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A Microbiological Study of Earthworm Casts

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SUMMARY

Microbiological, physical and chemical changes were followed in worm casts ageing in the field. Filamentous fungi and yeasts increased in number rapidly after the cast was produced, but not bacteria or actinomycetes which were initially numerous. Measurements of hyphal length confirmed the increased growth of fungi. Ageing casts showed a declining respiratory activity, possibly because the bacteria formed resting stages. Aggregate stability increased rapidly as casts age, probably due to increasing amounts of fungal hyphae. Polysaccharide content of casts was much greater than that of soil, but did not vary with changes in stability. Total and mineral nitrogen levels of casts were greater than those of soil; the major part of the inorganic nitrogen occurred as ammonia which was rapidly converted to nitrate.

INTRODUCTION

Earthworms feeding on soil and plant material set in train microbiological changes in the ingested food that continue when the cast is formed. Most microbiological investigations on worm casts have been concerned with the number of micro-organisms present. Stockli (1928), Kollmannsperger (1952, 1956), Ruschmann (1953*a*) and Schultz & Felber (1956) all reported more organisms in the gut or casts of earthworms than in the surrounding soil. Teotia, Dudley & McCalla (1950) found more bacteria but fewer fungi in casts than in soil and Rommell (1935) and Nef (1957) suggested that the feeding habits of earthworms alter the balance of soil population in favour of bacteria, so producing mull rather than mor conditions. Contrary to the findings of most workers, Day (1950) with *Lumbricus terrestris* in pot experiments found no significant difference between the numbers of bacteria in casts and soil. The present work examines changes in the microbial status of casts as they age under field conditions.

METHODS

The casting habits of earthworms vary; some species form casts on the soil surface, others within the soil in cracks, and some use cast material to line their burrows. Of the common field species in Britain, *Allolobophora terrestris* (Sav.) forma *longa* (Ude) and *A. nocturna* (Evans) are the main surface-casting types. Other species may form surface casts at times, but the habit is not general.

Casts are built up progressively by the frequent addition of small amounts of fresh material, so that different parts of a large cast are of different ages. This fact

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was taken into account when making observations on ageing casts. The casts used were produced by *Allolobophora terrestris* forma *longa*; casts and soil came from Great Field IV at Rothamsted. An area was freed from cast material and thereafter fresh casts were collected each day. These were bulked in groups of five and left in marked positions in the field and collected for examination as required. All soil samples were obtained from an area close to where the casts were collected.

Counting methods and media. These were the same as previously described (Parle, 1963), and included the use of selective media for cellulolytic and chitinolytic organisms. Proteolytic organisms were counted on a gelatin medium containing 200 g. powdered gelatin in 1000 ml. water, sterilized by steaming on three successive days.

Yeasts were identified by the methods of Lodder & Kreger-van Rij (1952), from poured plates. Fungal hyphae were measured by the method of Jones & Mollison (1948) using a 45 × objective and 10 × eyepiece with a micrometer scale.

Respiration was studied in the Warburg apparatus, using a slurry of the material to overcome differences in moisture content in casts of different ages (Chase & Gray, 1957).

Total nitrogen was estimated by the Kjeldahl method and mineral nitrogen by the methods of Bremner & Shaw (1955).

Bacterial polysaccharides were extracted by the method of Forsyth (1947) as modified by Chesters, Attoe & Allen (1957).

Aggregate stability was measured by the sodium saturation technique of Emerson (1954).

RESULTS

Numbers of micro-organisms in fresh casts and their variation with age

The only work so far reported on the change in microbial numbers with cast age is by Stockli (1928) who found that the numbers increased over the first 7 days. In an attempt to investigate this further, counts were made on casts collected after 1, 3, 5 and 20 days.

The counts in Table 1 confirm previous work (Parle, 1963) which showed a large increase in the microbial population passing through the worm gut. Rather higher soil populations were found than in the earlier work, probably because the soil organic matter increased during autumn. As the casts aged, the recovery of yeasts and filamentous fungi increased and actinomycetes appeared to increase toward the end of the period; bacterial numbers remained high. Irregular results were obtained with the counts of cellulose decomposers, but these appeared to be more numerous in fresh casts than in soils. Chitinolytic and proteolytic bacteria were fairly abundant in soil and casts and tended to decrease as the casts aged (except for chitinolytic bacteria at 20 days).

Fungi in ageing casts. The most notable feature of the cast material was the large increase in the number of yeasts and filamentous fungi with age; such an increase has not been reported before.

The most commonly occurring species of yeasts from cast material were: *Cryptococcus diffluens* (Zach.); *C. laurentii* (Kuff.); *Candida humicola* (Daszewska); *Trichosporon pullulans* (Lidner). Less frequently were found: *Candida curvata* (Diddens et Lodder); *Debaryomyces hansenii* (Zopf.); *Trichosporon cutaneum* (de

Table 1. *Numbers of micro-organisms in soil and in casts of* Allolobophora terrestris

(Mean log numbers per g. dry weight.)

Yeasts	Fungi	Actino-mycetes	Bacteria	Cellulose decomposers	Chitin decomposers	Gelatin decomposers
			No. of micro-organisms in soil			
5·20 (±0·069)	6·18 (±0·093)	7·60 (±0·170)	8·88 (±0·167)	1·48 (±0·452)	7·61 (±0·175)	7·30 (±0·268)
			No. of micro-organisms in casts			
			Fresh			
5·69	6·68	8·10	10·38	4·06	7·85	6·69
			One-day			
5·77	7·13	9·88	10·70	1·39	7·73	7·45
			Three-day			
5·80	6·88	8·41	10·16	3·39	7·51	6·74
			Five-day			
5·87	6·83	7·69	10·58	1·74	7·13	6·95
			Twenty-day			
6·17 (±0·115)	7·11 (±0·155)	9·81 (±0·284)	10·81 (±0·279)	0·00 (±0·756)	8·04 (±0·293)	2·43 (±0·448)

Table 2. *Fungi from casts of different ages*

Fungi	Soil	Fresh cast	Age in days: 1	2	3	10	15	20	30	50
	Percentage distribution									
Expt. 1										
Absidia	—	—	3·7	3·7	3·7	8·6	3·7	—	—	—
Cephalosporium	5·6	14·8	9·3	7·4	9·2	7·4	5·6	5·6	5·6	4·8
Cladosporium	11·1	3·0	3·7	5·6	5·6	5·6	7·4	5·6	—	3·2
Fusarium	—	—	—	3·7	—	—	3·7	—	—	—
Gliocladium	7·4	7·4	16·7	1·8	11·1	13·0	—	3·7	14·8	16·0
Oidiodendron	1·8	35·1	25·9	37·0	40·7	46·3	37·0	40·7	37·0	40·0
Penicillium	46·3	14·8	22·2	13·0	7·6	7·4	16·7	22·2	14·8	16·0
Trichoderma	5·6	—	1·8	—	1·8	—	11·1	—	3·7	—
Sterile	13·0	8·5	9·3	7·4	9·2	—	14·7	7·4	14·8	12·8
Unidentified	9·3	13·0	7·4	20·4	11·1	14·7	—	14·8	9·3	7·2
Expt. 2										
Cephalosporium	—	13·8	—	5·0	—	3·8	7·7	—	—	—
Cladosporium	1·8	28·9	—	5·0	11·5	9·7	15·4	5·8	7·7	3·8
Diplococcium	—	—	—	30·8	26·9	40·4	15·4	18·5	23·1	30·9
Fusarium	6·1	—	—	—	—	—	—	—	—	—
Geotrichium	5·4	—	11·8	3·8	—	—	—	3·8	3·8	5·8
Gliocladium	6·1	14·6	6·1	7·7	—	—	15·4	—	19·3	11·5
Oidiodendron	—	—	—	—	—	3·8	—	—	—	—
Penicillium	34·2	28·9	28·6	11·5	26·9	24·6	30·7	23·1	23·1	17·4
Trichoderma	1·4	—	—	—	11·5	—	—	4·6	3·8	11·5
Verticillium	—	—	12·5	9·2	3·8	7·7	7·7	13·5	7·7	3·8
Sterile	20·0	13·8	23·2	15·5	9·7	10·0	7·7	13·5	3·8	11·5
Unidentified	25·0	—	17·8	11·5	9·7	—	—	17·3	7·7	3·8

Beurm, Gougherot et Vaucher), and *Rhodotorula glutinis* (Fres.). Too few isolates were made to show whether any species were selected as casts age. None of the species isolated ferments sugars except *D. hansenii* which ferments glucose and sucrose weakly. These results are in accord with those of di Menna in New Zealand (personal communication) who rarely finds fermenting yeast species in soil.

Martin, Anderson & Coates (1942) examined changes in fungal populations as organic matter decomposed and found that the predominant fungal species depended on the availability of the energy source. This might also be expected in ageing casts. Casts of different ages and produced at different periods were sampled and plated on Lochhead's soil extract agar containing 30 μg. aureomycin/ml. Fungi appearing on the plates were identified after incubation for 14 days. Results of two such experiments done at different times (Table 2) show the relative abundance of the various groups as a percentage of the total isolates from a single sample.

On the occasion of the first count in November there was a preponderance of one genus, Oidiodendron, in the cast material of all ages, although very few were isolated from the soil. Oidiodendron occurs in association with decaying wood and litter (Dennis & Wakefield, 1946; Smith, 1946; Tribe, 1957) but is not common in soil. On the occasion of the second counts, starting 2 months later, Oidiodendron was found only once and Penicillium and Diplococcium species predominated. This suggests that no particular group of fungi is selected in the cast, but that the composition of the fungi population differs from time to time, possibly depending on the flora of the plant material recently eaten by the worm. Neither Oidiodendron nor Diplococcium was recovered from litter collected from the experimental area, but Penicillium and Cladosporium species were common.

Table 3. *Length of fungal hyphae per g. dry weight of casts of* Allolobophora terrestris

Sample	Length (m./g.)	Sample	Length (m./g.)
Expt. 1		Expt. 2	
Soil	79·0 ± 16·6	Fresh cast	120·8 ± 14·9
Fresh cast	133·4 ± 7·6	2-day cast	158·1 ± 16·2
5-day cast	284·2 ± 11·8	5-day cast	151·4 ± 13·3
10-day cast	434·8 ± 15·2	10-day cast	273·1 ± 14·5
15-day cast	607·3 ± 29·0	15-day cast	384·8 ± 15·1
20-day cast	528·5 ± 25·4	20-day cast	182·1 ± 12·2
25-day cast	408·4 ± 19·0	25-day cast	248·9 ± 13·5
45-day cast	415·4 ± 18·3		

The lengths of fungal hyphae in casts and soil

In much of the work on soil fungi, and in all that on fungi associated with earthworms, the dilution plating technique has been used, in spite of serious objections to this method for quantitative work. Hinson (1954) and Warcup (1955, 1957) showed that in many soils most colonies arising on dilution plates come from spores and not from growing fungi. To obtain some measure of the true fungal content of casts, total lengths of fungal hyphae were measured in material of different ages. Lengths were corrected for dry weight of the sample and results of two separate experiments are given in Table 3.

Good reproducibility was obtained within samples probably because the fine nature of the cast allowed even dispersal. The length of fungal hyphae increased greatly with age over a period of 15 days after the casts were formed. Some of this increase came from germinating spores and considerable amounts of young deeply staining material were seen. After 15 days the hyphal length declined slowly, because the fungi disintegrated.

Changes in microbial activity of ageing casts

Where there are large fluctuations in bacterial numbers, soil respiration is a valuable index of microbial activity (Chase & Gray, 1957; Katznelson & Stevenson, 1956; Rovira & Greacen, 1957 and Drobnik, 1958). Approximately 1 g. net weight of material to be examined was weighed into a Warburg flask and 2 ml. distilled water added; 0·2 ml. 20 % KOH was placed in the centre well. Duplicate flasks were prepared for each sample and hourly readings were made for 8 hr. at 25°. Flasks were shaken at 120 strokes/min. All values were corrected for the dry weight of the sample. Results are shown in Fig. 1, where each point represents the average oxygen uptake of duplicate flasks over the 8 hr. period.

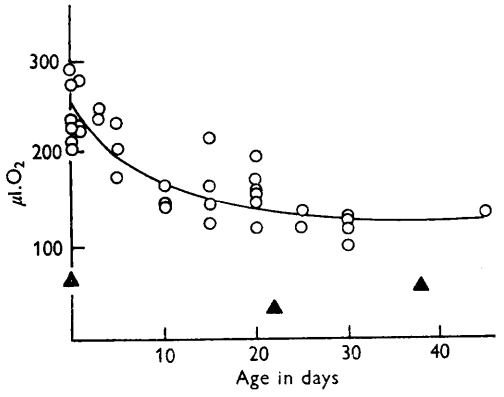

Fig. 1

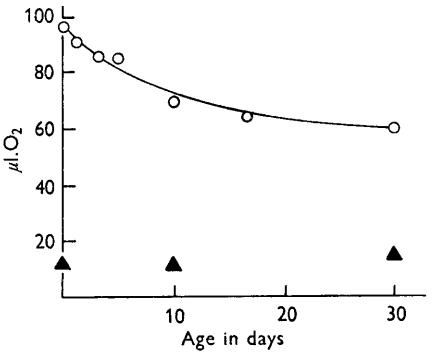

Fig. 2

Fig. 1. Oxygen uptake in 8 hr. by cast material of different ages. ○, Cast; ▲, soil.

Fig. 2. Oxygen uptake in 1 hr. by cast material of different ages in the presence of excess glucose. ○, Cast; ▲, soil.

The gradual fall in respiratory activity may be caused either by the number of respiring organisms decreasing or by the depletion of energy sources. To find which was primarily concerned, further experiments were made on the same cast material with added carbohydrate. After equilibration in the Warburg flask, 0·5 ml. of 0·005 M-glucose was added from a side arm to provide an excess energy source and readings were made at 10 min. intervals for 1 hr. This short time was chosen to minimize effects of microbial reproduction on oxygen uptake. Figure 2 shows that the pattern of oxygen uptake resembled that in the previous experiment, again indicating a decline in the number of actively respiring organisms with age of cast. This decline does not correspond with any of the population changes noted earlier and will be considered in the discussion.

Cellulose decomposition

The method used to count cellulolytic organisms gave irregular results and this group was studied further using the Warburg apparatus. Two sets of flasks were used, a control set and one containing 0·5 ml. of a cellulose suspension. Flasks were incubated with shaking for 96 hr., readings were taken for 12 hr. during the day and the manometer taps opened overnight.

Figure 3 shows ml. of oxygen used by casts of different ages. The number of cellulose decomposers fell as the casts aged, but activity was still greater than that of the soil after 40 days.

The nitrogen status of worm casts

Several workers have compared the chemical decomposition of worm casts with soil. It has been claimed that nitrification is enhanced in casts (Joshi & Kelkar, 1952; Day, 1950) and that casts contain more nitrate, amino acid and total nitrogen than soil (Lunt & Jacobson, 1944; Wittich, 1953; Shrinkhande & Pathak, 1951).

Total nitrogen in five sets of bulked casts is shown to be double that on two soils from the same area (Table 4).

Mineral nitrogen in casts was determined on 15–20 g. samples of material. The

Table 4. *Nitrogen contents of casts of* Allolobophora terrestris *and of soil*

	Sample	Total N (mg./g. dry wt.)
Cast	1	5·41
	2	5·52
	3	5·85
	4	5·99
	5	6·01
Soil	1	2·29
	2	2·34

Table 5. *Mineral nitrogen/g. dry weight of cast material*

Age (days)	Total mineral N (μg.)	NH_3-N (μg.)	NO_3-N (μg.)	NH_3-N (%)
0	282·3	272·8	9·5	96·6
	340·4	330·5	9·9	97·0
1	370·4	330·5	39·8	89·2
	404·3	362·5	41·8	89·7
2	256·7	239·1	17·5	93·2
	263·4	244·3	19·1	92·8
5	344·4	295·2	14·3	85·7
	339·4	294·9	13·1	86·9
7	278·7	222·3	56·4	79·8
	244·3	206·5	37·8	84·5
10	327·8	273·8	54·0	84·4
	262·1	237·6	24·5	90·7
20	134·6	82·3	52·3	61·1
	201·8	130·9	70·8	64·9

$CaCO_3$ excreted by the worm interfered with the determination and excess CO_2 was therefore first removed by placing soil extracts in a vacuum desiccator for 1 hr. before analysis; any loss of volume was made up with fresh extracting solution.

Table 5 shows the changes in amounts of nitrate and ammonia in casts of different ages. The percentage of total mineral nitrogen occurring as ammonia is also given. Each value is the average of duplicate analyses in different sets of casts. Because much cast material was required for each analysis the figures in the table represent the analyses on different samples collected on consecutive days. The results show a steady decline in content of ammonium nitrogen and an increase in nitrate. Nitrification begins immediately, as would be expected with such high levels of ammonia in the soil, much of the nitrogen lost from the cast during the 20-day period may have been nitrate.

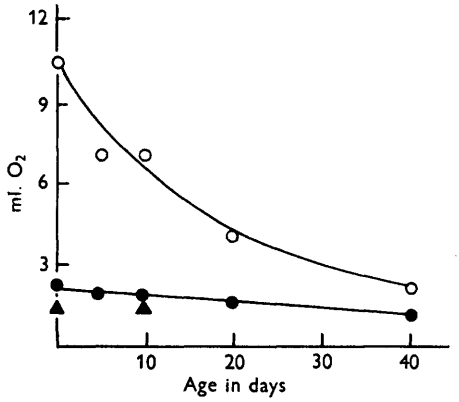

Fig. 3

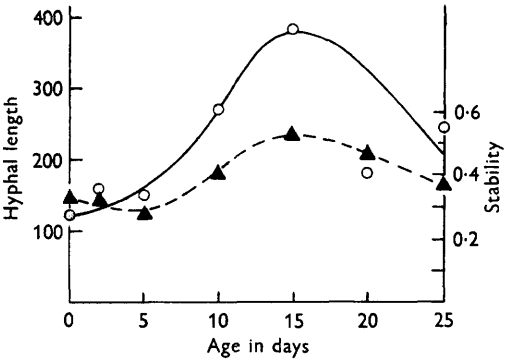

Fig. 4

Fig. 3. Oxygen uptake by casts of different ages in 96 hr. with cellulose as substrate. ○, Cellulose; ●, control; ▲, soil.

Fig. 4. Length of hyphae in m./g. and aggregate stability in ageing casts. Stability determined by ratio of permeability before and after sodium saturation. ○, Hyphae; ▲, stability.

The structural stability of casts

Fungi increase greatly in ageing casts and to investigate their possible stabilizing role, measurements of aggregate stability were made on casts of different ages, containing different amounts of fungal hyphae. Fungi are claimed by Martin & Waksman (1940) and McCalla (1945) to stabilize soil crumbs. Each set of measurements required approximately 15 g. of 1–2 mm. air-dried crumbs. To provide these, 60–80 g. wet weight of casts were collected daily and aged under normal field conditions. When required the samples were air-dried to constant weight, carefully broken and sieved to obtain the 1–2 mm. fraction. Three 5 g. samples were used to measure stability and some of the remaining material was used to prepare Jones & Mollison slides.

Figure 4 shows that stability alters as the casts age, rising to a peak at 15 days and then diminishing. The changes in stability are clearly correlated with length of fungal hyphae, indicating that the fungal content of casts largely determines their structural stability.

Bacterial polysaccharides and cast stability

Swaby (1948) and others suggested that bacterial gums play a part in increasing the aggregate stability of worm casts, and an attempt was made to examine this factor. Casts were collected from the same area and aged in the same way as for the previous experiment; 25 g. wet weight of casts was treated to extract polysaccharides which were then freed from inorganic impurities, dried at 50° and weighed.

Table 6 shows that much more polysaccharide was extracted from casts than from soil. The amounts of polysaccharide extracted at different times, however, were not correlated with cast stability. The 30-day-old cast may have lost polysaccharide because of decomposition by micro-organisms.

Table 6. *Weight of polysaccharide extracted/g. dry wt. of cast and percentage carbon in extracted material*

Cast age in days	Polysaccharide (mg./g. sample)	Carbon %
0	4·9	34·9
2	5·2	37·9
5	5·5	38·2
10	3·8	36·2
15	5·5	39·2
20	4·9	—
30	1·4	40·0
Soil	0·75	—

DISCUSSION

The high number of actinomycetes and bacteria in casts confirm earlier work, but these showed no consistent changes as the casts aged. The decline in microbial respiration with increasing age of casts, while microbial counts remained unchanged, suggests that the bacteria and actinomycetes formed resting stages. Schultz & Felber (1956) considered that bacteria in casts occurred mainly as spores. Counts and direct measurements showed that the fungal population increased greatly as casts aged a result contrary to those of Teotia *et al.* (1950) and Swaby (1949). Some reports (Rommell, 1935, and Nef, 1957) suggest that earthworms induce a predominantly bacterial population in soil by destroying fungal hyphae. My results do not accord with this view. The number of fungi found in fresh casts was not significantly different from the number in soil. Germinating fungal spores were commonly seen in fresh casts but only rarely in soil. The fungistatic factor found in soil by Jackson (1958) may not occur in casts and so allow spores to germinate. Although the stimulation of fungal growth was brief, lasting for about 15 days, the continuous ejection of cast material by worms would probably permanently increase the activity of the soil fungal population. The methods used do not show that worm activity favours the selection of any particular group of fungi, but more detailed examination is required.

Freshly formed casts are already stable. Such casts contain much polysaccharide which may play some part in stabilizing the aggregate. The suggestion by Swaby (1948) and others that such gums are water-soluble and will have little effect on aggregation is questionable, because little is known of their properties in soil and

they may be altered substantially by the extraction procedure; more gum is extracted by sodium hydroxide than by water. As the casts age, the stability of the 1–2 mm. fraction increases; the length of fungal hyphae also increases, and the increased stability may come from mechanical strengthening by fungi.

Results confirm that cast material contains more nitrogen than the surrounding soil, which is readily understandable when the selective feeding habits of worms are considered. Nye (1955) showed that when worms are kept in pots and all the nitrogen accounted for, their activity does not increase total nitrogen of the system. However, it is evident from analyses of mineral nitrogen in casts that worms change the character of ingested nitrogen, and this is probably important in the nitrogen cycle. Ammonia in fresh casts forms about 96 % of the extractable mineral nitrogen and this is rapidly converted to nitrate. Barley & Jennings (1959) showed that *Allolobophora caliginosa* increased available nitrogen by 6 %. With such high levels of mineral nitrogen as occur in casts there is little likelihood of atmospheric nitrogen fixation, and the antibiotic activity of Nocardia against Azotobacter noted by Ruschmann (1953*a*, *b*) has little significance. Moisture conditions under which worms work seldom favour the action of denitrifying organisms (Bremner & Shaw, 1958). The major contribution of earthworms in the nitrogen cycle in soil appears to be to increase the rate of mineralization of organic nitrogen.

In general, the value of earthworms in soil seems to lie in the fact that they increase the rate at which soil organic matter breaks down and in so doing may improve soil physical conditions for plant growth.

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