



Trade Science Inc.

# BioTechnology

*An Indian Journal*

**FULL PAPER**

BTAIJ, 7(4), 2013 [154-158]

## Impact of organic manure amendment on soil physicochemical, biological and enzymatic properties

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### ABSTRACT

In this study, physico-chemical, microbial and enzymatic properties of soil composed with organic manure was studied. Soil composed with organic manure underwent changes in all measured physicochemical and microbiological parameters like higher water holding capacity, moisture content, electrical conductivity, organic content and microbial populations than the control soil. Nearly two fold improvements in microbial populations including both bacterial and fungal populations were observed organic manure amended soil than the control. Soil cellulase activity was improved in organic manure soil. With increasing the soil incubation period the soil cellulase activity also enhanced up to 14th day interval and thereafter declined in both control and test soil. Improved physicochemical, microbial and enzymatic parameters in organic manure amended soil are an indication of improvement of soil fertility.

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### KEYWORDS

Organic manure;  
Physico-chemical and  
biological properties;  
Soil cellulase.

### INTRODUCTION

One of the major concerns in today's world is the pollution and contamination of the soil. The use of chemical fertilizers and pesticides has caused tremendous harm to the environment. Organic fertilizer differs from chemicals in that they feed plants while adding organic material in the form of biocompost or organic manure (tree/vegetative waste, municipal/vegetable waste) to the soil. Organic farming or natural farming technology is necessary to support the developing organic, sustainable and non-pollution agriculture. These methods are cost effective and ecofriendly in nature Soil is an excellent natural medium and soil enzymes play key biochemical

functions in the overall process of organic matter decomposition in the soil system<sup>[1]</sup>. Amendment of solid organic manure or biocompost increases the Physico-chemical microbial and enzyme activities of soil. Soil microorganisms and enzymes are highly involved in the degradation of soil organic matter and nutrient cycling. They catalyze several important reactions which are necessary for the life processes of microorganisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling<sup>[2]</sup>. The activities of these enzymes in soils undergo complex biochemical processes and play an important role in agriculture and particularly in nutrient cycling<sup>[3,4]</sup>. In soil, cellulose is available primarily in the

form of litter (dead plant leaf material) or lignocellulosic agricultural waste that is relatively recalcitrant due to the high lignin content of terrestrial plants. A lack of fixed nitrogen and other nutrients may secondarily limit microbial growth, and the low moisture content of soils<sup>[5]</sup>. The enzyme cellulase is a complex enzyme it play an important role in bioconversion of cellulose to simple soluble reducing sugars. The cellulose in soils are derived mainly from plant debris and incorporated into the soil, the limited amounts may also originate from fungi and bacteria in soils. Growth and survival of microorganisms important in most agricultural soils depends on the carbon source contained in the cellulose occurring in the soils whether in the form agricultural waste or cattle feed waste<sup>[7]</sup>. The Organic phosphate is converted by an enzyme the phosphatase is extra cellular enzyme plays an important role between biologically unavailable phosphorus and available phosphorus<sup>[8]</sup>. The inorganic phosphorus availability was controlled by soil organic matter which is influence microbial activity. The phosphatase activity was correlated with organic phosphate and microbial populations<sup>[9]</sup>. It plays key role in soil ecosystem and it is good indicator of soil fertility<sup>[10]</sup>. The soil pH influences the release and stability of phosphatase<sup>[3,11]</sup>. An attempt was made in this study to observe the influence of organic manure on soil physico-chemical, biological and enzyme particularly cellulase the key enzyme for degradation of cellulosic waste in organic manure.

## MATERIALS AND METHODS

### Collection of sample

The organic manure in the form, of forest tree waste leaf, litter decomposed waste was collected from near the local forest area of Tirupati, Andhra Pradesh, India. The soil without organic manure was treated control was collected from adjacent site of biocompost and these two samples were air-dried and mixed thoroughly to increase homogeneity and shifted through < 2 mm sieve.

### Analytical methods for characterization of soil

The physico-chemical properties of organic manure amended and normal soil were analyzed by standard methods APHA<sup>[12]</sup>. Water holding capacity of soil

sample were measured by finding amount of distilled water added to soil sample to get saturation point and then sixty per cent water holding capacity of soil sample was calculated by the method<sup>[13]</sup>.

### Enumeration of bacteria

Bacterial populations in control and test soils were enumerated from soil sample on nutrient agar medium with the following composition. (g/L): Peptone - 5.0, NaCl- 5.0, Beef extract- 3.0, Agar agar- 20.0, Distilled water- 1000 ml, PH- 7.2.. After preparation of medium, 20 ml of sterile medium was aseptically transferred to sterile Petri plates and allowed for solidification. After solidification of the medium 100 $\mu$ l aliquots of soil suspension was speeded uniformly with the help of sterile glass spreader. The plates were incubated in an incubator at 37 °C for 3 days. After incubation, bacterial colonies grown on plates were counted by Queby colony counter. Bacterial colonies are sub cultured on nutrient agar slants for further studies.

### Enumeration of fungi

Fungal populations in both control and test soils were enumerated on Czapeck-Dox agar medium. After preparation of medium, 20 ml of sterile medium was aseptically transferred to sterile Petri plates and allowed for solidification. After solidification of the medium 0.1 ml aliquots of soil suspension was speeded uniformly with the help of sterile glass spreader. The plates were incubated at room temperature (28°C $\pm$ 30°C) for 7 days. After incubated, fungal colonies grown on plates were counted.. The fungal colonies grown on the medium are sub cultured on the Czapeck-Dox agar slants for further studies.

### Cellulase enzyme assays

For assay of soil cellulose five grams of test sample (Organic manure supplemented) and control sample were transferred to test tubes and maintained at 60% water holding capacity at room temperature in the laboratory (28 $\pm$ 4°C) at regular intervals 0, 7, 14, 21, 28 days of incubation. Duplicate soil samples of each test and control were drawn with at periodic intervals to determine the cellulase enzyme. The effect of addition of to the soil organic manure to the soils studied by incubating the soil sample at 5, 10 percentages with control soil sample. The soil samples were transferred

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to 250 ml Erlenmeyer flasks and 1 ml of toluene was added. After 15 min, 6ml of 0.2M acetate buffer containing carboxy methyl cellulose added to soil samples containing conical flasks were plugged with cotton and incubated for 30 min at 30°C for cellulase activity. After desired incubation, soil extracts were passed through whatman filter paper and the filtrate was assessed by the method<sup>[14]</sup>.

## RESULTS AND DISCUSSIONS

### Physico-chemical properties organic manure soil

Soil fertility mediated by microorganism is dependent on maintenance of physico-chemical and biological characteristics in soil. Analysis soil with vermicompost underwent changes in all measured parameters than control soil. Soil composed with vermicompost exhibited improved physical and chemical properties. This compost imports black colour to soil. Higher water holding capacity from 0.26 - 0.42ml/g, moisture content, and higher electrical conductivity 0.3.9-1.89  $\mu$ Mhos/cm were observed in the control and compost soil respectively. These improvements in compost soil may be due to the deposition of organic manure in the form of vermicompost. These results were confirmed by the previous studies<sup>[17,18,19,20,21]</sup> organic effluents had increased the electrical conductivity to the soil. Similar reports made Pradeep and Narasimha 2011 (Leather effluents)<sup>[22]</sup>, Radha et al 2012, (Abattoir)<sup>[23]</sup> disposal effluents from Leather and Abattoir wastes improved the physicochemical properties of soil. In contrast, soil polluted with cement industries had low water holding capacity and electrical conductivity<sup>[24]</sup>. Slightly improved condition of pH (7.11) in compost soil was recorded in the present study. Similarly, Lalithakumari *et al.*<sup>[25]</sup>, Sparling et al.<sup>[17]</sup> and Nizamuddin, et al.<sup>[21]</sup> reported that discharges of dairy products like milk residues from dairy industry increased the soil pH. In contrast, Zande *et al.*<sup>[26]</sup> reported that the discharges of cane sugar residues from sugar cane industry reduced the soil pH. Higher organic matter content (8.9%) measured in vermicompost soil than the control with 5.4. Higher organic content of soil may be due to the decomposed form of vermicompost in the soil. Zande *et al.*<sup>[26]</sup>, Dodor and Tabatabai<sup>[27]</sup>, Nizamuddin *et al.*<sup>[21]</sup> made similar reports on the discharge of organic efflu-

ents like, dairy effluents, increase of organic matter. Improvement in total contents of nitrogen (0.9-1.25) phosphorous (65-178) organic carbon (5.7-12.9) and potassium (129-327) in observed in compost than the control (TABLE.1). Higher contents of these chemical properties of organic manure or biocompost may be due to the decomposed form of organic manure in the soil. Similarly, Narasimha *et al.*, (cotton ginning industry)<sup>[18]</sup>, Kaushik *et al.*, (Distillery)<sup>[28]</sup> made similar reports on the discharge effluents from agro based industries were improved the soil total phosphorous in contaminated soil. Nizamuddin<sup>[21]</sup>, reported that discharge of effluents from sugar and dairy industry enhanced the potassium content and nitrogen content in the soil.

**TABLE 1 : Physico-Chemical Properties of Organic manure and Control Soil.**

Properties	Control soil	Organic manure soil
Color	Grey	Black
pH	6.9	7.11
Electrical conductivity ( $\mu$ Mhos/cm)	0.33	1.92
Water holding capacity (ml/g of soil)	0.26	0.54
Organic matter (kg/g of soil)	4.6	9.2
Total nitrogen (Kg/h)	0.9	1.25
Phosphorus (kg/h)	65	178
Carbon (Kg/h)	5.7	12.9
Potassium (kg/h)	129	327

\*Values represented in the figure are mean of two separately conducted experiments.

**TABLE 2 : Microbial Population in Organic manure and Control Soil**

Parameter	Control soil (CFU/g soil)	Organic manure (CFU/g soil)
Bacteria	36 x 10 <sup>4</sup>	102 x 10 <sup>4</sup>
Fungi	4 x 10 <sup>4</sup>	13x10 <sup>4</sup>

\* Microbial population was counted in the form of CFU/g soil.

\*Activity measured in liberation of micromole of tyrosine/g soil

\*Values represented in TABLE are mean of Triplicates

### Microbial properties

The microorganisms play a vital role in nutrient cycling and soil fertility. Bacteria and fungi synthesize and secrete soil enzymes such as cellulase enzyme. This enzyme constitutes an important part of the soil matrix as extra cellular enzymes<sup>[29]</sup>. Micro flora of organic soil

was enumerated and listed in the TABLE 2. Higher microbial populations in compost were noticed and counted in terms of colony forming units. In the present study, number of bacterial ( $102 \times 10^4$ ) and fungal ( $13 \times 10^4$ ) population were observed in the compost soil than the control soil. The higher bacterial and fungal population may be due to suitable pH and deposition of decomposed organic matter or organic manure in the soil. In contrast irrigation of soil contaminated with effluents from agro based industries such as dairy, sugar cane and cotton mill industries improved the soil microbial population<sup>[17,18,21,30]</sup>.

**TABLE 3 : Cellulase Activity in Organic Manure and Control Soil**

Incubation (in days)	Control soil	Organic manure soil
0	36	66
7	58	148
14	112	358
21	36	92
28	19	44

Activity measured in liberation of micromole of glucose/g soil  
\*Values represented in TABLE are mean of Triplicates

The cellulase activity in soil amended with organic manure was studied and listed in TABLE 3. Microorganisms and their enzymes are the indicators for the crop yield and soil fertility. With influence of soil incubation period cellulase activity was improved up to 14<sup>th</sup> day interval further the activities decreased at 21<sup>th</sup> to 28<sup>th</sup> day of interval in both inoculated and uninoculated soils. This trend was common in Organic manure -treated, and with and without substrates treated soils. Compared with the uninoculated soil 2fold higher cellulase activity was observed in two treated soil than the controls. The cellulase activity at 14<sup>th</sup> day interval was higher than remaining intervals in both substrate and non-treated soil. For instance the cellulase activity in test soil (0) day interval was 66 µg/g of soil whereas at 14<sup>th</sup> day interval tremendously higher enzyme activity was observed that is 358 µg/g of soil. Decreased cellulase activities were observed at 21 and 28 days of intervals. In case of control soil this trend was reduced up to 80-90% at all intervals. The cellulase activity in normal soil that is without combination treated soil was recorded to have 3 fold higher enzyme activities was observed in organic manure treated soil then control soils (TABLE 3) Similar report was made

others, Kannan and Oblisamy<sup>[31]</sup>, (paper and Pulp) Rajasekhar Reddy<sup>[32]</sup> (Cotton ginning Industry) Jyothsna Devi<sup>[33]</sup> (Dairy Industry) and Discharge of these agro based industrial effluents consisting of lignocellulosic organic waste improved the soil cellulase activity.

## CONCLUSIONS

Analysis of soil with organic manure improved the physicochemical, biological and parameters like water holding capacity moisture content, PH, electrical conductivity, organic contents and microbial populations including bacteria and fungi than the controls soil. Soil enzymes like protease and cellulase activities were improved in soil amendment with organic manure than control soil. With increasing the soil incubation period the soil enzyme activities also improved in both control and test soil. Nearly threefold higher cellulase activity was observed in organic manure soil than control. Improved physicochemical and microbial population and enzyme activities treated soil is an indication of improvement in soil fertility.

## REFERENCES

- [1] R.G.Burns; Extracellular enzyme-substrate interactions in soil, In: Microbes in their natural environment J.H.Slater, R.Witttenbury, J.W.T.Wimpenny (Eds), Cambridge University press London, 249-298 (1983).
- [2] R.P.Dick; Soil enzyme activities as indicators of soil quality, In: J.V.Doran, D.C.Coleman, D.F.Bezdick, B.A.Stewart (Eds), Defining Soil Quality for a sustainable Environment Soil Science Society of American society of Agriculture Madison, 107-124 (1994).
- [3] M.A.Tabatabai; Soil enzymes. In: R.Weaver, S.W.Angle, P.Bottomley (Eds.), Methods of Soil Analysis. Part 2: Microbiological and Biochemical Properties. Soil Science Society of America, Madison, 775-833 (1994).
- [4] R.P.Dick; Soil enzyme activities as integrative indicators of soil health, In: C.E.Pankhurst, B.M.Doube, V.V.S.R.Gupta (Eds.), Biological Indicators of Soil Health, CAB International, Wallingford, 121-156 (1997).
- [5] Lynch; Straw as fermentation raw material. Brit. Crop.Prot.Council Monograph.32. Biotechnol Appl. Agric., 35-41 (1985).

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- [6] P.A.Richmond; Occurance and functions of native cellulose. In: Biosynthesis and biodegradation of cellulose C.H.Haigler, P.J.Weimer (Eds), 5-23 (1991).
- [7] S.P.Deng, M.A.Tabatabai; Cellulase activity of soils. *Soil BioBiochem.*, **26**, 1347-1354 (1994).
- [8] T.W.Speir, D.J.Ross; Soil phosphatase and sulphatase. In: Soil enzymes R.G.Burns (Ed.), Academic Press, New York, 197-250 (1978).
- [9] J.C.Tarafdar, A.Jungk; Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biol.Fert.Soils*, **3(4)**, 199-204 (1987).
- [10] W.A.Dick, M.A.Tabatabai; Potential uses of soil enzymes. In: Jr. F.B.Metting (Ed.), *Soil Microbial Ecology: Applications in Agricultural and Environmental Management*, Marcel Dekker, New York, 95-127 (1991).
- [11] V.Acosta-Martinez, M.A.Tabatabai. Enzyme activities in a limed agricultural soil. *Biol.Fertil.Soils*, **2000(31)**, 85-91 (2000).
- [12] APHA-AWWA-WEF: Standard methods for the examination of water a waste water 20<sup>th</sup> Edn. American public health association, America water works association, Water Federation, Washington, DC, (2000).
- [13] C.M.Johnson, A.Ulrich; Determination of moisture in plant tissues. *Calif. Agro.Bull.* In: *Soil and Plant analysis for tree culture* S.A.Wilde (Ed.), Obortage publishing Co. Oxford, **766**, 112-115 (1960).
- [14] S.K.Pancholy, E.L.Rice; Soil enzymes in relation to old field succession, Amylase, Cellulase, Invertase, Dehydrogenase and Urease. *Soil Sci.Soc Am.Proc.*, **37**, 47-50 (1973).
- [15] N.Nelson; A photometric adaptation of Somogyi method for determination of glucose, *J.Biol.Chem.*, **153**, 375-380 (1952).
- [16] V.F.Kuprevich, T.A.Shcherbakova; Comparative enzymatic activity in diverse types of soil, In: A.D.McLaren, J.Skujins (Eds.), *Soil Biochemistry*, M.Dekker New York, **2**, 167-201 (1971).
- [17] G.P.Sparling, L.A.Schipper, J.Russel, Changes in soil properties after application of dairy factory effluent to New Zealand volcanic ash and pumice soils, *Aust.J.Soil Res.*, **39**, 505 (2001).
- [18] G.Narasimha, G.V.A.K. Babu, B.Rajasekhar Reddy; Physicochemical and biological, properties of soil samples collected from soil contaminated with effluents of cotton ginning industry. *J.Env.Biol.* **20**, 235-239 (1999).
- [19] M.Poonkothai, R.Parvatham; Bio-Physical chemical assessments of automobile waste water. *J.Ind.Poll Contl.*, **21**, 59-62 (2005).
- [20] C.Xiao, M.Fauci, D.F.Bezdicek, W.T.McKean, W.L.Pan; Soil microbial responses to potassium-based black liquor from straw pulping, *Soil Science Society of America Journal*, **70**, 72-77 (2005).
- [21] S.Nizammudin, A.Sridevi, G.Narasimha; Impact of dairy factor effluents on soil enzyme activities. *Eco. Env. & Cons.*, **14(1)**, 89-94 (2008).
- [22] M.Reddi Pradeep, G.Narasimha; Effect of leather industry effluents on soil microbial and protease activity, *J.Enviro.Biol.*, **33**, 39-42 (2012).
- [23] S.Radha, V.J.Nithya, A.Sridevi, G.Narasimha; Effect of abattoir effluents Disposal on soil microbial and enzyme activities *Arch.Appl.Biol.Res.*, **13**, 41-4 (2011).
- [24] M.Shanthi, *Soil Biochemical Processing Industrially Polluted Areas of Cement Industry*. M.Phil thesis. Sri Krishnadevaraya University, Anantapur, India, (1993).
- [25] B.Lalitha Kumari, M.A.singaracharya; Characterization of dairy industry effluent amended soils in Warangal, Andhra Pradesh, *J.Indian Bot.Soc.*, **77**, 63-66 (1998).
- [26] G.K.Zende; Sugar industry by product and crop residues in increasing soil fertilizer, *Soil and crop productivity in sugar cane agro industrial alternations*, 351-369 (1996).
- [27] D.E.Dodor, M.A.Tabatabai; Effect of cropping systems on phosphatases in soils, *journal of plant Nutrition and sois Sciences.*, **1**, 133 (2003).
- [28] K.Kaushik, R.Nisha, K.Jageeta, C.P.Kaushik; Impact of long and short term irrigation of a sodic soil with distillery effluent in combination with Bio-amendments. *Bioresource Technology*, **96(17)**, 1860-1866 (2005).
- [29] R.L.Sinsabaugh; Enzymic analysis of microbial pattern and process, *Biology and Fertility of Soils*, **17**, 69-74 (1994).
- [30] P.B.Degens, L.A.Schipper, J.J.Claydon, J.M.Russell, G.W.Yeats; Irrigation of an allophonic soil with dairy factory effluents for 22 years. Responses of nutrient storage biota. *Australian journal of soil Ressearch.*, **38**, 25-35 (2000).
- [31] K.Kannan, G.Oblisami; Influence of pulp and paper mill effluents on soil enzyme activities, *Sol.Biol.Biochem*, **22**, 923-927 (1990).
- [32] B.Rajasekhar Reddy, G.Narasimha, G.V.A.K.Babu; Cellulolytic activity of fungal cultures, *J.Scientific & Indus.Research*, **57**, 617-620, (1998).
- [33] V.Jyothsna Devi, G.Narasimha; Influence of dairy water on soil physicochemical, biological and enzymatic properties *Pollution Research*, **26(4)**, 711-714 (2007).