Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 95, No. 3, June 1986, pp. 341–351.

Biological activity of earthworm casts: An assessment of plant growth promotor levels in the casts

R V KRISHNAMOORTHY and S N VAJRANABHAIAH*

Department of Zoology and *Department of Crop Physiology, University of Agricultural Sciences, Bangalore 560 065, India

MS received 27 March 1985; revised 31 August 1985

Abstract. Biochemical analyses and auxin and cytokinin bioassays were performed to test the biological activity of wormcasts. Both cellulose paper pulp and soil casts of *Lampito mauritii* were rich in ammonia, urea, organic carbon content, organic matter, soluble phosphorus and ionic potassium levels. The total nitrogen content of the soil remained unaffected by worm activations. The casts of *Lampito mauritii*, *Pheretima elongata*, *Pontoscolex corethrurus* and *Ocnerodrilus occidentalis* had greater urea levels relative to ammonia levels in contrast to those of *Drawida barwelli*, *Octochaetoides beatrix* and *Perionyx excavatus*. Worm activations of the leaf compost amended-soils by these species reduced the total phenol levels to varying degree depending on the species examined. Aging and exposures to light reduced the activity levels of these plant growth promotors in the casts of *L. mauritii*. A positive correlation between the worm density at the site of soil sampling and the plant growth promotor levels in the samples was obtained. The origin of these promotors from the gut microflora of the worm and their subsequent release into the environment were discussed.

Keywords. Tropical earthworms; earthworm casts; composition of wormcasts; plant growth promotors in wormcasts; biological activity of wormcasts.

1. Introduction

In tropical biotopes the wormcast production ranges from 13–278 tons ha^{-1} y^{-1}, (Ljungstrom and Reinecke 1969; Lavelle 1978; Dash and Patra 1979; Krishnamoorthy 1985). The casts can be an excellent soil adjuvant besides their value as biofertilizer (Tomlin 1983). The casts maintain more nutritive materials like oxidizable carbon, nitrogen Ca, Mg, K, Na and P than the soil (Dash and Patra 1979; Kale and Krishnamoorthy 1981). The conversion of soil organic matter into vermicompost or casts reduces the total nitrogen content of the ingested material. The NPK value of the casts is always lower than that of an acceptable standard fertilizer (Tomlin 1983). Several trials performed in the past have indicated (Keshavamurthy 1978; Reinecke and Visser 1980) that the vermicompost or wormcasts could promote the lush growth of plants. It is believed that this property may be due to the occurrence of plant growth promotors in the casts which might be a resultant of interactions existing between the microflora and the earthworm's gut (Satchell 1983). Hence, an intensive study of wormcast composition and the levels of plant growth promotors in the casts were made.

2. Material and methods

2.1 *Collection of worms and cultures*

The worms were collected from woodland and grassland areas of Bannerghatta, about 25 km south of Bangalore. They were stocked in culture pots with garden soil mixed

with activated dung manure (3:1 proportion) as food. Five hundred adult (clitellate) worms of each species were left in a plastic box by 15 × 20 cm square by 10 cm height (1:2 w/w) containing 1 kg decomposing leaf litter compost-amended garden soil. The floor of each box was made of bolting cloth with 35 mesh pores as described earlier (Satchell and Martin 1984). After introducing the worms, the boxes were covered with a black cloth to prevent interference of light on worm activity. Prior to introduction, the worms were allowed to gut-void for 8 hr into a clean Petri dish and the excrements were separated. A similar box containing the soil but without worms served as control. After 3 days of worm activation at 25 ± 2°C the worms were hand sorted and separated from their castings and soil in the box. The contents of the control box and worm activated box were mixed and used for analysis.

Cultures of each species were left for one week for one hundred gut-voided worms to work through finely shredded paper pulp weighing 250 g taken in a plastic box as described above. A box containing the paper pulp without worms served as control. One gram of phytin (calcium inositol hexaphosphate) was mixed to each of 40 g of paper pulp. Samples of fresh faecal material and of the control medium equivalent to 1 g dw were then collected for the study.

2.2 *Worm densities and soil samples*

Worm densities in different biotopes around Bangalore were quantified by quadrat sampling technique (Lewis and Tayler 1968). Soil samples of the same biotopes were collected at random from sub soil layer (5–10 cm below the surface) and used for assays.

2.3 *Extraction of plant growth promotor substances*

One kg castings, pooled from 4 to 5 paper pulp culture sets or soil casts collected from the field afresh, were boiled in 2 l distilled water on a water bath for 30 min and filtered through a cheese cloth. Dilute phosphoric (1 : 10 v/v) acid was added drop by drop until the pH of the filtrate dropped down to 3·00. Then 200 ml ethyl acetate was mixed. The ethyl acetate fraction was separated by centrifugation and then removed by aspiration. The debris was extracted with ethylacetate in the same way and the fractions were combined and neutralised by partitioning with 5% aqueous $NaCO_3$ in a separating funnel. The ethyl acetate fraction was concentrated under low pressure and the concentrate was chromatographed.

2.4 *Thin layer chromatography of growth promotors*

Silicated thin layer chromatograms of 50 μl ethyl acetate fractions were approximately run for 45 min under red light in a darkroom to check the purity of auxin and cytokinins. Marker plots were made with indol acetic acid and benzyl adenine (both obtained from Sigma Co.). The plates were developed in isopropanol-water-conc. ammonia (9 : 1 : 1 v/v) for 45 min and the spots were visualised by charring at 105°C after spraying 6N H_2SO_4. The identified regions on the uncharred and dried plates were scrapped and eluted into 5 ml distilled water acidified with a drop of (1 : 1000 v/v)

dilute HCl just before their use for bioassays. Otherwise they were preserved dry on the plate under darkness.

2.5 *Chemical analyses*

The wormcasts and soil samples were analysed for the organic constituents according to the methods presented in table 1.

2.6 *Corrections for the synergistic effects of potassium in the sample bioassays*

The potassium levels of both the field and purified samples were determined flame photometrically. Since it is known that the endogenous potassium levels of the sample, synergistically affect the bioassays (Ezekiel *et al* 1978; Green and Muir 1979), simultaneous bioassays were run for the corresponding endogenous potassium levels. Thus the promotor level in samples obtained through bioassays were corrected after measuring the cotyledon responses for the observed potassium level of the sample.

2.7 *Growth promotion of wheat seedlings*

The shoot elongation and shoot wet weight biomass of the wheat seedlings (*Triticum aestivum* Linn.) in different soil amendments with wormcasts were measured according to the methods described by Arditti and Dunn (1969).

Table 1. Methods followed for the studies on the composition of wormcasts and soils.

Constituent estimated	Method followed	Author(s) referred
Nitrogen	Macro-kjeldahl	Allen *et al* (1974)
Carbon	Dry ashing	Allen *et al* (1974)
Phosphorus (inorganic)	Molybdovanadate-colorimetric	Wilde *et al* (1972)
Oxidizable organic matter	Walkley and Black-modified titrimetric	Jackson (1967)
Exchangeable potassium	Flame photometry	Mehlich (1956)
Ammonia	Colorimetric (after Nesslerization)	Oser (1965)
Urea	Diacetyl monoxime-colorimetric	Oser (1965)
Total phenols	Colorimetric	Hartenstein and Hartenstein (1981)
Auxins	Wheat coleoptile bioassay	Setty and Wheeler (1968)
Cytokinins	Cucumber cotyledon bioassay	Udayakumar and Sastry (1973)

3. Results

The casts of *Lampito mauritii* Kinberg were rich in ammonia, urea, oxidisable organic matter, exchangeable potassium and phosphorus, total nitrogen, and plant growth promoting substances (table 2). Phenol and organic carbon contents were lesser in the casts than in surrounding soils. The control (wormless) soil had a C/N ratio of 5·26, and this was lowered due to casting activity of worms to about 0·82. Similarly, the control soil had a urea/ammonia (U/A) ratio of 0·87, which was altered to 3·34 due to worm activities, indicating that the urea content of the soil increased. The total phenol content of the soil was reduced significantly by the casting activity. Both cytokinin and auxin activities were increased in soils by the worm activities (table 2).

The activities of individual species brought significant changes in the U/A ratios, phenol levels and plant growth promotor substances in the leaf compost-amended soils (table 3). Compared to wormless control, the worm activated soils showed a significant reduction in the phenol levels. Initially, the wormless control soil had a $0{\cdot}56 \pm 0{\cdot}03$ mg phenols per 100 g dw soil. This level was brought down to 17–55% by the activities of different earthworm species (table 3). The activities of *Perionyx excavatus* E. Perrier reduced the phenol levels more than those of any other species compared; and of *Octochaetoides beatrix* Beddard reduced to a poorer extent. Species differed with reference to the urea and ammonia contents of their castings (table 3). The castings of *L. mauritii* Kinberg had a higher U/A ratio than those of other species examined; and those of *O. beatrix* Beddard had the lowest ratio (table 3). Besides, urea, ammonia and phenols, the wormcasts also contained plant growth promotors such as cytokinins and auxins (table 2). The rates of production of these substances were evaluated by estimating the total excretory output of them through casts in a day. Such an analysis (table 3) indicated that *Pheretima elongata* E. Perrier released greater quantities of them through casts than any other species examined. *Drawida barwelli* Beddard and *O. beatrix* Beddard released them at the lowest rate. The release of these promotors through castings varied in various species in the following descending order: *Pheretima elongata* E. Perrier — *Perionyx excatatus* E. Perrier — *Pontoscolex corethrurus* Fr. Mull — *Ocnerodrilus occidentalis* Eisen — *Lampito mauritii* Kinberg — *Octochaetoides beatrix* Beddard.

Table 2. Composition of wormcasts of *L. mauritii*.

Constituent	No. of observations	μg g dry wt^{-1} ($\bar{X} \pm$ S.D)	
		Control soil	Castings
Ammonia nitrogen	10	163 ± 13	268 ± 14
Urea nitrogen	8	143 ± 11	896 ± 18
Total nitrogen	4	1180 ± 66	2649 ± 86
Organic carbon	6	6209 ± 136	2181 ± 178
Oxidizable organic matter	6	692 ± 14	928 ± 29
K^+	5	163 ± 16	216 ± 28
P_i	5	179 ± 12	252 ± 18
Phenols	6	4100 ± 10	2400 ± 30
Cytokinins	5	$(0{\cdot}04 \pm 0{\cdot}01) \times 10^{-3}$	$(3{\cdot}43 \pm 0{\cdot}12) \times 10^{-3}$
Auxins	5	$(1{\cdot}11 \pm 0{\cdot}02) \times 10^{-3}$	$(43{\cdot}27 \pm 6) \times 10^{-3}$

Table 3. U/A ratios and phenol levels in the casts and the rate of plant growth promotor output by some tropical earthworms.

Species	Percent decrease in phenols by worm activations	U/A ratio of castings ($n = 8$)	Rate of production[a] (nano grams day^{-1} worm^{-1})	
			Cytokinins[b]	Auxins[c]
Wormless control soil	0·0	0·76 ± 0·05	Nil	Nil
L. mauritii	41·5	3·34 ± 0·16	4·3 ± 0·016 (5)	54 ± 6 (5)
P. elongata	26·2	2·96 ± 0·24	82·3 ± 7·16 (6)	419 ± 16 (6)
P. corethrurus	30·1	1·16 ± 0·09	67·6 ± 2·12 (5)	302 ± 14 (5)
P. excavatus	55·9	0·82 ± 0·13	78·1 ± 0·52 (6)	316 ± 21 (6)
O. occidentalis	23·8	1·34 ± 0·11	5·1 ± 0·76 (4)	76 ± 8 (4)
D. barwelli	31·7	0·96 ± 0·08	3·2 ± 0·12 (6)	48 ± 5 (6)
O. beatrix	17·1	0·86 ± 0·07	3·9 ± 0·11 (6)	42 ± 7 (6)

[a] No. of experimental observations are given in parenthesis.
[b] Benzyladenine equivalents.
[c] Indol acetic acid equivalents.

The growth promotor levels in soils of different biotopes showed a direct positive correlation with the population density of earthworms inhabiting those soils (table 4, figure 1). Greater levels were found in biotopes with higher densities of populations. These results substantiate the fact that more the worm activations, the greater will be the levels of growth promotor substances in the inhabiting soils.

The plant growth promotor activities of the wormcasts were found to be fairly stable (maintaining about 90% initial activity) for about 3 weeks under constant moisture and darkness (table 5). Aging of the casts despite these conditions destroyed the activity, and it was found that nearly 60–70% of the activity was lost in the casts aged for 10 weeks (table 5). Exposures to sunlight for 72 hr destroyed nearly 99% of the activities of cytokinins and auxins in the casts (table 6). Exposures for 15 hr generally retained 90% of the activity. Exposure timings showed inverse correlations (log Y (moisture) = 0·2034–0·0189 x (exposure), $r = -0{\cdot}9922$) with the moisture level in the casts. Moisture level of the casts showed inverse correlations with percent loss of cytokinin activity (y(cytokinin) = $4{\cdot}1604 - 4{\cdot}5675x$ (moisture), $r = -0{\cdot}8857$), as well as with that of auxin activity (y(auxin) = $5{\cdot}3606 - 4{\cdot}442x$ (moisture) $r = -0{\cdot}8935$.

The influence of wormcasts on the growth promotion of the wheat seedlings was examined and the data were presented in table 7. There was some shoot elongation and shoot wet weight biomass increase in the seedlings in acid washed sand medium. This growth promotion increased slightly in a medium of mixture of garden soil and sand (1:2 proportion). When the garden soil, sand and the wormcasts were mixed in 1:1:1

Table 4. Levels of plant growth promotors in the soils of earthworm inhabited biotopes.

Biotope	Worm Density No. per M^3	Nano gram per kg dw soil	
		Cytokinin[a]	Auxin[b]
Farm garden	52	1·96	14·48
Watered farm garden	86	4·86	68·91
Irrigated garden	162	5·26	84·34
Mango orchard	202	9·32	126·43
Woodland	273	11·36	206·68
Grassland	322	19·46	221·49
Desiduous forest floor	293	18·41	242·31
Ragi field (dryland)	65	2·36	42·86
Sugarcane field	78	3·63	52·56
Tank edge	121	4·26	63·18

[a]Benzyl adenine equivalents.
[b]Indol acetic acid equivalents.

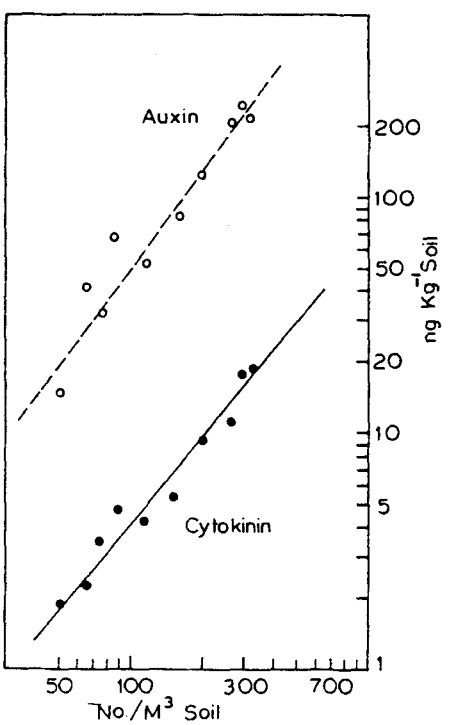

Figure 1. The relationship between the worm density of the biotope and the levels of plant growth promotors of the soil. The direct positive correlations obtained have yielded significant coefficients i.e. ($\log y = 1{\cdot}2512 \log x - 0{\cdot}7506$) $r = 0{\cdot}9669$ for auxins, and ($\log y = 1{\cdot}1615 \log x - 1{\cdot}6974$) $r = 0{\cdot}9688$ for cytokinins.

Table 5. Loss of plant growth promotor activities in the paper pulp wormcasts of *L. mauritii* on aging under constant moisture and darkness conditions at (26 ± 2°C) room temperature.

Weeks after casting	Loss of activity with reference to initial level (%)	
	Cytokinins	Auxins
0	0	0
1	2	1·5
2	4	4
3	9	7
4	15	18
5	26	21
6	35	34
7	47	41
10	69	58

Table 6. The effect of sunlight on the moisture level and plant growth promotor activities in the wormcasts of *L. mauritii*.

Exposure to sunlight (hr)	Moisture levels in the casts (%)	Loss of activity with reference to initial level (%)	
		Cytokinins	Auxins
0	24·5	0	0
10	19·4	4·4	8·6
15	11·2	6·1	9·5
24	9·2	30·7	32·3
30	5·3	51·4	54·8
48	3·3	92·5	88·9
52	2·5	98·1	97·6
72	1·1	99·5	98·9

proportion, the shoot elongation increased by 52 % and the wet weight biomass by 62 %. When sand and wormcasts were mixed in 1 : 2 proportion, the growth promotion increased further by about 72–76 %. In pure wormcast medium, the growth promotion increased still further by about 82–89 %. The wormcasts had a higher NPK ratio than the garden soil-amended medium. It could be seen from the data of table 7, that an increase in the proportion of the wormcasts despite retaining the NPK ratio constant, increased the growth promotion of the seedlings. It could be envisaged, therefore that a quantitative increase of wormcasts increased the plant growth promotor activities though not the NPK ratio.

Table 7. The effect of casts of *L. mauritii* on the growth of wheat (*T. aestivum*) seedlings during the first 15 days after sowing.

Dry weight proportion of the soil amendments				Growth promotion: Increase over the control (%)	
Acid washed sand	Wormless garden soil[a]	Worm activated garden soil casts[b]	NPK of the amendment	Shoot elongation	Shoot wet weight biomass
3[d]	0	0	0:0:0	0 (6)	0 (6)
1	2	0	0·77:0·12:0·11	0·5 ± 0·3[c] (4)	0·6 ± 0·2 (6)
1	1	1	0·83:0·08:0·09	52 ± 13 (12)	62 ± 16 (9)
1	0	2	0·85:0·07:0·08	76 ± 5 (9)	72 ± 11 (7)
0	0	3	0·85:0·07:0·08	82 ± 3 (7)	89 ± 9 (6)

[a]NPK, 0·77:0·12:0·11.
[b]Cast composition as in table 2; NPK 0·85:0·07:0·08.
[c]Mean ± S.D. of observations given in parentheses.
[d]Control amendment.

4. Discussion

Wormcasts are generally characterized by higher Eh (due to aerobic metabolism), lower pH (due to CO_2 and oxalic acids), cation exchange capacity (due to production of humic and fulvic acid), lower phenol and nitrogen contents (due to metabolism of microflora of the gut), and higher concentration of nucleic acids and phosphorus (due to the growth of microbial mass) (Hartenstein and Hartenstein 1981). It is now irrevocably accepted by the worm biologists that casting involves a pass-through of most of the soil organic matter ingested with some increase in mineralisation but probably no less than that which would occur in wormless soil *in situ*. Numerous indices have been evolved in the past such as C/N ratios, humic/fulvic acid (H/F) ratios etc to measure the extent of mineralisation that occurred due to worm activity (Kale and Krishnamoorthy 1981). Hartenstein and Hartenstein (1981) have used the content of phenols as suitable measure of humification. As humification proceeds the phenol content of the soil generally decreases. The decreases in the concentration of phenols in the present study may be explained due to the likely metabolism of microflora of the gut which are capable of decomposing simple phenolic compounds (Neuhauser *et al* 1978). Species differences with references to decrements of phenolic content as envisaged in the present study will characterize their gut microflora. Some phenols present in the soil medium will also condense into humic and fulvic acids during production and aging of the wormcasts. This possibility however cannot be ruled out. Many classes of phenols inhibit the functions of plant growth hormones (Sembdner *et al* 1980) and in the light of this fact the reduction in phenol levels in the casts, has a biological value in promoting the plant growth.

Whether the earthworm secretes digestive enzymes is still an enigma. But the accumulating evidence suggest that besides its own digestive enzymatic set up, the microflora in its gut secrete most of the enzymes that play a major part of digestion (Satchell and Martin 1984; Satchell *et al* 1984). Therefore, the castings consist not only of the defaecated products of worms, but also of the intermediary metabolites of the microflora in the worms gut.

Parle (1963) observed a steady decline in the ammonia nitrogen content of the casts as they age, and consequently the nitrogen content of them increases steadily. It is conceivable that the ammonia is only an intermediary product of nitrogen metabolism of the microflora harbouring the worm's gut. The species specific variations found in the present study with reference to U/A ratios of the casts possibly would reflect the nature of the nitrogen metabolism of the microflora existing in the gut of the worm species. However, the origin of urea and ammonia in the casts is not of much importance in so far as one is concerned with the end product which has an applied objective i.e. the production of stabilized residues as a plant growth medium.

Near the soil surface the castings increase the chances of germination of seeds that are already burried in soil (Grant 1983). The present results offer evidence that plant growth promotors help the rapid growth of the plants despite the adversaries of high ammonia content of the castings.

Both cytokinins and auxins are photolabile and relatively unstable *in vivo* as well as *in vitro* systems (Sembdner *et al* 1980). In an endogenous system of plant they are destructible metabolically by the plant cell enzymes but *in vitro* they are destroyed by the specific enzymes of the soil microbes. Many microorganisms secrete them (Sembdner *et al* 1980). Not withstanding these properties, the present results have demonstrated that the wormcasts maintain the activity of these promotors for a considerable period under proper moisture level and darkness. The maintenance of these levels in the wormcasts seem to be age-dependent and moisture-specific.

The results also indicated that wormcasts have all the nutrients that are required for a plant but claims on their relative proportion to qualify the casts as a field fertilizer are still immature. The data of table 7, particularly demonstrate that the wormcasts have a greater biological value of promoting the growth of wheat seedlings than having a NPK fertilizer value. The total nitrogen content of the casts increased more than that of the surrounding medium in the present study. Changes in mineral contents observed in the study due to worm activation corroborates the earlier findings (Lee 1983). However, it should be envisaged that the cast composition varies with soils and with the levels of their humification (Lee 1983). When the entire culture medium was examined the wormcast composition differed with that of wormless medium. When individual castings were collected and compared, the situation depicted a different picture. The fresh leaf-litter amendment in the present study had a NPK ratio of 0·77:0·12:0·11. Due to worm activation in the form of castings, this ratio was changed to 0·72:0·15:0·13. In Canada, Tomlin (1983) noticed a NPK ratio of 0·61:0·08:0·16 in the wormcasts. The field fertilizers have generally a higher NPK formulation. Compared to them the castings of *L. mauritii* have a limited fertilizer value. However, it has been shown that plants grown in such vermicompost show a lush growth (Keshavamurthy 1978). The lush growth may be due to the occurrence of plant growth promotors besides due to the readily available or assimalable nitrogen present in the casts (present results). These growth promotors may be of microbial origin but the rate of their output through casts differs from species to species. The occurrence of growth promotors is further

emphasized by the direct relationship noticed between the promotor levels and worm densities in soils.

Acknowledgements

We thank Dr R Narayana DI(BS & H), for facilities and Mr J Abraham for technical assistance. We are thankful to Professor M Udayakumar, Crop Physiology Department, for helpful discussions.

References

Allen S E, Grimshaw H M, Parkinson J A and Quarmby C 1974 *Chemical analysis of ecological materials* (New York: John Wiley and Sons) p. 138

Arditti J and Dunn A 1969 *Environmental plant physiology—Experiments in cellular and plant physiology* (New York: Holt, Rinehart and Winston Inc) p 312

Dash M C and Patra U C 1979 Wormcast production and nitrogen contribution to soil by a tropical earthworm population from grassland site in Orissa, India; *Rev. Ecol. Biol. Sol.* **16** 79–83

Ezekiel R, Sastry K S K and Udayakumar M 1978 Benzyladenine and Potassium induced changes in protein in excised cucumber cotyledons under normal and stress conditions; *Indian J. Exp. Biol.* **16** 522–523

Grant J D 1983 The activities of earthworms and the fates of seeds; in *Earthworm Ecology, from Darwin to Vermiculture* (ed) J E Satohell (London: Chapman and Hall Ltd) pp 107–122

Green J F and Muir R M 1979 An analysis of the role of potassium in the growth effects of cytokinin, light and abscisic acid on cotyledon expansion; *Physiol. Plant.* **46** 19–24

Hartenstein R and Hartenstein F 1981 Physicochemical changes effected in activated sludge by the earthworm *Eisenia foetida*; *J. Environ. Qual.* **10** 377–382

Jackson M L 1967 *Soil Chemical Analysis* (New Delhi: Prentice Hall of India Pvt. Ltd.) p 497

Kale R D and Krishnamoorthy R V 1981 Enrichment of soil fertility by earthworm activity; in *Progress in Soil Biology and Ecology in India* (ed) G K Veeresh (Bangalore: University of Agricultural Sciences) Technical Series No. 37, pp 64–68

Keshavamurthy P 1978 *Seasonal migrations in earthworms in relation to soil composition*, Ph. D. thesis, Bangalore University, Bangalore

Krishnamoorthy R V 1985 A comparative study of wormcast production by earthworm populations from grassland and woodland sites near Bangalore, India; *Rev. Ecol. Biol. Sol.* **22** 209–219

Lavelle P 1978 Les vers de terre de la savane de lampto (Cote d' Ivoire) Peuplements, populations at fonctions de l' ecosysteme; *Publ. Lab. Zool. EHS* **12** 301

Lee K E 1983 Earthworms of tropical regions- some aspects of their ecology and relationship with soils; in: *Earthworm Ecology from Darwin to Vermiculture* (ed.) J E Satchell (London: Chapman and Hall Ltd) pp 179–193

Lewis T and Taylor L R 1968 *Introduction to experimental ecology* (London: Academic Press) p 401

Ljungstrom P O and Reinecke A J 1969 Variations in the morphology of *Microchaetus modestus* (Microchaetidae: Oligochaeta) from South Africa with notes on its biology; *Zool. Anz.* **182** 216–224

Mehlich A 1956 Flame photometric methods for exchangeable potassium; *J. Assoc. Off. Agric. Chem.* **39** 330–343

Neuhauser E F, Hertenstein R and Connors J 1978 The role of soil macroinvertebrates in the degradation of vanillin, cinnamic acid and lignins; *Soil Biol. Biochem.* **10** 431–435

Oser B L 1965 *Hawk's Physiological Chemistry* 14th edition (New York: McGraw Hill Book Co) pp 1218–1239

Parle J N 1963 A microbial study of earthworm casts; *J. Gen. Microbiol.* **31** 13–23

Reinecke A J and Visser F A 1980 The influence of agricultural land use practices on the population densities of *Allolobophora trapezoides* and *Eisenia rosea* in southern Africa; in *Soil Biology as related to Land Use Practices* (ed.) D L Dindal (Washington DC: EPA) pp 310–324

Satchell J E 1983 Earthworm microbiology; in *Earthworm Ecology from Darwin to Vermiculture*; (ed.) J E Satchell (London: Chapman and Hall) pp 351–364

Satchell J E and Martin K 1984 Phosphatase activity in earthworm faeces; *Soil Biol. Biochem.* **16** 191–194

Satchell J E, Martin K and Krishnamoorthy R V 1984 Stimulation of microbial phosphatase produced by earthworm activity; *Soil Biol. Biochem.* **16** 195

Sembdner G, Gross D, Leibisch H W and Schneider G 1980 Biosynthesis and metabolism of plant hormones; in *Hormonal Regulation of Development—I (Molecular aspects of plant hormones)* (ed.) J MacMillan (Berlin: Springer Verlag) pp 336–347

Setty K G H and Wheeler A W 1968 Growth substances in roots of tomato (*Lycopersicon esculentum* Mill.) infected with root knot nematodes (*Meloidogyne* spp); *Ann. Appl. Biol.* **61** 495–501

Tomlin A D 1983 The earthworm bait market in North America; in *Earthworm Ecology from Darwin to Vermiculture* (ed.) J E Satchell (London: Chapman and Hall) pp 331–338

Udayakumar M and Sastry K S 1973 A bioassay for cytokinins using cucumber cotyledons; *Indian J. Exp. Biol.* **11** 564–565

Wilde S A, Voigt G K and Iyer J G 1972 *Soil and Plant analysis for tree culture* (Calcutta: Oxford Publ. House)