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Influence of organic manure on soil microbial and enzyme activities

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ABSTRACT

The effect of organic manure on the native soil was investigated in the present study. Supplementation of organic manure to the soil alters the physico-chemical, biological and enzyme properties of the soil. These alternations include decreases in soil P^H from 7.9 to 7.4, increase in electrical conductivity 0.36 to 1.21 (µmohs/cm), water holding capacity 0.36 to 0.38 ml/g of soil of control and test soils respectively. There is increase in soil texture like sand, phosphorous and potassium in the test soil. Enzyme activities such as cellulase and dehydrogenase were assessed in both the soil samples with and without amendment of respective substrates (carboxy methyl cellolose and tri phenyl tetrazolium chloride). Accumulation of end products, glucose and formazone from the substrates in the soil were estimated at periodic intervals. Celluase and dehydrogenase enzyme activities were relatively higher in soil amended with organic manure and respective substrate than control.

KEYWORDS

Organic manure;
Physico-chemical properties;
Microbial population;
Enzymes activities.

INTRODUCTION

Organic agriculture is a holistic production management system which promotes and enhances agro system health, including biodiversity, biological cycles and soil biological activity. Organic agriculture is a system that relies on ecosystem management rather than external agricultural inputs. It is a system that begins to consider potential environmental and social impacts by eliminating the use of synthetic inputs. It is a system that begins to consider potential environmental and social impacts by eliminating the use of synthetic inputs replaced with site specific management practices that maintain and increase long term soil fertility and prevent pest and diseases. Organic manure is a broad sense includes compost from rural and urban wastes, crop residues, agro industrial biowastes and green manures apart from the commonly used farm yard manure. Organic manure improves soil porosity and water holding capacity and microbial environment replenishes essential micro nutrients in soil, increases the utilization efficiency of the applied fertilizers and favours micro nutrient availability to the plants. Soil management influences soil microorganisms and soil microbial processes through changes in the quantity and quality of plant residues entering the soil and its spatial distribution. Soil biological activity is dependent on climatic factors and geological parent materials. Soil biological activity is greatest in the root surface rizosphere region of plants. Soil microbial biomass and soil microbial activity are highly

correlated with soil quality which can be used as vital indicators of soil fertility and productivity. The turnover and mineralization of organic substances, nutrient transformations, and cycling of organic wastes in soil are all dependent on the metabolic functions of soil microorganisms[1,2]. Soil enzymes are derived primarily from microorganisms, plants roots and soil animals and they are indicators of soil biological activity. Soil enzymes are important for catalyzing innumerable reactions necessary for life process of microorganisms in soils, decomposition of organic residues, cycling of nutrients and formation of organic matter and soil structure. Soil microbial enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system[3,4]. They are used as sensors in studies on the influence of soil treatments on soil fertility. They are important in catalyzing several important in catalyzing several important reactions for the life processes of microorganisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic wastes, organic matter formation and nutrient cycling[5]. All soils contain a group of enzymes that determine soil metabolic process[6] depend on its physical, chemical, microbiological and biochemical properties. Those microbial secreted enzymes constitute an important part of the soil matrix cellular enzymes also called "Abiontic enzymes"[7]. Some enzyme activities can be more influenced by type of organic matter than the quantity of organic matter. Enzyme activities were associated with active microorganisms in soil which are the major source of soil enzymes. Nutrients availability also influence the enzyme activities as various microorganisms control their enzyme production in response to nutrient availability[8]. Soil enzyme activities are often used an indices of microbial growth and activity in soils. Cellulases are a group of enzymes that catalyse the degradation of cellulose, polysaccharides build up of β-1,4 linked glucose units[9]. Activity of cellulases in agricultural soils is affected by several factors. These include temperature, soil $p^{H,}$ water and oxygen contents, the chemical structure of organic matter and its location in the soil profile horizon[9]. Cellulase enzymes play an important role in global recycling of the most abundant polymer, cellulose in nature. Decomposition of cellulose enzymes in the soil is for microbial use and improves soil health in agricultural ecosystem.

MATERIALS AND METHODS

Collection of organic manure

Organic manure soil which contains tress wastes and litter (test sample), control sample (native soil) was collected from biofarming field, Pulicherla, Chittoor (Dist), Andhra Pradesh, India.

Analytical methods for characterization of soil

The Physico-chemical properties of organic manure s and control sample soils were carried out[10].

Biological properties

The biological properties including bacterial and fungal populations both soils were enumerated by soil serial dilution method.

Enzyme assays

Celluase assay: For assay of soil cellulase 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,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 30^0C for cellulase activity. After desired incubation, soil extracts were passed through whattman filter paper and the filtrate was assessed by the method[11].

Dehydrogenase assay

For Dehydrogenase activity, both soil samples were treated with 0.1 gm calcium chloride and 1 ml 0.18m M triphenyl tetrazolium chloride (TTC) as substrate. One ml of distilled water was added in place of TTC, it was served as control. The effect of organic manure on soil microbial and enzyme activity was assessed by incubating various concentrations of organic amended

(test) sample that is 5%, 10% with control soil at different days of incubation 0, 7, 14, 21 days. Determination of dehydrogenase activity in samples in the presence of soil sample on the reduction of 2, 3, 5-triphenyl tetrazolium chloride (TTC). Soil samples were treated with 0.1 gm of 0.18m M TTC incubated at 30^0 C. The triphenylformazone formed was extracted with methanol from the reaction mixture and assayed at 485 nm in spectrophotometer.

RESULTS AND DISCUSSON

Physico-chemical properties

The impact of organic manure on soil physical and chemical properties was studied and tabulated in the TABLE 1.

The soils treated with organic manure showed altered Physico-chemical properties then the control (TABLE 1). For instance higher WHC, electrical conductivity, organic contents were observed in the test sample than the control soil. Higher water holding capacity and electrical conductivity was observed in organic manure treated soil than control soil. The water holding capacity and electrical conductivity increased from 0.36ml/g to0.38ml/g and 0.31umhos/cm to 1.21umhos in test and control soils respectively. Increased Water holding capacity in this study may be due to the accumulation of organic residues in organic manure in test sample. (TABLE 1) These results were in conformity with Sparling *et al* (Dairy industry)[12], Narasimha et al (cotton ginning industry)[13,14]. Xiao et al[15] (Black liquor straw pulp) had increased electrical conductivity. In contrast Shanti (1993)[16] reported, soil polluted with cement industry had low water holding capacity and electrical conductivity. The P^H of the soil was represented in (TABLE 1) There was slight variation in P^H from 7.4 to7.9 upon based on the organic residues. Similar reports were made by Zende et al[17] that discharging of cane sugar residue from sugar cane industry reduced the soil P^H. In the present study higher organic content was observed in the organic manure (TABLE 1). The total content of phosphorous in test and control sample was 183kg/h and 35kg/h respectively. Narasimha et al[13] made similar reports that the discharge of effluents from cotton ginning mill enhanced the soil total phosphorus contents in the organic manure treated soil than the control. Potassium content in test and control sample was 827kg/h and 291kg/h respectively. Similarly Narasimha et al[18] and Nizamuddin et al[19] reported that discharge of effluents from sugar and diary industry enhanced the soil total potassium by 2 to 4 folds.

TABLE 1 : Physico-chemical properties of soil treated (Organic manure)/ non treated (control)

PROPERTIES	ORGANIC MANURE TREATED SOIL (TEST)	NATIVE SOIL (CONTROL)
P^H	7.9	7.4
Water holding capacity (ml/g of soil)	0.38	0.36
Electrical conductivity (μ mhos/cm)	1.21	0.36
Texture		
Phosphorous (kg/h)	183	35
Potassium (kg/h)	827	291
Carbon	High	Low

Biological properties

Biological parameters such bacterial and fungal populations were improved due to supplementation of organic manure in test soil than the control (TABLE 2). For instance 4 fold bacterial and 2 folds higher fungal populations were observed in test soil than the control (TABLE 2).

TABLE 2 : Microbial population* in the Organic manure and control soil

Type of organism	Test sample	Control sample
Bacteria	$120x10^4$	$12x10^4$
Fungi	$11x10^4$	$5x10^4$

***Microbial population was measured in the terms of colony forming units CFU/g of soil.**

Soil microbial biomass and soil microbial activities are as the indicators of soil fertility. The turnover and mineralization of soil substance, nutrient transformation and microbial population, affects the soil fertility Sparling et al.[12]. Higher bacterial and fungal populations were observed in test soil than control soil. For instance test soil contains $120x10^4$, $11x10^4$ bacterial, and $12x10^4$, $5x10^4$ fungal populations observed in test and control soils respectively. Higher bacterial population in test soil may be due to higher Organic content (Humas) in soil. Similarly, Narasimha[13], reported that microbial popu-

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lations were increased with discharge of effluents from cotton ginning mill to the soil. Jenkinson and Ladd[20] also reported that biomass generally increased by the application of organic matter which may have overcome chemical fertilizers both in terms of organic matter which may have overcome chemical fertilizers both in terms of sustainability and from environmental conditions. In contrast irrigation with dairy effluents enhanced the soil microbial and enzyme activities.

The cellulase activity in soil inoculated with organic manure was studied and listed in Figure 1. Microorganisms and their enzymes are the indicators for the crop yield and soil fertility. Direct inoculation of microbial population may reflect the soil fertility in terms of improvement of enzyme. With influence of soil incubation period protease activity was improved up to 7th day interval further the activities decreased at 14th 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 widely no folds higher cellulase activity was observed in two treated soil than the controls. The cellulase activity test (treated soil) in substrate treated and untreated soils was also studied here also nearly two fold higher enzyme activity were observed in glucose treated soil than untreated soil. The cellulase activity in soil supplemented with 10% was shown in Figure (1b) with increasing the soil incubation day's cellulase activates also increased up to 7th day declined at further incubation days. 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 substrate soil at initial (0) day interval was 28 µg/g of soil whereas at 14th day interval tremendously higher enzyme activity was observed that is 46 µg/g of soil. Similar trend was observed at remaining days of interval. In case of control soils this trend was reduced up to 80% The cellulase activity in normal soil that is without combination treated soil was recorded to have 4 fold higher enzyme activities was observed in organic manure treated soil then control soils (Figure 1a)

The cellulase activity in both soils at 5% organic manure treated was also observed. Here also with increase in the concentration of organic manure to the soils slightly higher the cellulase activity was recorded

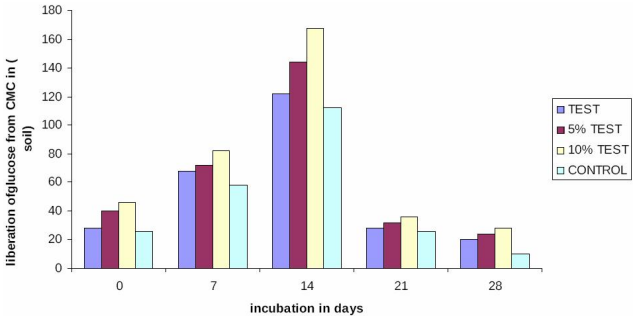
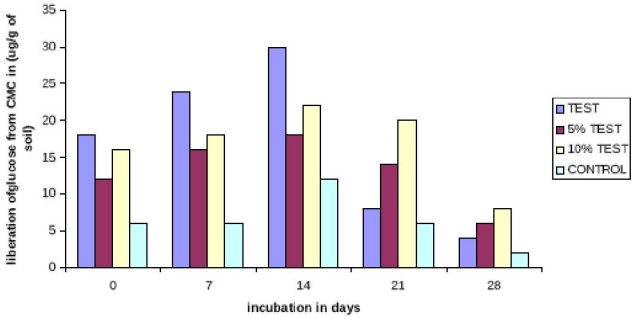


Figure 1(a) : Cellulase activity in organic manure and control soil (with substrate)



.*Activity in terms of liberation of glucose from CMC/gm of soil.

. Values represented in table are mean of duplicate.

Figure 1(b) : Cellulase activity in organic manure and control soil (with out substrate)

in organic manure treated soil then control soil. Per instance the cellulase activity in organic manure soil at 5% was 144 µg of glucose liberated from substrate where as 168µg per gram soil at 10%. Similar trend was followed at remaining days of intervals but in control no considerable higher activities observed (Figure 1a).

The cellulase activity at 5% of organic manure soil was investigated and shown in Figure 2a. Like previous reports with increasing in the concentration of organic manure the soil the cellulase activity also improved at 10% level concentration. For instance the cellulase activity in the soils treated with 10% organic manure treated soil was 168µg per gram of soil where as 144 µg, 122 µg in 5% and test soil concentration respectively with increase in the soil incubation days soil cellulase activity was also improved up to 14th day interval further the activity was calculated in 14, 21 and 28 days of intervals in 10% organic treated soils compared with control soil at different concentration of organic there was no considerable higher activities among the various concentration of soil. Similar report was made oth-

ers, Kannan and Oblisamy[21], Narasimha[13] and Discharge of effluence from agro based industries improved soil protease activity in contrast dust generated from cement industries decreased in the soil protease activity Shanti[16] the percentage of increased in the protease activity in the present study may be due to the direct inoculation of indigenous microorganisms to the soil. Increased proteolytic activity in casein treated soils may be due to the high availability of suitable substrates and increased in proteolytic microorganisms in the soils. Soil protease activity was calculated with the number of soil bacteria protease activity was enhanced not only with the addition of proteins but also with the addition of sugar. Similarly activity was decreased under alkaline conditions. According to the Narasimha et al[13] discharged effluence from cotton ginning mill improved the soil cellulase activity. The dehydrogenase activities of test and control soil were studied and shown in Figure 2. With increasing the incubation period the urease activity improved up to 14th day interval further the activity was declined. Two fold higher urease activity was observed in test sample interval 42µg of form zone /g of soil where as in control 10µg of form zone / g of soil like other soil enzymes cellulase and dehydrogenase activity also increased in the first where and there after declined in both soil examples. The similar traced was observed in urease enzyme activities even in the presence of buffer in both soil ureased buffering condition soil sample treated work organic manure treated soil exhibited above 2-3 fold higher urease activity over control. For instance the dehydrogenase activity of 10µ g of forma zone/g of soil in control soil where as 8µ g of forma zone/ g of soil in organic treated soil at 14th day interval.

Dehydrogenase activity at test, 5 and 10 percentage concentration of organic manure was studied and shown Figure (2a, b) there is a considerable lower dehydrogenase activity was observed in 5% organic manure treated soil where as two fold higher activity was observed in 10% Organic manure treated soil. Among the concentration tested in the present study higher dehydrogenase activity was recorded at 10 % organic manure treated soil. Dehydrogenase activity of vermiash compost produces with incorporation of fly ash in Figure 2(a, b) Dehydrogenase activity in Organic manure and control samples in different incubations as by using different concentrations.

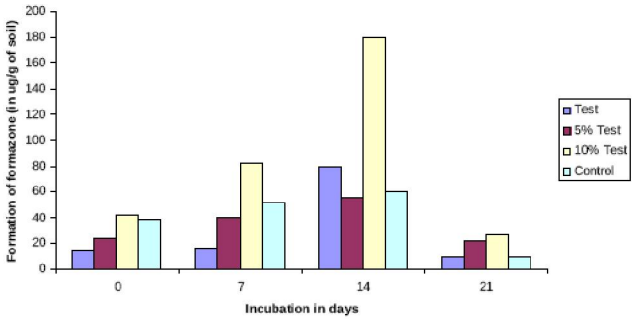
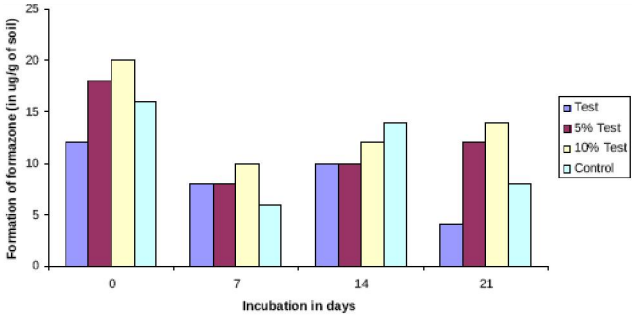


Figure 2(a) : Dehydrogenase activity in organic manure and control soil (with substrate)



. Activity in terms of liberation of formazone from TTC/gm of soil.

. Values represented in table are mean of duplicate.

Figure 2(b) : Dehydrogenase activity in organic manure and control soil sample (without substrate)

Different ratios showed significantly higher activity compared to control. A similar result attributed in worm casts was the result of higher microorganisms' present warm casts. The addition of low metal sludge's, composts and other organic materials has been found to increase the activities of dehydrogenase, phosphatase and β-glucosidase, Chander and Brookes[22]. Soil dehydrogenase system is due to rather wide group of enzymes which transfer electrons to available acceptors including triphenyl tetrazolium chloride. Its activity appears to be more dependent on the metabolic state of microbial population of the soil rather than the activity of the specific free enzymes acting on particular substrate. Soils irrigated with effluent waste water from pulp and paper mills[21]. The increased enzyme activities were attributed to the enhanced microbial activity in soils, stimulated by the addition of nutrients and organic matter contained in the sludges and composts. exhibited relatively high dehydrogenase activity than soil without corresponding irrigation[21,23,24]. According to Brezezinska[25] higher dehydrogenase activity in eutric histosol soil irrigated with municipal waste water was

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recorded. Addition of biomethanated distillery spent wash to dry black and red soils[26] and of distillery effluents[27] enhanced dehydrogenase activity. In the same way dehydrogenase activity was also increased by the addition of fly ash to soil[28]. According to various studies[29,30] increase of organic carbon in soils through addition of sewage sludge resulted in enhancement of dehydrogenase activity. Dehydrogenase activity was relatively higher in soils with higher organic matter[31]. Similarly high dehydrogenase activity in soils with effluent discharges from cotton ginning mill in the present study can be attributed to high organic content and low pH for proliferation of fungi reported elsewhere[13].

CONCLUSION

In the present investigation the results clearly indicates that supplementation of organic manure to the native soil improved the microbial populations and stimulated activity of cellulase and, dehydrogenase, Both soil enzymes exhibited maximum activity on 7th and 14th day interval followed by decreased in their activities at lateral intervals of incubation in comparison to control soil. Improvement in microbial population and soil enzyme activities in organic manure supplemented soil is an indication of improvement of soil health interns of improvement in soil fertility.

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