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Microbial and enzyme activities in cattle dung composed soil

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ABSTRACT

In this study, physico-chemical, microbiological and soil enzymes such as cellulase and phosphatase activities were studied in soil composed with cattle dung. Analysis of soil with cattle dung revealed that compost soil underwent changes in all measured physicochemical, biological and enzymatic parameters like lower water holding capacity (0.4ml/g of soil) moisture content, (0.2%), higher PH (8.9), electrical conductivity (3.6μMhos/cm), organic content (7.9kig/h) and microbial populations, (bacteria, 22×10^4 CFU/g soil) and (fungi 2×10^4 CFU/g soil) were observed in the compost soil. Enzyme activities such as cellulase and phosphatase also increased with increasing the incubation period of soil composed with cattle dung.
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KEYWORDS

Cattle dung;
Physico-chemico-biological properties;
Cellulase;
Phosphatase activities.

INTRODUCTION

Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system[1]. Soil 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 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[5]. 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[7]. The phosphatase is extra cellular enzyme which hydrolysis the organic phosphates to inorganic phosphate and plays an important role between biologically unavailable phosphorus and available phosphorus[8]. 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[9]. It plays key role in soil ecosystem and it is good indicator of soil fertility[10]. The soil pH influences the release and stability of phosphatase[3,11]. An attempt was made in this study to observe the influence of cattle dung on soil physco-chemical, biological and enzyme properties.

MATERIALS AND METHODS

Collection of soil sample

The soil composed with cattle dung was collected from coastal area of Andhra Pradesh, India. The compost soil was 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 cattle dung soil was analysed by standard methods APHA [12]. 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[13].

Enumeration of bacteria

Bacterial populations in soil 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 100µl 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 3days. 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 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.

Soil incubation and enzyme assays studies

Five grams of compost soil samples except two grams for phosphatase were placed in 100 ml conical flasks for determining soil enzymes cellulase and phosphatase activity. Water was added to each flask to get 60% water holding capacity (WHC) and maintained throughout incubation period. The flasks were incubated at room temperature a. Soil samples were withdrawn after 7, 14, 21 and 28 days of incubation to determine the soil enzyme activities.

Cellulase assay

After incubation of soil samples at derived intervals, the soil cellulase enzyme activity was determinate by the method described[14]. Cellulase assay was carried out by the following manner. To the soil samples 0.5 ml of toluene was added and the contents in the flasks were maintained thoroughly. After 15 minutes, 10ml of 0.2 M acetate buffer (pH 5.9) was added followed by 10 ml of 1% Carboxy Methyl Cellulose (CMC) as a substrate. The flasks were incubated for 30 minutes at room temperature. After incubation 50 ml of distilled water was added and soil extracts were filtered through Whatman filter paper, the volume of the filtrate was made up to 100 ml with distilled water. Reducing sugar content in the filtrate was determined by the method[15]. Control was prepared without addition of substrate.

Phosphatase assay

Soil phosphatase was determined by incubating soil at different time intervals (0, 7, 14, 21, 28, days). After incubation of soil samples was withdrawn to determine the enzyme activity of phosphatase as detailed earlier[16]. The samples were treated with 6 ml of molybdate buffer (pH 6.5) and the substrate 2 ml of 0.03 M PNPP (para-nitro phenyl phosphate) disodium salt and incubated for 30 minutes at room temperature for one hour. After that the flasks were placed on ice before the soil extracts were filtered through Whattman filter paper. To the filtrate, one ml of 5M $CaCl_2$ and 4 ml of 0.5 M NaOH were added and the yellow color developed was read at 405 nm. Control was prepared without substrate.

RESULTS AND DISCUSSIONS

Physico-chemical properties of soil

Soil fertility mediated by microorganism is dependent on maintenance of physico-chemical characteristics in soil. These properties were represented in the TABLE 1. Analysis soil samples revealed that compost soil underwent changes in all measured parameters. Soil composed with cattle dung exhibited of physical and chemical properties. The compost soil from complex of cattle dung and hay which are decomposed by several microorganisms present in the soil. This compost made the soil unpleasant and imports black colour to soil. Lower water holding capacity with 0.4ml/g, moisture content, 0.2% and higher electrical conductivity 3.6µMhos/cm were observed in the compost soil. These improvements in compost soil may be due to the long term deposition of organic manure in the form of cattle dung. These results were confirmed by the studies[17-21] 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 alkaline condition (pH-8.9) in compost soil was recorded in the present study. Higher pH in the compost soil may be due to the flooding of sea water (alkaline) in to the compost soil. Lalithakumari *et al.*[25] and 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. Zande *et al.*[26] reported that the discharges of cane sugar residues from sugar cane industry reduced the soil pH. Higher organic matter content (7.90%) of soil may be due to the decomposition of cattle dung and hay into the soil. Zande *et al.*[26], Dodor and Tabatabai[27], Nizamuddin *et al.*[21] made similar reports on the discharge of dairy effluents, increase of organic matter enhanced soil enzyme activities. Total contents of nitrogen, phosphorous organic carbon and potassium in compost soil were 178 Kg/hec, 80 Kg/hec, 0.54 Kg/hec and 549 Kg/hec respectively (TABLE 1). Higher contents of these chemical properties of compost soil may be due to the decomposed compost soil. Compost is an organic matter; it is a mass comprising of a mixture of herbivore dung (source of nitrogen and nutrients) and hay (source of cellulose and hemicellulose). Narasimha *et al.,* (cotton ginning industry)[18], Kaushik *et al.,* (Distillary)[28] made similar reports on the discharge effluents from agro based industries were improved the soil total phosphorous in contaminated soil. Similarly Nizamuddin[21], reported that discharge of effluents from sugar and dairy industry enhanced the potassium content and nitrogen content in the soil.

TABLE 1 : Pysico-chemical properties of compost soil

Properties	Compost soil
Color	Black
Odor	Bad
pH	8.9
Electrical conductivity (μ Mhos/cm)	3.6
Moisture content (ml/g of soil)	0.4
Organic matter (kg/g of soil)	7.9
Total nitrogen (Kg/h)	178
Phosphorus (kg/h)	80
Carbon (Kg/h)	80
Potassium (kg/h)	549

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

Microbial properties

The microorganisms play a vital role in nutrient cycling and soil fertility. Bacteria and fungi synthesize and secrete soil enzymes such as phoshatase, cellulase, protease, etc extracellularly. These enzymes constitute an important part of the soil matrix as extra cellular enzymes[29]. Micro flora of compost soil was enumerated and listed in the TABLE 2. Higher microbial populations were noticed and counted in terms of colony forming units. In the present study, number of bacterial (22×10^4) and fungal (2×10^4) population were observed in the compost soil. The higher bacterial and fungal population may be due to alkaline pH and deposition of organic wastes 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[18,30,17,21].

Soil enzyme activities

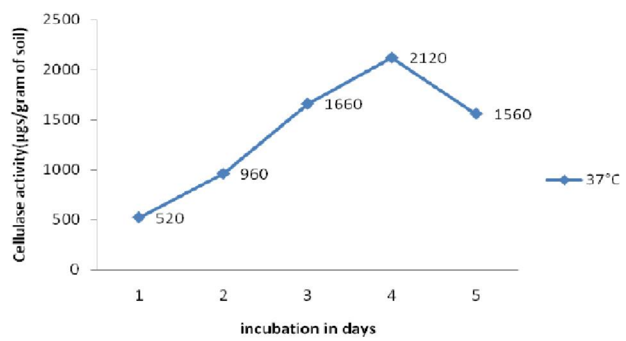
TABLE 2 : Microbial population in cattle dung soil

Parameter	Population in compost CFU/g of soil
Bacteria	$22x10^4$
Fungi	$2x10^4$

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

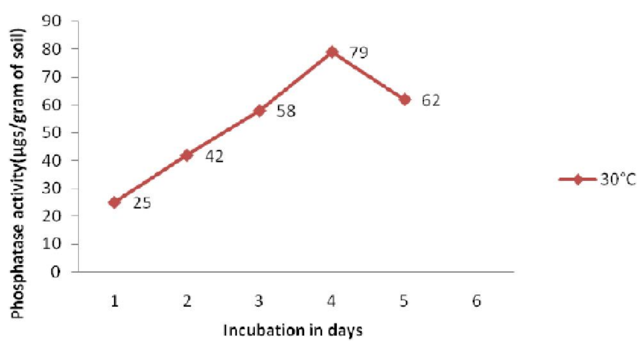
Compost soil sample was analyzed for their enzyme activities. There is a considerable interest in the study of enzyme activities of soils[31] such activities may reflect the potential capacity of a soil to form certain biological transformations of importance to soil fertility[32]. Soil enzymes are highly involved in the degradation of soil organic matter and nutrient cycling. The enzymes cellulase and phosphatase were play crucial role in catalyzing the hydrolysis and solubilizing the substrates glucose, starch, casein and PNPP. In this study, the cellulase activity was measured by incubating the compost soil samples at room temperature in the presence of substrate (1% CMC). The activity was measured in terms of liberating µgs of glucose from carboxy methyl cellulose per gram of soil. With increase in soil incubation period cellulase activity were also improved in compost soil up to 21 days and declined further interval (Figure 2). For instance the cellulase activity of compost soil incubated at 30ºC showed higher cellulase activity (Figure 2). For instance cellulase activity in soil organic waste composed soil increased from 520-2120 µg of glucose (nearly four fold) from '0' day to 21st day intervals, later it was declined at 28th day interval. Higher cellulase activity in the present study would be attributed to the presence of higher organic content and microbial population. The increment in the cellulase activity was correlated with fungal and bacterial number and moisture content of the compost soil. According to Narasimha[18]; Nizamuddin[21]; Nagaraj[33] Srilakshmi et al[34] discharge of effluents from cotton ginning mill, dairy, sugar industries and litter soil improved the cellulase activity.

The activity of phosphatase was measured by incubating the samples at 30ºC in the presence of substrate (PNPP). The Phosphatase activity was measured in terms of release of para-nitro phenol from PNPP. Like cellulase activity with increase in the incubation period of compost soil phosphatase activity also increased up to 21 days of interval there after it was declined. For instance Phosphatase activity in soil organic waste composed soil increased from 25 µgs of PNP at '0' day and after two fold increasing of enzyme activity at 7th day and three fold increasing at 21st day intervals, later it was declined at 28th day interval (Figure 3). According to studies Narasimha, *et al*.[35], discharge of effluents from cotton ginning industry increased the soil phosphatase, dehydrogenase amylase and invertase. Similarly Stimulation of phosphatase activity observed in irrigated soil with waste water from pulp and paper mill was reported kannan et al[36], the high phosphatase activity in cattle dung soil may be due to high organic matter content (TABLE 1) and microbial populations TABLE 2, in the present study.



*** Activity in terms of liberation of glucose from Carboxy methyl cellulose /g of soil; * Values represented in the table are mean of two separately conducted experiments**

Figure 1: Cellulase activity in soil composed with cattle dung



***Activity in terms of liberation of PNP from PNPP / g of soil; *Values represented in the figure are mean of two separately conducted experiments**

Figure 2: Phosphatase activity in soil composed with cattle dung

CONCLUSIONS

Analysis of soil with cattle dung altered the physicochemical, biological and enzymatic parameters like lower water holding capacity moisture content and higher PH, electrical conductivity, organic contents and microbial populations including bacteria and fungi, were observed in the cattle dung composed soil. Higher enzyme activities such as cellulase and phosphatase also measured in soil. Improved soil microbial and enzyme activities in cattle dung soil is an indication of improvement in soil fertility.

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