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Mineralization of phosphorus by faecal phosphatases of some earthworms of Indian tropics

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Abstract. The casts of earthworms contain greater amounts of extractable inorganic phosphate than underlying soils. Casts contain higher phosphatase activity than that occurs in undigested soil, which increases the inorganic phosphorus released by mineralization. Of the 4 species compared, *Perionyx excavatus* showed a higher faecal phosphatase activity. Composition of casts vary with species. pH increases and nutrients accumulate in worm-activated soils. Based on the data on surface cast production, it is estimated that grasslands around Bangalore show a phosphorus turnover of about 55 kg ha^{-1} y^{-1}, and the woodlands, 38 kg ha^{-1} y^{-1}.

Keywords. Faecal phosphatase activity; earthworm cast composition; phosphorus turnover in soils; phosphorus mineralization.

1. Introduction

It is now established that alkaline phosphatases are excreted in earthworm faeces as wormcasts (Satchell and Martin 1984; Satchell *et al* 1984). The wormcasts are not only richer in soluble inorganic phosphates but also in exchangeable phosphorus (Sharpley and Syers 1976; Mansell *et al* 1981), indicating the occurrence of active dephosphorylations of phosphorylated compounds in the gut of the worm. The mineralization and mobilization of phosphorus in soils are very much needed especially when farm yard manures are used in agriculture, as they contain most of the P in immobilizable organic form (McAuliffe and Peech 1949). Earthworms' role in P mobilization may thus be relevant and manifest in the disposal of organic wastes as their casts containing phosphatases may help dephosphorylating the immobilizable phosphorylated organic complexes in the soils. A higher phosphatase activity in undigested soil has been demonstrated in the wormcasts of some temperate worms (Satchell and Martin 1984; Satchell *et al* 1984). However, such studies have not been extended to tropical situations. The present paper describes to what extent the earthworms around Bangalore would contribute to the P mineralization in soils.

2. Materials and Methods

2.1 *Maintenance of worms and culture*

Cultures of 4 species of earthworms, viz. *Lampito mauritii* Kinberg, *Perionyx excavatus* E Perrier, *Pontoscolex corethrurus* and *Pheretima elongata* E Perrier were kept at room temperature in boxes (10 × 16 × 6 cm each) containing garden soil amended (pH 7·45, 20% moisture) with cow dung from a manure heap in 9:1

proportion. Ten adult (clitellates) worms of each species were introduced in each box and covered with a black cloth to reduce the effects of light on surface active worms. Wormcasts from the cultures were collected for comparison with control cultures of the same soil amendment maintained under the same conditions but without worms. Freshly deposited, unfrozen, pooled casts from 6 to 7 boxes and soil materials were used for analyses.

2.2 *Cultures on paper waste*

Paper waste sludge prepared in the laboratory by soaking and beating blotting paper strips was found to be palatable and habitable to worms. It formed a phosphate-free culture medium. The paper waste paste containing approximately 86% water, was sterilized to minimise microbial growth. Forty g aliquots of this paste was placed in a small polythene box ($8 \times 10 \times 3$ cm) containing 20 adult worms or no worms (control). One g of phytin (calcium inositol hexaphosphate) to each 40 g of paper waste was added. The medium can be regarded as simulating the worms' natural diet in so far as paper waste contains mainly of water, cellulose and minerals (ash). The paper waste culture was maintained at desired temperature in a BOD incubator for desired period of time.

2.3 *Phosphatase activity*

Samples of fresh wormcasts or paper wastecasts and of the control media equivalent to 0·5 g dw were collected for phosphatase activity. They were incubated in 20 ml universal buffer solution (BDH) adjusted to the desired pH in a waterbath at 26°C or at desired temperature for 30 min to extract the enzyme. Then it was filtered through a muslin cloth (sterilized and folded 4 times) in a Buchner funnel. Phosphatase activity in the filtrate was determined by the disodium phenylphosphate method (Hoffman 1968) using dilutions of phenol for the standard curve calibration. The phosphatase activity was estimated as μg phenol liberated in 3 h per unit dw wormcast. The enzyme activity was assayed with the buffer providing a pH range of 2–10. The assays were repeated to obtain concurrent results.

2.4 *Development of faecal phosphatase activity*

The paper waste-phytin (2·5%) cultures of *P. excavatus* in 6 boxes were maintained in a BOD incubator. At weekly intervals, one box was removed and the faecal phosphatase in the residue was extracted into the buffer solution pH 8·5, and the activity was measured at the same incubation temperature. The enzyme in the residues obtained at 20, 25, 28 and 30°C were assayed at respective temperatures. Residues without the worms, assayed at 30°C served as controls.

2.5 *Chemical analyses*

The total organic carbon content of soil and casts was determined by the formula $1{\cdot}8\ C\% = 100\% - \%\text{ash}$ (Allen *et al* 1974). The total nitrogen content was determined by Kjeldahl procedure on samples of about 0·5 g (Jackson 1967). The total Ca, Mg, K and Na were estimated after digesting the material with (2 ml/g) concentrated HCl. The digested material was filtered and assayed. The exchangeable (ionic) Ca, Mg and K were extracted with double distilled water, and filtered through a Whatman No. 1 paper. The filtrate was approximately diluted for determination of the cations by atomic absorption spectrophotometry with $LaCl_3$ to overcome PO_4 interference. The NH_4^+–N and NO_3^-–N of soil and casts were extracted with 1 M KCl at a solution: solid ratio 20:1, filtered through a Whatman No. 1 paper and estimated according to Strickland and Parsons (1968). The Cl^- contents of the water extracts were determined by $AgNO_3$ titration (USEPA 1979). The total phosphorus was extracted with Olsen's extractant (0·5 M $NaHCO_3$ buffered at pH 8·5), and the inorganic P, with distilled water and estimated colorimetrically by ascorbic acid-molybdic acid method (USEPA 1979). The difference between the total P and inorganic P values was considered to give the organic P content. Dry weights of samples were obtained as loss in weight at 105°C overnight. Ashing of samples was done in a muffle furnace set at 450°C for 20 h. Loss on ignition values were calculated on determining the ash content. All results were calculated and expressed on dry weight basis.

3. Results

The composition of wormcasts varied with species (table 1). The wormcasts were rich in certain components when compared to wormless control soils. The casts of *P. excavatus* were rich in total nitrogen, ammonia nitrogen and nitrate and phosphate contents; whereas the casts of *L. mauritii* were rich in urea content. The

Table 1. Composition of casts of some earthworm species.

Percentage composition	Control soil	*L. mauritii*	*P. elongata*	*P. excavatus*	*P. corethrurus*
pH	7·45	8·10	8·15	8·25	8·05
Organic C	0·612	0·292	0·298	0·276	0·279
Total nitrogen content	0·091	0·193	0·189	0·208	0·174
C/N ratio	6·72	1·51	1·57	1·32	1·60
Total Ca	0·81	1·39	1·28	1·36	2·01
Total Mg	0·32	0·29	0·31	0·30	0·28
Total K	0·516	0·49	0·52	0·48	0·49
Total P	0·067	0·083	0·091	0·162	0·093
Total Na	0·113	0·120	0·115	0·117	0·121
Exchangeable Ca	0·126	0·184	0·172	0·209	0·398
Exchangeable Mg	0·153	0·231	0·284	0·279	0·261
Exchangeable K	0·099	0·386	0·391	0·381	0·396
NH_4^+-N	0·002	0·012	0·018	0·092	0·026
NO_3^--N	0·0005	0·006	0·0058	0·0092	0·0064
Cl^-	0·73	0·729	0·719	0·730	0·726
PO_4^-	0·0469	0·0699	0·0678	0·1397	0·0711
Urea	0·072	0·155	0·138	0·098	0·089
Total organic P (OP)	0·02	0·0128	0·0227	0·0219	0·021
OP/Pi ratio	0·42	0·18	0·33	0·16	0·28

Values are dry weight percentages.

casts of *P. elongata* were rich in organic phosphorus content. They were more alkaline than the surrounding soil. They were poorer in organic carbon and richer in total nitrogen contents. The C/N ratios were lower in the wormcasts than in the soil. It was also noticed that wormcasts contained more total Ca and P contents but the total Mg, K and Na contents remained more or less unchanged during casting. Total exchangeable cation (like NH_4^+-N, Ca, Mg and K) concentrations increased in the casts, suggesting that the soil organic matter-bound ions are freed during the passage of soil through the gut. The exchangeable anion concentrations like those of NO_3^--N, and PO_4^- were greater in the casts, whereas that of Cl^- remained unaffected. Furthermore, it was found that casts were rich in urea content. The total organic phosphorus content was not changed. Consequently, the organic P/inorganic P ratio reduced in the casts. The extent of these changes obviously (table 1) varied with species. It is evident from the data of table 1 that among the 4 species compared, the casts of *P. excavatus* showed a discrete but the highest degree of these variations.

The effects of casting activity on phosphorus mineralization by the 4 species selected for the study were compared (table 2). The casts of paper waste sludge of two species viz. *L. mauritii* and *P. excavatus* contained more of water-soluble P and mineral contents. More of total P and Pi were extractable from these casts. The reduction on loss-on-ignition values in the casts, showed the decomposition of organic matter present in soils during passage of the soil in the worm's gut. The increases in total extractable P over the control can perhaps be attributed to increased concentration of the mineral matter. However, in the culture medium of *P. excavatus* an increase of 26·15% in the total extractable phosphorus existed which was somewhat greater than that occurring in other cultures of other species. This observation indicates the occurrence of a higher level of mineralization in the worm.

Paper waste sludge maintained for 6 weeks was eaten and casted out by worms.

Table 2. Effects of earthworm faecal phosphatase activity on phosphorus mineralization in paper waste sludge maintained for 6 weeks and difference from controls (% in parenthesis).

	$\mu g\ P\ g^{-1}$ culture residue dw/20 worms/6 weeks				
Fraction	Control	*L. mauritii*	*P. elongata*	*P. excavatus*	*P. corethrurus*
Water-soluble P	15 ± 1·56†	25 ± 1·92* (+66·66)	16 ± 1·38^{NS} (+6·6)	27 ± 2·08* (+80·0)	17 ± 1·28^{NS} (+13·33)
Total extractable P	260 + 9·81	318 ± 9·81* (+22·30)	271 ± 3·87^{NS} (+4·23)	328 ± 12·36* (+26·15)	279 ± 6·84^{NS} (+7·30)
Extractable inorganic P	182 + 11·37	268 ± 14·81* (+47·25)	202 ± 9·67^{NS} (+10·98)	283 ± 13·79* (+55·49)	212 ± 8·35* (+16·48)
Loss on ignition (%)	48 + 2·66	38 + 1·93* (−20·83)	32 + 1·69* (−33·33)	41 + 1·26* (−14·58)	38 + 2·03* (−20·83)
Mineral content** (%)	45 + 1·92	51 + 2·37* (+13·33)	53 + 1·62* (+17·77)	51 + 2·89* (+15·55)	51 + 3·16^{NS} (+13·33)

†Mean + SD of 7 sets of experimental observations.
*Difference significant ($P < 0·01$).
**Ash content.
NS, Not significant ($P > 0·01$) with reference to controls by Students mean difference '*t*' test.

The castings of *L. mauritii* and *P. excavatus* when compared to controls were found to be rich in water-soluble P, total extractable P and extractable inorganic P (table 2). Loss of ignition values were lowered in the casts of almost all species compared but the latter had more mineral content (table 2). These results show that mineralization of phosphorus is more in the casts of at least two species than in raw wormless paper sludge. The paper waste contained phytin which was mineralized both by intestinal as well as faecal phosphatases when the former passes through the gut. Mineralization is more in cultures with *P. excavatus* than in cultures with other 3 species (table 2). The faecal phosphatase activity in different species varied but all of them exhibited two peaks of activity with reference to the pH of the incubating medium. The wormless (unbeaten) raw food exhibited only one peak between pH 3 and 4 (figure 1). The other pH activity peak was smaller than this peak and had fallen around pH 9 in the casts of almost all species. It is believed that the peak between pH 3 and 4 might be due to microbial phosphatases (Satchell and Martin 1984) and it is present in the raw food without worms. The other peak found at pH 9 may be due to the phosphatase released by worms' gut (figure 1). The activities of both the phosphatases were higher in the casts of *P. excavatus* than in the casts of other 3 species (table 3). These observations suggested that *P. excavatus* has greater potentialities to mineralize the organic matter. Mineralization by *P. excavatus* in garden soil cultures was found to be greater (table 4) than that in paper sludge cultures with phytin. Increase in mineral residue is also greater in

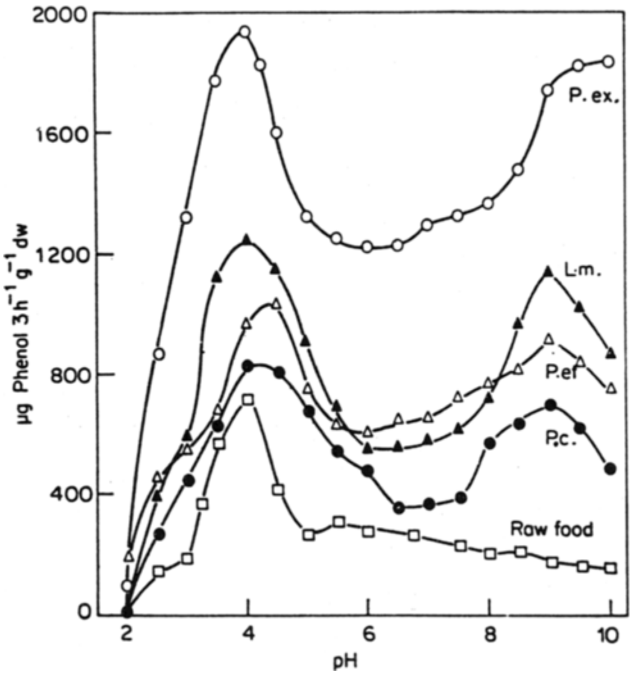

Figure 1. Phosphatase activity measured at 26°C in the casts (faecal casts) of 4 earthworm species cultured in paper waste sludge and 2·5% phytin. P.ex, *P. excavatus* Lm, *L. mauritii*, Pel, *P. elongata*; Pc, *P. corethrurus*; Raw food, contained paper waste sludge and phytin without worms (control).

Table 3. Faecal phosphatase activity in the casts of some earthworm species on paper waste sludge substrate for 6 weeks.

	μg phenol g dry wt^{-1} 3 h^{-1}	
Culture residue	at pH 3/4	at pH 9/9·5
Wormless control (raw food)	776 ± 21	182 ± 16
L. mauritii	1251 ± 82	1146 ± 79
P. elongata	964 ± 18	926 ± 19
P. excavatus	1942 ± 32	1753 ± 38
P. corethrurus	838 ± 21	712 ± 15

Values are mean ± SD of 5 samples.

Table 4. Effects of *P. excavatus* activity on the mineralization of paper sludge and garden soil in a period of 6 weeks.

	Paper sludge with phytin		Garden soil	
Parameter	Wormless control	Worm-activated	Wormless control	Worm-activated
pH change	6·8→6·8	6·8→6·5	7·2→7·2	7·2→8·0
Body weight growth (%)	—	− 12·83	—	+ 2·38
Mortalities (%)	—	3–4	—	Nil
Pi (μg/g dw)	189	242	486	689
Mineral content* of the residue (%)	43	52	35	58

*Ash content.

worm-activated garden soil, but the body weight-growth of the worm appeared to be retarded in cultures with paper sludge and phytin (table 4). The worm activities in soils caused a noticeable pH increase while in paper sludge cultures there was a considerable pH-drop (table 4). Worm cast deposition in soils probably may alkaline the soils. About 3 to 4% mortality was noted in paper sludge cultures in contrast to none in soil cultures (table 4). The phosphatase activity (at pH 9) of the paper sludge casts of *P. excavatus* showed a linear and direct increase as the concentration of phytin in the medium increased (figure 2). Phytin contains organic P and as the substrate concentration increased, the worms gut phosphatase activity increased.

A culture time of 3 weeks seemed to develop a significant faecal phosphatase activity. Furthermore, it was found that phosphatase activity in the paper waste casts of *P. excavatus* was dependent on temperature at which the culture was maintained as well as on the time of culture (figure 3). At higher temperatures the activity was found to be greater and stable, and with culturing time the activity exhibited a sigmoid curve (figure 3). Wormless controls exhibited poor activity at all temperatures and culture time. Three weeks old culture at any temperature seemed to produce casts with nearly 70–90% of total phosphatase activity (figure 3) obtainable by the culture. These results illustrate the extent of mineralization rates of P by the faecal phosphatases of worms which especially in tropical environments vary with season, and day and night periods.

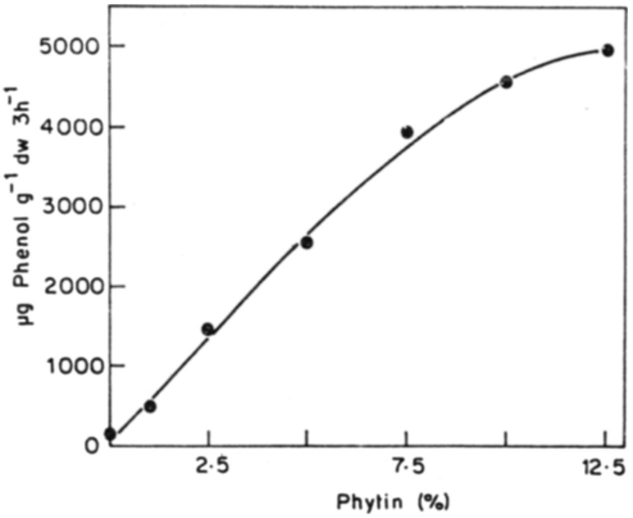

Figure 2. Phosphatase activity in the faecal casts of *P. excavatus* cultured for 4 weeks in paper waste sludge, at pH 8·5, temperature 25°C and varying phytin concentration.

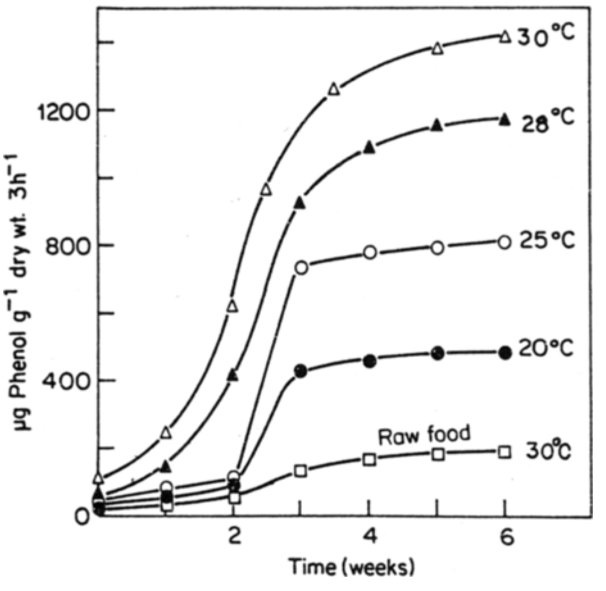

Figure 3. Development of phosphatase activity measured at weekly intervals and cultured in the casts of *P. excavatus* in paper waste sludge with 2·5% phytin in a BOD incubator set to the temperature shown in the figure and assayed at the same temperature at pH 8·5.

4. Discussion

The alkalinity of the cast soil observed here may be an indicator of beneficial effects of the worms in alkalizing the soils. The wormcast soils in north-east India had shown significantly higher pH than the normal soil (Bhadauria and Ramakrishnan

1989). However Nijhawan and Kanwar (1952) noted a lower pH value for the worm cast soils of Punjab; which is in contrast with the present observations. The lowering of C/N ratios on casting observed in the present study however, corroborates the findings of Nijhawan and Kanwar (1952), but contradicts those of Shrikhande and Pathak (1948). Lowered C/N ratios in wormcasts however, indicate the existence of proportionately higher nitrogen available in the cast soil than in parent soil. Concentration of exchangeable elements like Na, Mg and K may be due to decomposition of organic matter, occurring during the passage of soil through worm's gut. Increase in Ca status of the cast may be due to calcium-oxalate-decomposing bacteria (Jakoby and Bhat 1958).

The enhancement of phosphatase activity in the worm-activated media described here are consistent with the observations of Graff (1970) and Sharpley and Syers (1976) on phosphorus mineralization by earthworm populations in field. The data are also consistent with the observations of Satchell and Martin (1984) on *Eisenia fetida*, and *Dendrobaena venata* in the laboratory. However, the present study suggests that among the species viz. *L. mauritii*, *P. corethrurus*, *P. elongata* and *P. excavatus* examined, the latter one bears greater potentialities to bringforth mineralization of organic P to inorganic P.

The wormcast phosphatase activity at pH 3–4 may be mainly a microbial activity (Satchell and Martin 1984). However, the possibility that the activity at pH 9–10 does not indicate the microbial activity cannot be excluded. However, in view of the many references existing on the functions of alkaline phosphatases in earthworms (Haase 1969; Dev and Vyas 1972) it seems more likely that these peaks indicate earthworm intestinal phosphatases. Overall, this study suggests that phosphatase activity in worm faeces may be substantially increased directly by the worm's own enzymes and indirectly by stimulation of the microflora present in the gut (Satchell *et al* 1984). The phosphatases in the wormcasts while their passage through the gut, start mineralizing the organic phosphorus into inorganic P which is the ultimate form of P required by the plants. The faecal phosphatases as evidenced in the present results, are fairly stable and more responsive to the temperature of the assay. Especially, in tropical conditions where soil temperatures are higher than those of sub-tropics, the P mineralization rates by the faecal phosphatases could be stable and higher.

The cast composition in the 4 species examined (table 1 and figure 1) varies widely. The increase in extractable inorganic P of 55% in the culture residues of *P. excavatus* seems somewhat greater than in those of other species (table 2), and the fact could be attributed to a higher level of mineralization occurring in the residue. This is confirmed by the increase in water soluble P which was about 80% in *P. excavatus*.

The presence of bacterial and faecal phosphatases in the wormcasts which are laid in soils may promote mineralization of soil organic phosphorus. This may be a minor role but essentially a direct metabolic reaction in the decomposition of plant material (Barley 1961). Based on the estimates of surface cast production in grasslands and woodlands around Bangalore (Krishnamoorthy 1985) and assuming that all those casts, are due to *P. excavatus* it is estimated that about 55 kg ha^{-1} y^{-1} inorganic P can be obtained in grasslands and about 38 kg ha^{-1} y^{-1} in woodland soils through mineralization. Out of these 19·79 kg of Pi ha^{-1} y^{-1} may be obtainable by wormcast phosphatase specific activity alone. The latter figure was arrived at, by considering the activity of the faecal enzyme under the conditions prevailing in the field. These figures are obviously higher than the 9 kg Pi ha^{-1} y^{-1} reported for New Zealand pastures (Sharpley and Syers 1976). It must be emphasized, however that the Pi concentration in surface casts although higher than in underlying soil, is actually lower than that present in plant litter. For example, 59–83% of the total P in the litter from New Zealand pastures was estimated to be in the form of inorganic P (Gillingham *et al* 1976). By decomposing the plant litter in the soils, the surface casting earthworms thus help accelerating the rate of cycling of P in pasture and grassland situations.

The present results apparently also indicated that wormcasts have all major components that are essential (NPK) to regard them as field fertilizers. The data of table 1 particularly demonstrate that wormless parent soils (i.e. the controls) had a NPK ratio of 91:47:99 which on (worm) activation in the form of castings turned to 193:70:386 by *L. mauritii*, 189:68:391 by *P. elongata*, 208:140:381 by *P. excavatus* and 174:71:396 by *P. corethrurus*. These worm activations are comparatively better than those observed in the soils of north-east India (Bhadauria and Ramakrishnan 1989). Besides, the high urea and nitrogen contents relative to Pi content of the casts (table 1) obviously liken the status and significance of them as natural field fertilizers.

References

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

Barley K P 1961 Abundance of earthworms in agricultural land and possible significance in agriculture; *Adv. Agron.* **13** 249–268

Bhadauria T and Ramakrishnan P S 1989 Earthworm population dynamics and contribution to nutrient cycling during cropping and fallow phases of shifting agriculture (Jhum) in North-East India; *J. Appl. Ecol.* **26** 505–520

Dev B and Vyas I 1972 Histoenzymatic pattern of alkaline phosphatase in the different components of the alimentary canal of the common earthworm *Barogaster annandalei* (Stephenson), and its possible physiological significance; *Acta Morphol. Neerl. Scand.* **9** 355–369

Gillingham A G, Syers J K and Gregg 1976 Phosphorus cycling in steep hill county pastures; *N. Z. Soil News* **24** 127–128

Graff O 1970 Phosphorus content of earthworm casts; *Landbauforsch. Voelkenrode* **20** 32–36

Haase E 1969 On histophysiology of the intestine of the earthworm; *Zool. Anz.* **33** 535–539

Hoffman G 1968 Eine Photometrische method zur bestimmung der phosphatase-aktivitat in Boden; *Z. Pflanzenernaehr. Dueng. Bodenk* **118** 161–172

Jackson M L 1967 *Soil chemical analysis* (New Delhi: Prentice-Hall of India)

Jakoby W B and Bhat J V 1958 Microbial metabolism of oxalic acid; *Bacteriol. Rev.* **22** 75–80

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

Mansell G P, Syers J K and Greog 1981 Plant availability of phosphorus in dead herbage ingested by surface casting earthworms; *Soil Biol. Biochem.* **13** 163–167

McAuliffe C and Peech M 1949 Utilization of phosphorous in farm manures; *Soil Sci.* **68** 179–184

Nijhawan S D and Kanwar J S 1952 Physico-chemical properties of earthworm castings and their effect on the productivity of soil; *Indian J. Agric. Sci.* **22** 357–373

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

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

Sharpley A N and Syers J K 1976 Potential role of earthworm casts for the phosphorous enrichment of run-off waters; *Soil Biol. Biochem.* **8** 341–346

Shrikhande J G and Pathak A N 1948 Earthworms and insects in relation to soil fertility; *Curr. Sci.* **17** 327–328

Strickland J D H and Parsons T R 1968 A practical handbook of the sea water analysis; *Bull. Fish. Res. Board. Can.* **167** 311

USEPA (U S Environmental Protection Agency) 1979 *Methods for chemical analysis of water and wastes*, USEPA - Rep. EPA - 600/4–79–020 USEPA, Cincinnati, Ohio, USA