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.

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[1]. 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[2]. The activities of these enzymes in soils undergo complex biochemical processes and play an important role in agriculture and particularly in nutrient cycling[3,4]. 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. 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. 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. 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. It plays key role in soil ecosystem and it is good indicator of soil fertility. The soil pH influences the release and stability of phosphatase. 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 physic-chemical properties of organic manure amended and normal soil were analyzed by standard methods APHA. 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.

**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 µl aliquots of soil suspension was speeded uniformly with the help of sterile glass spreader. The plates were incubated at 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±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±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...
to 250 ml Erlenmeyer flasks and 1 ml of toluene was added. After 15 min, 6 ml 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 whattman filter paper and the filtrate was assessed by the method[14].

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 composted with vermicompost exhibited improved physical and chemical properties. This compost imports black colour to soil. Higher water holding capacity from 0.26 - 0.42 ml/g, moisture content, and higher electrical conductivity 0.32-1.89 μMhos/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[17,18,19,20,21] organic effluents had increased the electrical conductivity to the soil. Similar reports made Pradeep and Narasimha 2011 (Leather effluents) [22], Radha et al 2012, (Abattoir) [23] 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 [24]. Slightly improved condition of pH (7.11) in compost soil was recorded in the present study. Similarly, Lalithakumari et al. [25], Sparling et al. [17] and Nizamuddin, et al. [21] reported that discharges of dairy products like milk residues from dairy industry increased the soil pH. In contrast, Zande et al. [26] 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. [26], Dodor and Tabatabai [27], Nizamuddin et al. [21] made similar reports on the discharge of organic effluents 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) [18], Kaushik et al., (Distillery) [28] made similar reports on the discharge effluents from agro based industries were improved the soil total phosphorous in contaminated soil. Nizamuddin [21], reported that discharge of effluents from sugar and dairy industry enhanced the potassium content and nitrogen content in the soil.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Control soil</th>
<th>Organic manure soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Grey</td>
<td>Black</td>
</tr>
<tr>
<td>pH</td>
<td>6.9</td>
<td>7.11</td>
</tr>
<tr>
<td>Electrical conductivity (μ Mhos/cm)</td>
<td>0.33</td>
<td>1.92</td>
</tr>
<tr>
<td>Water holding capacity (ml/g of soil)</td>
<td>0.26</td>
<td>0.54</td>
</tr>
<tr>
<td>Organic matter (kg/g of soil)</td>
<td>4.6</td>
<td>9.2</td>
</tr>
<tr>
<td>Total nitrogen (Kg/h)</td>
<td>0.9</td>
<td>1.25</td>
</tr>
<tr>
<td>Phosphorus (kg/h)</td>
<td>65</td>
<td>178</td>
</tr>
<tr>
<td>Carbon (Kg/h)</td>
<td>5.7</td>
<td>12.9</td>
</tr>
<tr>
<td>Potassium (kg/h)</td>
<td>129</td>
<td>327</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control soil (CFU/g soil)</th>
<th>Organic manure (CFU/g soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>36 x 10⁴</td>
<td>102 x 10⁷</td>
</tr>
<tr>
<td>Fungi</td>
<td>4 x 10⁴</td>
<td>13 x 10⁴</td>
</tr>
</tbody>
</table>

* 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 [29]. 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 (102×10⁴) and fungal (13×10⁶) 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 [17,18,21,30].

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

<table>
<thead>
<tr>
<th>Incubation (in days)</th>
<th>Control soil</th>
<th>Organic manure soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>36</td>
<td>66</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>148</td>
</tr>
<tr>
<td>14</td>
<td>112</td>
<td>358</td>
</tr>
<tr>
<td>21</td>
<td>36</td>
<td>92</td>
</tr>
<tr>
<td>28</td>
<td>19</td>
<td>44</td>
</tr>
</tbody>
</table>

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 14th day interval further the activities decreased at 21st to 28th 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 14th 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 14th 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 than control soils (TABLE 3) Similar report was made others, Kannan and Oblisamy [31], (paper and Pulp) Rajasekhar Reddy [32] (Cotton ginning Industry) Jyothsna Devi [33] (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 physic chemical and microbial population and enzyme activities treated soil is an indication of improvement in soil fertility.

**REFERENCES**


