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Physico-chemical properties and enzyme activities in forest soil

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ABSTRACT

An assessment was made on an impact of forest litter on soil physic-chemical, microbial and enzyme activities. The experimental results indicated that, most of the physicochemical properties of soil such as silt, clay, and electrical conductivity, water holding capacity, organic matter and total nitrogen contents, microbial populations including bacterial, fungal Actinomycetes and soil enzymes activities such as protease and cellulase activities were signiûcantly improved in forest litter soil than the normal soil. With increasing in soil incubation period the soil enzyme activities such as protease and cellulases also improved up to 21st intervals there after declined in both test and control soils. Nearly two fold bacterial (210 x 10^4 CFU/g soil) and three fold fungal (12 x 10^4 CFU/g soil) actinomycetes (11 x 10^4 CFU/g soil) populations were observed in forest litter soil than the normal (113 x 10^4, 4 x 10^4, 2 x 10^4 CFU/g) Higher microbial population and enzyme activities is an indication of improvement of soil health in forest soil.

KEYWORDS

Forest soil;
Physico-chemical biological properties of soil;
Enzyme activities.

INTRODUCTION

Forest ecosystem produces a lot of organic matter in the form of leaves, twigs, branches, fruits and reproductive parts, such as flow-ers, seeds, spores[1,2]. Plant residues added to the soil are transformed into CO_2, microbial material and relatively stable humus components[3]. Cellulose is the abundant component of plant bio-mass. It is found in nature almost exclusively in plant cell walls and also produced by some bacterial species[4]. Perpetual renewal of plant biomass via the process of photosynthesis ensures an inexhaustible supply of such organic matter. Plant biomass rich in cellulose is one of the foreseeable and sus-tainable sources of fuel, animal feed and feedstock for chemical synthesis[5]. Cel-lulose has enormous potential as a renewable source of energy[6]. Therefore, the degradation of cellulosic biomass repre-sents an important part of the carbon cycle within the biosphere[7]. Bioconversion of cellulosic biomass to fermentable sugars through biocatalyst cellu-lases derived from cellulolytic organisms has been suggested as a feasible process and offers potential to reduce the use of fossil fuels and reduce environmental pollution. Any process which could ef-ficiently and economically convert cellulosic material to glucose would be of important in-dustrial significance. Cellulase provides a key opportunity for achieving tre-mendous benefits of biomass utilization[8]. Bacteria and fungi are well

known agents of decomposition of organic matter, in general, and of cellulosic substrate in particular[4]. Soil organic matter content is a function of organic matter inputs (residues and roots) and litter decomposition. It is related to moisture, temperature and aeration, physical and chemical properties of the soils as well as bioturbation (mixing by soil macro fauna), leaching by water and humus stabilization (organo mineral complexes and aggregates). Land use and management practices also affect soil organic matter[9]. Soil enzyme activities are sensitive to stress suffered by the ecosystems[10]. Accumulations and activities of soil enzymes of different origins are inûuenced by several factors (such as temperature, moisture, soil organic matter, nutrient content, pH)[11]. The plant–soil interactions has revealed that plant species can have signiûcant impacts on soil physicochemical properties (e.g., soil water content and pH) and on the quality of substrate for soil microbes (e.g., total carbon (C), nitrogen (N), and phosphorus (P) concentrations, C/N ratio, and the phenolic concentrations in soil) beneath the plant species through their litter quality and quantity[12,13]. Soil physicochemical properties and substrate quality have been used to explain differences in decomposition processes (mineralization of soil organic matter) beneath different plant species. Soil microbial communities play a key role in nutrient cycling and recent studies suggest that microbial composition and function can fundamentally alter soil decomposition processes independent of environmental drivers such as water content or soil temperature[14,15]. Forest tree litter containing varying amounts of Phosphorus can critically inûuence the microbial activities because the low concentration of available P in soils often limits not only plant productivity[16] but also microbial activities (e.g., soil respiration rate)[17]. The soil enzyme activity is an indicator of stress meeting ecosystems[10]. Extracellular enzymes play an outstanding role in litter decomposition and nutrient cycling whose processes are directly controlled by factors belonging to the given site such as temperature, moisture, nutrient availability and chemical properties of the litter[18]. In view of importance of vegetative tree waste or forest litter enzymes and the present work was carried out with impact of forest litter on soil microbial and enzyme activities.

MATERIALS AND METHODS

Collection of sample

The litter soil composed of dry leaves and barks of the trees was collected from forest area of Tirupati, Andhra Pradesh, India. The sample was air dried and mixed thoroughly to increase homogeneity for further studies.

Physico-chemical properties of soil

The physical and chemical properties of test and control soils were determined in accordance with standard analytical methods (APHA, 2000). The Mineral matters of soil samples such as sand, silt, clay contents were analyzed with use of different size of microbiological sieves[19]. The forest soil pH and electrical conductivity was determined by pH meter (Elico) and conductivity meter, respectively. Water holding capacity and organic carbon content of forest litter soil was quantified by the method[20]. Phosphorus and potassium contents were determined by the methods[21].

Enumeration of soil microorganisms

Forest soil microbial populations such as bacterial, fungal and actinomycetes were enumerated by serial dilution technique. For this method one gram of both forest and normal soil samples were serially diluted and 0.1 ml of soil suspension was spreaded with a sterile spreader on nutrient agar (pH 7.2), potato dextrose agar and starch casein agar medium for the growth of bacteria, fungi and actinomycetes respectively and plates were incubated in an incubator at $37^0$0C bacteria, actinomyetes and room temperature 26±4^0C for fungi. After incubation period, colonies formed on the surface of the medium were counted by Quebec colony counter.

Soil enzyme activities

(a) Assay of soil protease

Protease activities of both soil samples were determined by placing 5 gm of soil sample in each boiling test-tube with 60% water holding capacity at 28 + 4oC. Triplicates of both test and control soil samples were drawn after 0, 7, 14 and 21days of incubation to determine protease activity by the method[22]. Samples of 5 gm of soil were placed in 25ml of boiling test tubes; 10 ml of 2% casein in 0.1 M trisbuffer at pH7.5 was added

and were incubated for 24 hr. After incubation, to these, 4 ml of 17.5% trichloroacetic acid was added and the suspension was filtered by Whattman No. 1 filter paper. The amount of protein content was determined by following the method[23] by Elico digital spectrophotometer. Finally, protease activity was expressed in terms of microgram (ug) of tyrosine released per gram of soil per 24 hr.

(b) Assay of soil celluase

For assay of soil cellulase five grams of test sample forest litter soil 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, days of incubation. Duplicate soil samples of each test and control were drawn with at periodic intervals to determine the enzyme activities of cellulase studied by the method[24]. 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°C for cellulase activity. After desired incubation, soil extracts were passed through whattman filter paper and the filtrate was assessed by the method[25].

RESULTS

Soil samples analyzed for physico-chemical properties and results were represented in the TABLE 1. The soil texture of litter soil was 74% sand, 16% silt and 9 % clay. When compared to control soil, higher water holding capacity (1.44 ml/gm) and electrical conductivity (1.42 µ Mhos cm-1) were observed in litter soil. It may be due to the accumulation of organic wastes in the form of organic manure in the soil between the pore spaces of soil particles. The parameters like organic matter percentage, total nitrogen, phosphorus, potassium were higher in forest litter soil (test) soil than the Non forest (control) soil.

Improved microbial populations including bacterial, fungal and actinomycetes population were observed test soil than the control. For instance, 210 x 10^4 CFUg soil, 12 x 10^4CFU/g soil 11 x 10^4 CFU/g soil 113 x 10^4, 4 x 10^4, 2 x 10^4 bacterial fungal and actinomycetes were observed in test and control soil respectively. Nearly two fold bacterial and three fold fungal and actinomycetes populations were recorded and listed TABLE 2.

TABLE 1: Physico-chemical properties of forest and control soil

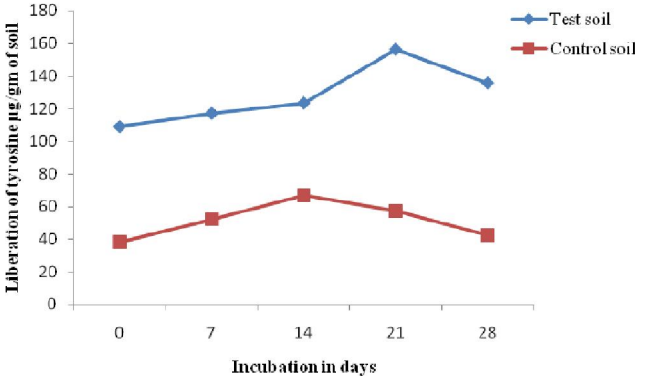
Properties	Forest litter soil (Test)	Normal Soil (Control)
Colour	Brown	Reddish brown
Odour	Normal	Normal
pH	6.8	7.2
Water holding capacity (ml/gm)	1.44	0.4
Electrical conductivity (µ Mhos cm-1)	1.42	0.13
Texture (%)		
Sand	74	57
Silt	16	36
Clay	9.0	7.0
Organic matter	67	30
Phosphorous (Kg/hec)	140	78
Potassium (Kg/hec)	165	123

***Values represented in the table are mean of triplicates of**

TABLE 2: Microiological properties of forest and control soil

Microorganisms	Forest soil (Test)	Non forest soil (Control)
Bacteria	210 x 10^4	113 x 10^4
Fungi	12 x 10^4	4 x 10^4
Actinomycetes	11 x 10^4	2 x 10^4

Values represented in the table are mean of duplicates
***Microbial population was measured in the terms of colony forming units CFU/g of soil**



***The activity measured in terms of tyrosine in µgm/gm of soil**
***Values are mean of duplicates ± S.D (Standard deviation)**

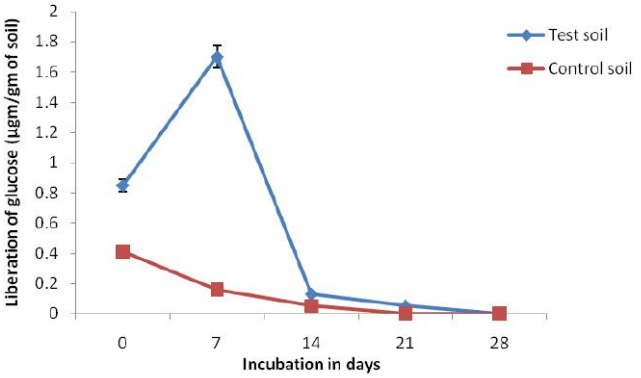
Figure 1: Protease activity in forest and control soil

In the present study the higher activity of protease was recorded in litter soil sample than in control soil sample. For instance, at 21-day interval, litter soil sample exhibited 156.33±0.46 µgm/gm of soil as against 57.22±0.14 µgm/gm of soil in respect of control soil

sample. The protease activity shown by litter soil sample increased in the range of 2 to 3 folds over control soil samples at all intervals. The high protease activity is mainly due to an increased growth of the microbial community in forest litter soil (TABLE 2).

DISCUSSIONS

In the forest litter soil with increasing in soil incubation period cellulase activity was improved up to 7th day interval and declined at further interval (Figure.2). In contrast in control the activity was gradually decreases from 0th day. For instance, the enzyme activity in test soil (litter) at Initial day interval was 0.85±0.04µg of glucose liberated /g of soil where as 1.7±0.07µg of glucose/g at 14th day interval and decreased to 0.05±0.005µg/g. Higher levels of enzyme activity were observed in the test soil than control at all incubations.



***The activity measured in terms of liberation of glucose in µgm/gm of soil; Values are mean of duplicates ± S.D (Standard deviation)**

Figure 2: Cellulase activity in forest and control soil

Litter aids in soil moisture retention by cooling the ground surface and holding moisture in decaying organic matter. A litter layer of decomposing biomass provides a continuous energy source for macro- and micro-organisms[9]. Similarly Narasimha et al (2012)[26], also reported in the IMO's treated soil improved the soil microbial populations, Narasimha et al.,[27] observed that soil microbial population increased with discharge of effluents from cotton ginning mill. The high microbiological activity in high-quality litter corresponds to observations[28]. Proteases in soils play a significant role in nitrogen mineralization, an important process in regulating the amount of plant available nitrogen for plant growth. The N mineralization in soil and litter was characterized by measuring extracellular protease activity. Protease activity seems to be mainly controlled by litter chemistry and rapid induction degradation cycles when litter is inserted in mineral soil[29].

Cellulose is the most abundant biopolymer and typically constitutes 20–30% of the plant litter mass[30]. Soil cellulase activity was measured by disappearance of substrates like cellulose powder, carboxy-methyl cellulose and appearance of reducing sugars quantitatively measured by spectrophotometer. Disturbance of micro flora in soil system due to pollution such as discharge of industrial effluents or accumulation of vegetative waste (litter) may adversely affect recycling of nutrients[31]. According to Joshi et al.,[32] cellulase activity was greatly increased in soils treated with cellulose as a substrate. Narasimha et al.,[27] reported that discharge of effluents (cellulosic waste, cotton seed lints) from cotton ginning mill improved the soil cellulase activity and microbial populations.

CONCLUSIONS

In the present study, a significant improvement was observed in forest soil in terms of physico-chemical microbiological (bacteria and fungi Actinomycetes) and soil enzyme activities such as protease than over the normal soil (control). This improvement was nearly two to three fold in microbial populations (bacterial fungal actinomycetes) and soil protease and cellulose activities in forest soil than the control respectively. Increasing of soil microbial population and enzyme activities in forest soil is an indication of improvement of soil quality and fertility.

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