Mechanism of P Solubilization in Vermicompost Treated Red Lateritic Soils

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Abstract

Red lateritic soils are typically low in total organic carbon (TOC) and available phosphorus (AP) content and continuous fertilization is required to obtain desired crop yield. In this experiment, cattle manure in three forms (air-dried, composted and vermicomposted) were applied to red lateritic soil to study their effect on TOC and AP content of soil and probable mechanism of P-solubilization as affected by these treatments were also studied. Vermicompost was the most effective to solubilize insoluble P in red lateritic soil (Alfisols) as compared to other organic amendments (air-dried cattle manure and compost). The highest SPA in vermicompost-treated soil attributed to the comparatively higher concentration of all the three SPA isozymes in these soils. The maximum P-solubilization in these soils might be attributed to the highest SPA and presence of several organic acids like citric, lactic and oxalic acids in vermicompost-treated soils. Since, vermicompost application also increased TOC, mineralizable N and exchangeable K content of soil, vermicompost could be considered as the most rational organic amendment to improve chemical properties of red lateritic soils.

Keywords: organic amendments; P-solubilization; acid phosphatase enzyme; isozyme study; organic acids.

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1. Introduction

Acid lateritic soils are very poor in organic matter owing to high temperature and rainfall and intense microbial activity (Biswas & Mukherjee, 2000). After nitrogen (N), phosphorus (P) is the second most limiting nutrient for crop production in majority of the arable soils especially red lateritic soils (Ochwoh *et al*. 2005) and crop yields are often adversely affected by low availability of P in soil. In most of the cases, continuous fertilization is required to obtain desired crop yield. Brady (2002) proposed that application of P-fertilizers in soil converts phosphate into secondary insoluble form and solubilization of P from these compounds actually determines the rate of P-release from fertilizer itself. Therefore, P-solubilizing potential of a soil plays a key role to supply phosphate-P to the field crops. Under this context, attentions are paid to increase the utilization of different kinds of organic wastes as organic amendment through composting. However, composting has some disadvantages like nutrient loss, cost of land, equipment and labour is required for composting and odour is also associated with composting (Eghball et al., 2002). In this perspective, vermicomposting has been recognized as an eco-friendly technique for converting organic wastes into nutrient-rich soil amendments.

Vermicomposting is controlled oxidative non-thermophilic decomposition of organic matter by mutual interaction between earthworms and microorganisms. Pramanik et al. (2007) revealed the presence of several enzymes in vermicompost. Application of vermicompost increased nutrient content, enhanced activities of different enzymes and activated microorganisms in soil (Lavelle & Martin, 1992). Transformation of nutrients in soil is enzyme mediated biochemical processes facilitated by microorganisms. Thein and Myers (1992) revealed that increasing microbial activity enhanced the P bioavailability in soil. Soil harbor several microorganisms capable of producing exo-cellular phosphatase enzymes (Ramachandran et al., 2007; Chen et al., 2006) and relative proportion of these microorganisms might play an important role in P-solubilization in soil. Biswas and Narayanasamy (1998) proposed that partial acidulation by organic acids increased P-solubilization from insoluble phosphates. Therefore, it could be hypothesized that production of organic acids and release of exo-cellular enzymes might be the prime mechanism for P-solubilization by soil microorganisms. In this experiment, cattle manure was applied in three different forms (air-dried, composted and vermicomposted) to study their relative effect on dynamics of total organic carbon (TOC) and available P (AP) content of soil. All the soils were analysed for their acid phosphatase (SPA) enzyme pattern and organic acids of vermicompost-treated soils were also detected. The objectives of this experiment were (i) to predict the most suitable form of organic manures for improving carbon and phosphorus status of lateritic soils, (ii) to evaluate the mechanism of P-solubilization by soil microorganisms and (iii) to study the relative effect of these factors in P-solubilization as affected by different treatments.

2. Materials and Methods

2.1 Experiment setup

The experiment was conducted in the agricultural farm of directorate of Water Management Research, Bhubaneswar, Orissa, India. Air-dried cattle manure (total phosphorus [TP] content 5.18 mg g^{-1}) was applied at 15 t ha^{-1}, and compost (TP content: 9.47 mg g^{-1}) and vermicompost (10.82 mg g^{-1}) were applied at 8.20 t ha^{-1} and 7.18 t ha^{-1}, respectively to supply equal amount of total phosphorus to the soil. The nutrient status of these three forms of cattle manures was presented in Table 1. During this study, soil moisture content was maintained near field capacity by periodical watering as and when required. Total organic carbon (TOC), mineralizable nitrogen and exchangeable potassium content of the soil were determined initially (on zero day) and at the end of study (after 75 days). Available phosphorus content was determined periodically (on 0, 7, 15, 30, 45, 60 and 75 days). All the microbiological and biochemical parameters were estimated after 75 days.

Table 1. Chemical properties of applied organic amendments

Cattle manure	TOC [a]	TN [a]	TP [a]	C/P ratio	TK [a]
Air-dried	360.7 ± 8.9	6.84 ± 0.41	5.18 ± 0.33	69.63	4.85 ± 0.29
Composted	249.6 ± 6.1	10.33± 0.67	9.47 ± 0.51	26.36	10.27 ± 0.41
Vermicomposted	236.1 ± 7.9	12.92 ± 0.72	10.82 ± 0.46	21.82	10.32 ± 0.50

TOC: total organic carbon, TN: total nitrogen, TP: total phosphorus and TK: total potassium
a: mg/g

2.2 Chemical analysis

Soil organic carbon content in soil was estimated by the standard dichromate oxidation method of Nelson & Sommers (1982). Mineralizable nitrogen was measured by alkaline distillation with permanganate (Jackson, 1973). Available phosphorus was estimated colorimetrically by acidic ammonium molybdate solution from sodium bicarbonate extract (Olsen & Sommers, 1982).

2.3 Population of phosphate-solubilizing bacteria (PSB)

The initial soil and organic amendment treated soils were tested for PSB count by dilution plate method (Trevors and Cook, 1992). Soil (1 g) was stirred (80 rpm for 2 min) with 100 mL sterile distilled water in a conical flask and allowed to settle for 15 min. The suspension was serially diluted 10^4 times for further analysis. To estimate the population of PSB, 500 μL of that solution was spread on Pikovskaya (1948)'s medium supplemented with streptomycin (100 mg L^{-1}) before pouring. The clear zone indicates the presence of cellulolytic fungi. To detect acid production by test strain, loop-full of PSB culture was incubated on solid Pikovskaya (1948)'s medium with 0.01% bromophenol blue indicator at 30^0C for 4 days.

2.4 Organic acids assay

Phosphate-solubilizing bacteria were grown in Pikovskaya's broth medium for thin layer chromatography (TLC) analysis of organic acids. After 3 days incubation, culture broth medium was centrifuged at 6000 rpm for 15 minutes to separate pellets and supernatant was analyzed by TLC for organic acids detection. The mixture of acetone, ammonium hydroxide, ethanol, chloroform and water (30: 11: 5: 3: 1, volume basis) was used as mobile phase for separating organic acids of culture medium. Methyl red (0.25 g) and bromophenol blue (0.25 g) were dissolved in 70% methanol and the resultant solution was used for staining TLC plates (Lee et al. 2001). In this experiment, pure acetic, citric, lactic, oxalic and salicylic acids were used as reference standards.

2.5 Enzyme assay

Acid phosphatase activity (SPA) of soil samples was estimated by colorimetric method (Tabatabai Bremner 1969). For measuring enzyme activity, p-nitrophenyl phosphate was used as substrate and yellow colour intensity of p-nitrophenol was measured at 420 nm. Protein concentration was determined by Lowry method using bovine serum albumin as the standard (Lowry et al. 1951).

Enzymes from soil were extracted by 0.5M sodium pyrophosphate solution. To purify phosphatase enzyme, proteins were precipitated in 30% acetone sulphate solution, redissolved in minimum volume of 50 mM phosphate buffer (pH 8.0) and dialysed against the same buffer for overnight. The dialysed protein solution was then analyzed by Fast Protein Liquid Chromatography (FPLC) using 50 mM tris-buffer solution (pH 8.0) at a flow rate of 1.0 ml min^{-1}. The retained proteins were eluted with a linear NaCl gradient (Fig. 1) and total 120 fractions were collected. Those fractions were then analyzed colorimetrically to obtain the active fraction(s) for acid phosphatase activity. Proteins in the enzyme fractions were analyzed by polyacrylamide gel electrophoresis (PAGE) and a-naphthyl acid phosphatase and fast-blue BB salts were used for staining the gel.

3. Results and Discussion

3.1 Changes in total organic C, mineralizable N and exchangeable K in soil

Total organic carbon (TOC), mineralizable N and exchangeable K content of soil were significantly ($P \leq 0.05$) increased due to application of air-dried, composted and vermicomposted cattle manures (Fig. 2). The highest TOC was recoded in compost-treated soil, though it was statistically at par with that of vermicompost-treated soil. Application of vermicompost registered the highest value of mineralizable N in soil and it was followed by that of that of compost, air-dried manure and control treatments, respectively. Though compost treated soil had the highest exchangeable K content, it did not differ significantly ($P \leq 0.05$) from that of other treatments.

3.2 Changes in available phosphorus (AP) dynamics in soil

Application of organic amendments in different form immediately decreased AP content of soil. The extent of this decrease, however, was varied depending on nature of treatment and was proportional to the C/P ratio of the treatments. Application of organic substrates in soil activates its microbial community and these activated microorganisms rapidly assimilate nutrients for their growth. In plots, received amendments having comparatively higher C/P ratio, microorganisms assimilate more P from soil reservoir to satisfy their requirement and that in turn leads to the more reduction in AP content of soil. Thereafter, AP content of soil was steadily increased due to mineralization of applied organic substrates (Fig. 3). The rate of increase in AP content was varied depending on the nature of applied organic amendment and after 75 days incubation, vermicompost treated soils recorded the highest AP content followed by that of compost and air-dried cattle manure, respectively.

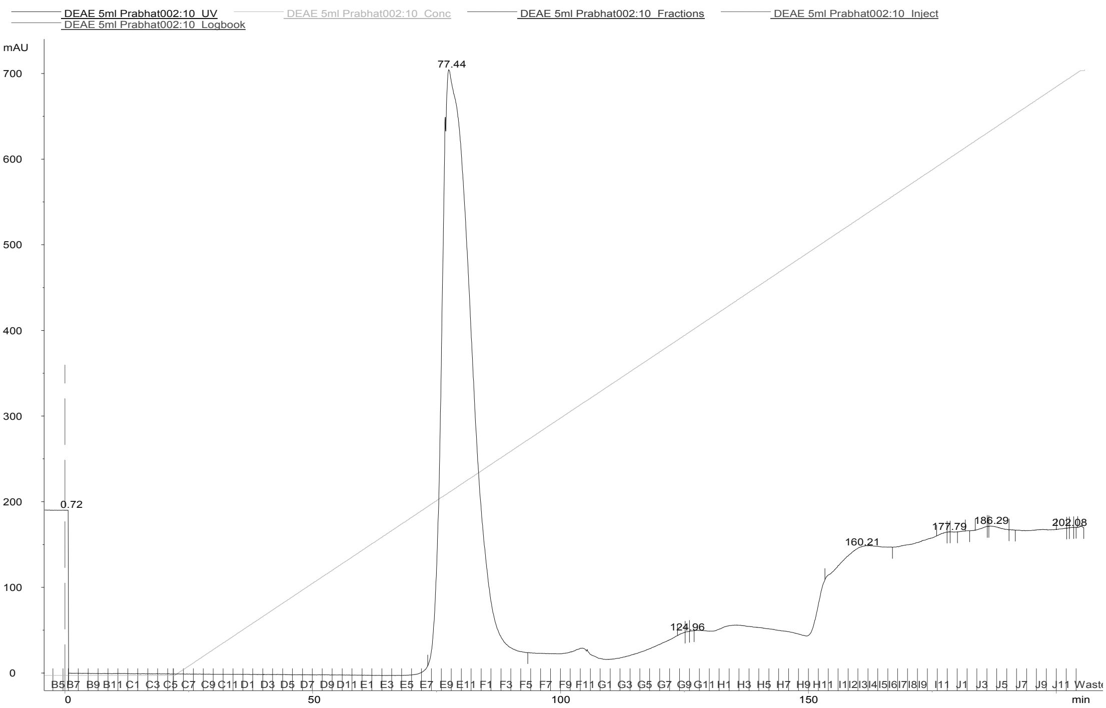


Fig. 1. FPLC chromatogram of proteins extracted from PSB broth culture.

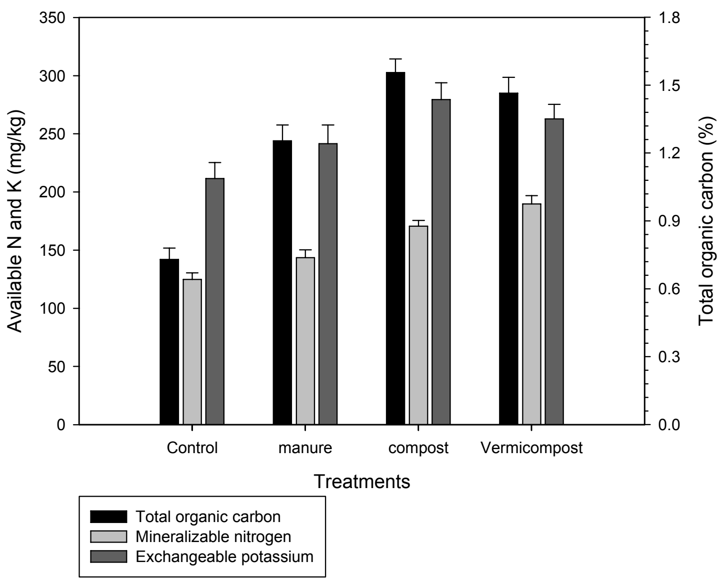


Fig. 2. Changes in total organic carbon, mineralizable nitrogen and exchangeable potassium content of soil as affected by different organic amendments.

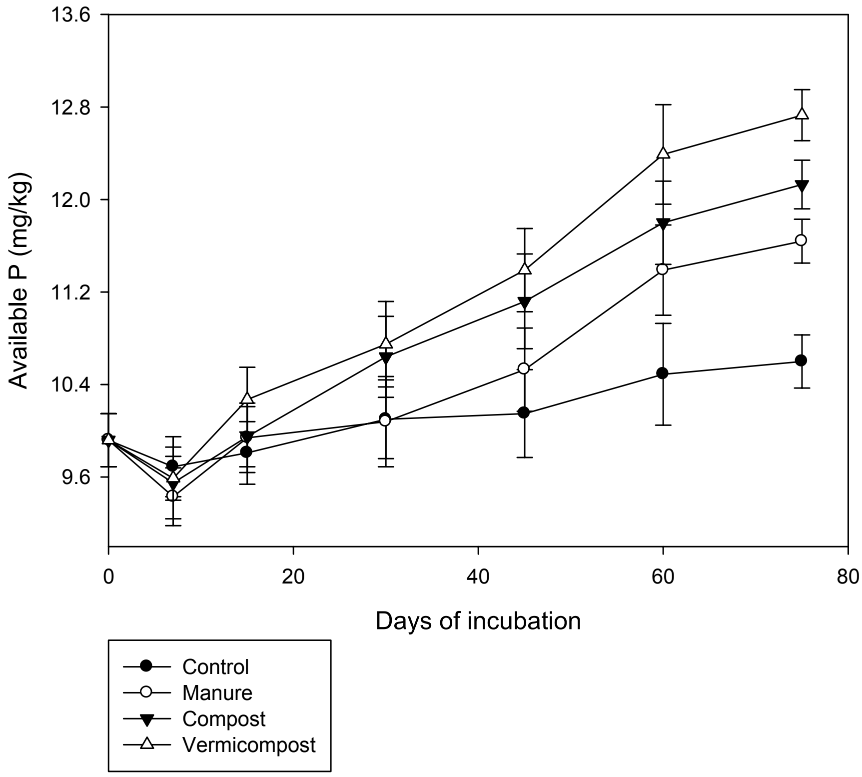


Fig. 3. Changes in available phosphorus content in soil as affected by different organic amendments.

3.3 Phosphate-solubilizing bacteria (PSB) in soil

Estimation of PSB by dilution plate method indicated that application of organic substrate significantly ($P \leq 0.05$) increased total count of PSB in soil. Both compost and vermicompost treated plots recorded significantly ($P \leq 0.05$) higher PSB count than that of cattle manure treated plots, though the values of compost and vermicompost treated soils did not differ statistically (Fig. 4).

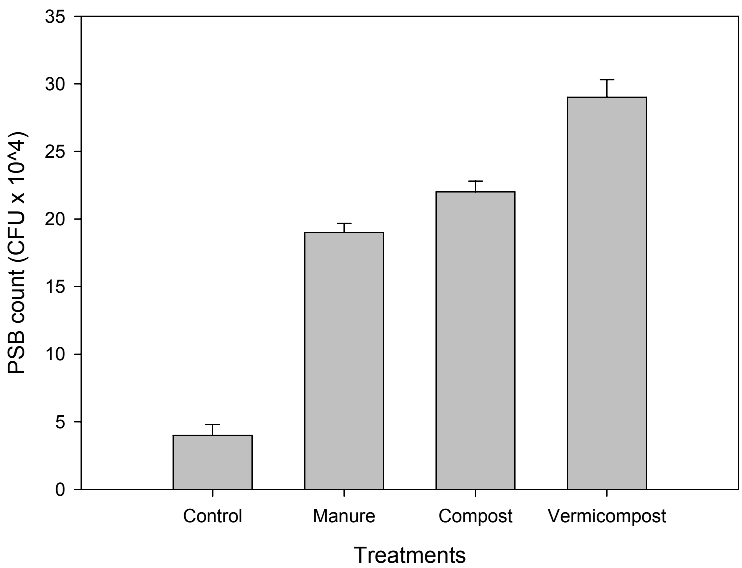


Fig. 4. Total count of phosphate-solubilizing bacteria (PSB) in soil as affected by different organic amendments.

3.3.1 Acid phosphatase activity (SPA)

Results indicated that SPA of soil was significantly ($P \leq 0.05$) increased due to organic amendment application. Both compost and vermicompost application recorded significantly ($P \leq 0.05$) higher SPA as compared to that of air-dried cattle manure treated soil, however, the values of compost and vermicompost treated soil were statistically at par. The SPA activity of enzyme solutions, extracted from organic amendments treated soils, followed the same trend as that of soil (Table 2). Acetone precipitation recorded 1.42-1.77 fold enzyme purification was achieved. After FPLC analysis, generally seventh to twelveth fractions had shown SPA activity. Results indicated that SPA enzyme could be purified 6.37-8.13 fold, based on the enzyme concentration of the sample. After purification, SPA of vermicompost-treated soil was significantly ($P \leq 0.05$) higher than that of other treatments and it was followed by that of compost, air-dried manure and control treatments.

Table 2. Acid phosphatase activity of soil as affected by different organic amendments

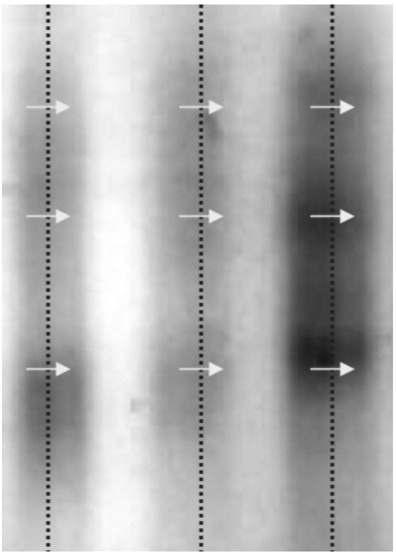
Treatment description		Total activity [a]	Total protein [b]	Specific activity [c]	Purification
Control	Soil	28.38 ± 2.91	119.4 ± 3.98	0.238	-
	Protein solution	17.06 ± 1.04	50.61 ± 2.50	0.34	1.42
	Combined fractions	1.71 ± 0.08	1.13 ± 0.09	1.51	6.37
Manure	Soil	43.25 ± 3.47	136.8 ± 5.18	0.316	-
	Protein solution	36.17 ± 1.29	69.44 ± 1.83	0.52	1.65
	Combined fractions	4.16 ± 0.13	1.73 ± 0.08	2.40	7.61
Compost	Soil	56.67 ± 2.86	156.4 ± 7.16	0.362	-
	Protein solution	44.81 ± 0.96	75.62 ± 2.05	0.59	1.64
	Combined fractions	7.42 ± 0.21	2.96 ± 1.97	2.51	6.92
Vermicompost	Soil	61.09 ± 3.64	167.0 ± 6.95	0.366	-
	Protein solution	54.69 ± 2.14	84.59 ± 2.47	0.65	1.77
	Combined fractions	9.67 ± 0.26	3.25 ± 2.65	2.98	8.13

a: μg p-nitrophenol hr^{-1} ml^{-1} soil, b: mg ml^{-1} soil and c: μg p-nitrophenol hr^{-1} mg^{-1} protein

PAGE of protein fractions, obtained after FPLC analysis, revealed the variation in isozyme pattern of SPA due to different organic amendment application (Fig. 5). Relative concentrations of SPA isozymes were presented in Table 3. The total band intensity of different treatments had shown significantly positive correlation ($r = 0.893^*$) with the SPA activity of purified protein fraction.

3.3.2 Analysis of organic acids

The yellow coloured ring in bromophenol blue medium indicated the release of acids by PSB. Ethyl acetate extracts of PSB, isolated from vermicompost treated soil, were analyzed by TLC (Fig. 6). Analysis indicated that the presence of several organic acid (like citric, lactic and oxalic acids) in the culture medium of PSB. Therefore, it could be concluded that PSB in vermicompost-treated soil produced these organic acids to solubilize insoluble P.

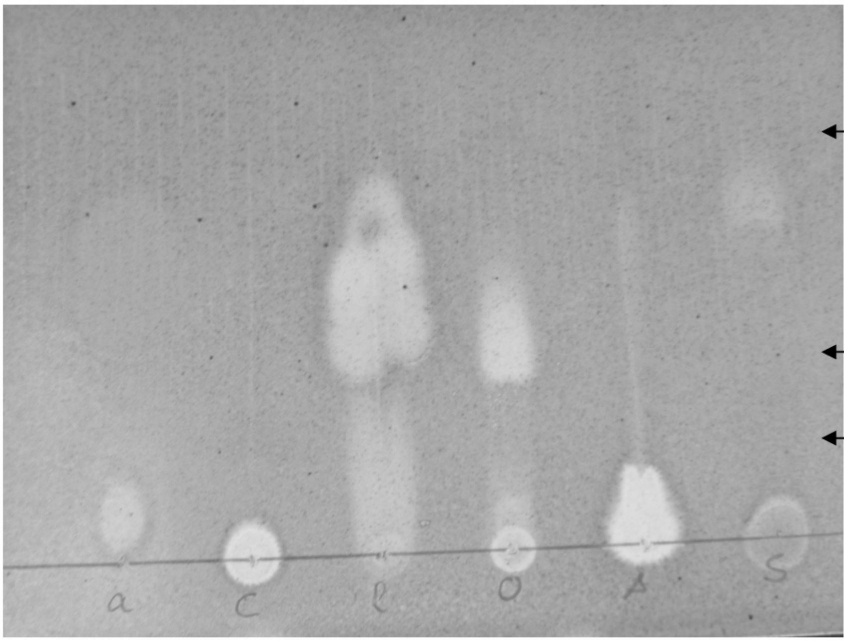


T_1: air-dried manure, T_2: compost and T_3: vermicompost

Fig. 5. Changes in acid phosphatase isozymes of soil as affected by different treatments.

Table 3. Relative band intensity of acid phosphatase isozymes as affected by different treatments

Cattle manure	Group I	Group II	Group III	Total intensity
Air-dried	17.04	21.59	40.15	78.78
Composted	13.11	20.22	60.42	93.75
Vermicomposted	49.37	33.62	81.49	164.48



a: acetic acid, c: citric acid, l: lactic acid, o: oxalic acid and s: salicylic acid
S: broth extract

Fig. 6. Detection of organic acids in ethyl acetate extract of PSB broth culture.

4. Conclusion

Vermicompost was the most effective to solubilize insoluble P in red lateritic soil (Alfisols) as compared to other organic amendments (air-dried cattle manure and compost). The highest SPA in vermicompost-treated soil attributed to the comparatively higher concentration of all the three SPA isozymes in these soils. The maximum P-solubilization in these soils might be attributed to the highest SPA and presence of several organic acids like citric, lactic and oxalic acids in vermicompost-treated soils. Since, vermicompost application also increased TOC, mineralizable N and exchangeable K content of soil, vermicompost could be considered as the most rational organic amendment to improve chemical properties of red lateritic soils (Aqualfs).

References

Biswas, D.R., Narayanasamy, G., 2006. Rock phosphate enriched compost: An approach to improve low-grade Indian rock phosphate. Bioresource Technology 97, 2243–2251.

Biswas, T.D., Mukherjee, S.K., 2000. Text book of soil science, 2nd edn. Sixth reprint, Tata-McGrawHill Publishing Company Ltd, New Delhi.

Brady, N.C., 2002. The Nature and Properties of Soil, 13th ed., Colier McMillan, London.

Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A., Young, C.C., 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied Soil Ecology 34, 33-41.

Eghball, B., Wienhold, B.J., Gilley, J.E., Eigenberg, R.A., 2002. Mineralization of manure nutrients. Journal of Soil and Water Conservation 57, 470–473.

Jackson, M.L. 1973. Soil chemical analysis. Prentice Hall Private Ltd, New Delhi, India.

Lavelle, P., Martin, A., 1992. Small-scale and large-scale effects of endogeic earthworms on soil organic matter dynamics in soils of the humic-tropics. Soil Biology and Biochemistry 12, 149–1498.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.L., 1951. Protein measurement with folin phenol reagent. Journal of Biological Chemistry 193, 265–275.

Nelson, D.W., Sommers, L.E., 1982. Total carbon and organic carbon. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of soil analysis, part 2, pp. 539–579. American Society of Agronomy, Madison, WI.

Ochwoch, V.A., Claassens, A.S., deJager, P.C., 2005, Chemical Changes of Applied and Native Phosphours during Incubation and distribution into different soil phosphorous pool. Community of Soil Science and plant analysis 36, 335-556.

Olsen, S.R., Sommers, L.E., 1982. Phosphorus. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of soil analysis, part 2, pp. 403-413. American Society of Agronomy, Madison, WI.

Pikovskaya, R.I., 1948. Mobilization of phosphorus in soil with the vital activity of some microbial species. Microbiologiya 17, 362-370.

Pramanik, P., Ghosh, G.K., Ghosal, P.K., Banik, P., 2007. Changes in organic-C, N, P and K and enzyme activities in vermicomposts of biodegradable organic wastes under liming and microbial inoculants. Bioresource Technology 98, 2485-2494.

Ramachandran, K., Srinivasan, V., Hamza, S., Anandaraj, M., 2007. Phosphate solubilizing bacteria isolated from the rhizosphere soil and its growth promotion on black pepper (*Piper nigrum* L.) cuttings. Developments in Plant and Soil Sciences 20, 325–331.

Thien, S.J., Myers, R., 1992. Determination of bioavailable phosphorus in soil. Soil Science Society of America 56, 814–818.

Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphatase for assay of soil phosphatase activity. Soil Biology and Biochemistry 1, 301 – 307.