by the amount of reducing material present in the solution, and the food supply, are two of the principal factors influencing the distribution of Protozoa in sewage filters.

2. The Protozoa considered occur throughout a wide range of pH values, but the optima for different species are different.

3. Where chemical compounds added to the solution affect the protozoan population adversely, it may be due either to the formation of deleterious oxidation products, or to the development of a bacterial flora which is inimical to the Protozoa.

REFERENCES.

(1) **Barritt, N. W. (1931).** "The biological filtration of dilute sucrose solutions." Biochem. J. 25, 1419–46.

(2) **Jenkins, H. S. (1931).** "The biological oxidation of carbohydrate solutions. Part I. The oxidation of sucrose and ammonia in sectional percolating filters." Biochem. J. 25, 147–60.

(3) **Ministry of Health (1929).** "Methods of chemical analysis as applied to sewage and sewage effluents." H.M. Stationery Office.

(4) **Noland, L. E. (1925).** "Factors influencing the distribution of fresh-water ciliates." Ecology, 6, 437–52.

(5) **Roux, J. (1901).** "Faune infusorienne des eaux stagnantes des environs de Genève." Mém. de l'Inst. Genève, 19.