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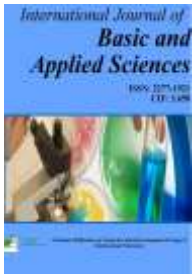

Full Length Research Paper

Role of Forest Litter on Soil Enzyme Activities

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ARTICLE DETAILS

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ABSTRACT

Effect of forest litter on soil physico-chemical, biological properties and enzyme activities was assessed. Except pH improved physico-chemical properties were noticed in forest soil accumulated with litter than control soil (without litter) which includes pH 4.8-5.1, WHC 0.30-0.33 ml/gram of soil, EC 0.09 – 0.10 µ Mhos/cm, Phosphorus 2- 9kg/h, Potassium 103-108 Kg/h. The biological properties such as bacterial and fungal population was enumerated and expressed in terms of CFU/g of soil. Two fold higher bacterial and four fold higher fungal population was found in litter soil than control soil. The enzyme activities such as cellulase, protease and dehydrogenase were measured with/without supplementation of substrates (1% CMC, 1% Casein and 0.18 m,M TTC respectively). With increase in soil incubation days enzyme activities increased up to 14th day interval and there after declined at 21" day interval. Forest soil accumulated with litter exhibited higher enzyme activities than control soil at all incubation days.

1. Introduction

Vegetation plays an important role in soil formation (Chapman and Reiss, 1992). Forest ecosystem contributes a lot of organic matter in the form of leaves, twigs, branches, reproductive parts, fruits etc., which after decomposition results in the formation of organic matter and release of nutrients (Tandel et al., 2009). Forest trees help improving soil fertility through biological nitrogen fixation, phosphorus solubilization and decomposition of organic matter in their Rhizosphere and non Rhizosphere zone. These processes play an important role in plant nutrition and maintaining soil fertility (Prasad and Mertia, 2005). The fertility of soil improves under the tree cover which checks soil erosion, adds soil organic matter, available nutrients and replenishes the nutrients through effective recycling mechanisms (Tripathi et al., 2009). Decomposition of leaf litter includes leaching, breakup by soil fauna, and transformation of organic matter by micro organisms and transfer of organic and mineral compounds. Decomposition of plant residues is influenced by substrate quality, decomposer community and environmental factors (Swift et al., 1979; Coleman and Crossley, 1996; Smith and Bradford, 2003). Plant tissues are the main sources of organic matter which influences physico-chemical characteristics of soil such as pH, WHC, texture and nutrient availability (Johnston, 1989). Physico-chemical characteristics of forest soils vary in space and time due to variations in topography, climate, physical weathering, processes, vegetation cover, microbial activities and several other biotic and abiotic variables. (Shishirpoudel and Jaysah, 2003).

Various soils exhibits enzyme activities but differ based on the respective crops and are related to microbial biomass, therefore changes in enzymes and microbial activities could alter the availability of nutrients for plants uptake (Dick et al., 1988 a) and these changes are potential sensitive indicators of soil quality. The term "soil microbial activity" implies to the overall metabolic activity of all microorganisms inhabiting soil including bacteria, fungi, actinomycetes, protozoa, algae and

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micro fauna (Nannipieri, 1990). The microbial activity plays a vital role in soil productivity, sustainability as it underpins a number of fundamental soil properties such as fertility and structure. The diversity and population of soil microorganisms and the enzymes produced will depend mainly on the chemical com- position of plant residues. The soil enzymes are sensor for soil degradation and microbial status (Wick et al., 1998; Aon and Colaneri, 2001; Baum et al., 2003).

The objective of the present investigation is to determine influence of available nutrients (litter) on soil physico-chemical, biological properties and soil enzyme activities which in turn represents soil quality.

2. Materials and Methods

2.1 Collection of soil samples

Soil sample composed with litter was collected from Thirtharameswara Reserve forest, Honnali, Davangere ,Karnataka India. The soil sample collected from adjacent site served as control. It was air dried and mixed thoroughly to in- crease homogeneity and shifted to < 2mm. sieves for determination of soil texture.

2.2 Analytical methods for physico-chemical characterization of soil samples

Mineral matter of soil samples such as sand, silt, clay contents were analyzed with the use of different sizes of sieves by following method of Alexander (1961). Cent percent water holding capacity of soil samples were measured by the method of Johnson and Ulrich (1960). Soil pH was measured in ELICO digital pH meter with Calomel glass electrode assembly. Electrical conductivity of soil samples were determined by the conductivity bridge quantified by the method of Chapman and Pretty (1961). Soluble phosphorus and potassium contents were determined by the method of Kuprevich and Shcherbakova (1972). The microbial populations such as bacteria and fungi in both the soil samples were enumerated by serial dilution technique.

2.3 Enzyme activities in soils samples with/without litter

Five gm of soil samples were transferred to test tubes. Samples were maintained at 60% water holding capacity at room temperature in the laboratory(28±4°C). Duplicate soil samples (with/without substrate) of test and control were withdrawn at periodic intervals (0, 7, 14 and 21 days) to determine the cellulase, protease and dehydrogenase activity followed by the method of Pancholy and Rice (1973), Cole (1977) and Chandrayan et al., (1980) and Casida et al., (1964). The soil samples were transferred to 250 ml. Erlenmeyer flasks and 1mL of toluene was added. After 15 min, 6mL of 0.2 M acetate buffer (pH 5.9) containing 1% carboxymethyl cellulose, 1% casein was added to the soil samples and flasks were plugged with cotton and incubated at 30 min, at room temperature. After desired incubation, soil extracts were passed through what man filter paper and cellulase and protease activities in the filtrate was measured by the method of Nelson-Somogyi (1944) and Folin-Lowry (1951) respectively. Dehydrogenase activity was determined by treating soils samples with 0.1 g calcium carbonate and 1 ml. of 0.18 mM TTC incubated at 30°C for 24 hours. The triphenyl formazan formed was extracted with methanol from the reaction mixture and assayed at 485 nm in spectrophotometer (ELICO, SL 171).

3. Results and Discussion

3.1 Physico-chemical characteristics of soil samples with/without litter

Soil fertility mediated by microorganisms is dependent on maintenance of physico-chemical properties of soil. Therefore the soil samples were analyzed for physico-chemical characteristics and results were represented in Table 1. Analysis of soil samples revealed that forest litter soil (test) underwent changes in all the measured parameters in comparison to control.

Table 1. Physico-chemical characteristics of soil samples with/without litter

Properties	Test (litter) soil	Control Soil
Colour	Reddish brown	Grey
Odour	Light pungent	Normal
pH	4.8	5.1
Water Holding Capacity(mg/l of soil)	0.33	0.30
Electrical conductivity (uMhos / cm)	0.10	0.09
Sand (%)	80.11	85.70
Slit (%)	14.60	9.92
Clay (%)	4.94	3.90
Phosphorus (kg /h)	9	2
Potassium (kg /h)	108	103

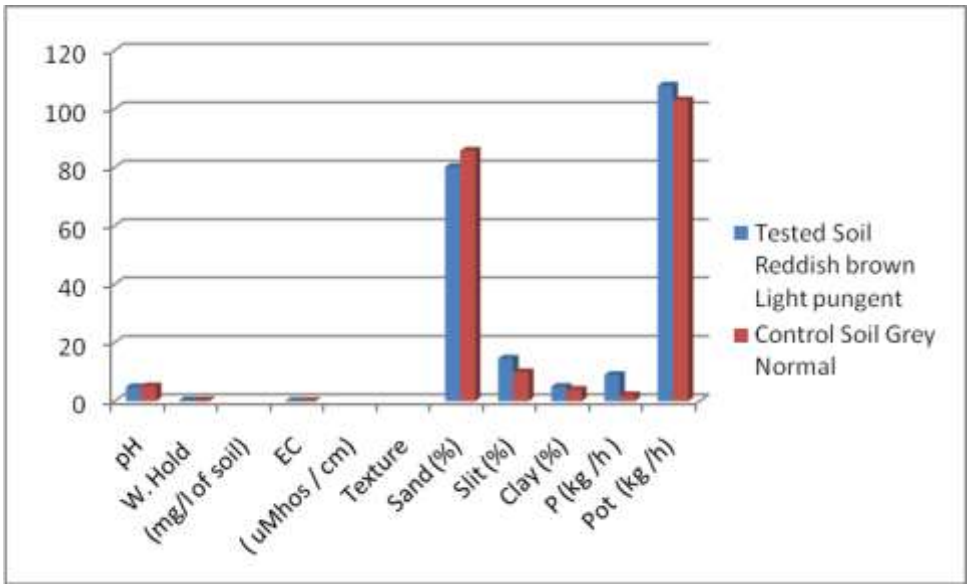

Fig:1 Graph shows the characteristics of soil samples with/without litter

Soil composed with forest waste (litter) exhibited texture different from that of corresponding control. Soil texture in terms of percentage of sand, silt and clay were 80.11, 14.60 and 4.94 in the test; 85.70, 9.92 and 3.90 in control soils (Table 1). The above results indicated that test sample had lower sand and higher silt and clay content in comparison to the control. The results of the present study seem to be in agreement with an earlier study, Oseniet al., (2007) reported that natural forest and aged plantation soils show similar particle size characteristics of sand, clay and silt.

Soil pH is one of the most indicative measurements of soil because it is an important factor for the survival of microorganisms (Evans et al., 1984). In the present study, the pH of litter sample was de- creased to 4.8 from 5.1. This change in pH may be due to the deposition of plant residues in the soil. Similar reports were made by Oseni et al., (2007) that pH of the natural forest soil was acidic, as acidity was observed to increase with increase in soil depth. The pH of soil ranged about 4.03 to 4.24 in Pinus densiflora forest soils and 4.38 to 4.65 in Quercus mongolica forest soils (Lee et al., 1998). An acidic pH of 5.25and 5.35 was recorded in oak and pine oriented forests (Prashant, 2010). Water holding capacity (WHC) and electrical conductivity (EC) of 0.33 ml/g and 0.10 µMhos/Cm was recorded in test soil where as 0.30 ml/g and 0.09 µ Mhos/Cm was re- corded in control. This improvement in EC and WHC in the test soil may be due to the long term deposition of organic manure in the form of plant residues. Phosphorous and potassium content in the test soil is 9 kg/h and 108 kg/h as against control 2 kg/h and 103 kg/h respectively (Table 1). Similar results were reported by Cavero et al., (1997), Clark et al., (1998), Poudel et al., (2002). Higher levels of total organic carbon, total nitrogen and soluble phosphorous were found in organic soils. High con- tent of available phosphorous (11.2 mg/kg) was observed in pine oriented than oak oriented forest areas (6.3 mg/kg), (Prashant, 2010). Concentrations of C. N and potassium increased significantly with increasing application rates of organic amendments (Supradip Saha et al., 2008). Amendment of sewage sludge to the soil improved total N and P contents (Subbaiah and Sreeramulu, 1979).

3.2 Counting of microflora in soil samples with/ without litter

Microorganisms are widely distributed in different types of environments like soil, water and air. In soil these organisms play an important role in maintaining soil fertility by recycling of nutrients through their biochemical processes. Amendment of sewage sludge (litter) to the soil generally raises microbial activity by increasing the soil organic matter. Be- cause of importance of soil microbial biomass in breakdown of organic matter in soil and decomposition in soil by proteolytic fungi and bacteria, micro flora of both soil samples were enumerated. Higher bacterial and fungal populations were observed in the test soil than the control (Table 2). The fungal populations were relatively higher in litter decomposed soil by nearly 3 folds than in control soil. For instance the fungal population in the test soil was 14x10° CFU/g of soil where as 3x10 CFU/g of soil in control (Table 2). Two folds higher bacterial population with 384x104 CFU/g in test soil was re- corded than in control soil with 158x10 CFU/g. In- crease in size of fungal and bacterial population ob served in the litter soil may be attributed to deposition of organic manure (mostly lignocellulosic wastes) and lower pH favourable for fungal organ- isms. These findings corroborate with observation of Oseni et al., (2007). The natural forest at 0-10Cm depth has the greatest number of both fungi and bacteria count. Similarly, Narasimha et al., (1999) and Nagaraj et al., (2009), reported that organic waste released from agro-based

industries improved the microbial populations. Higher microbial activity (Mader et al., 2002) and microbial biomass (Mader et al., 2002; Mulder et al., 2003) were found in organic soils. The higher microbial activities in rhizosphere soil in oak oriented forest soil might be due to increased supply of carbon and nutrients from dead root cells and rhizodeposition (Huxley, 1999; Kang et al., 2009) and less forest floor removal (Xiao et al., 2008).

3.3 Enzyme activities in soil samples with/without litter
1. Cellulase activity: Soil cellulase activity was measured by disappear

Table 2. Microbial populations in soil samples with/ without litter.

Parameter	**Test (litter) soil**	
Bacteria	384×10^4	158×10^4
Fungi	14×10^4	3×10^4

Microbial populations in terms of colony forming units (CFU/g soil)of substrates like cellulose powder, carboxymethyl cellulose and appearance of reducing sugars quantitatively measured by spectrophotometer (Levinson and Reese et al., 1950). 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. Therefore cellulase activity was measured with or without addition of substrate (CMC) and represented in Fig. 1. Cellulase activity was enhanced in soils with/without litter composition upon inclusion of substrate in the assay systems (Fig. 1). The enzyme activity was measured in terms of liberation of µg of glucose from CMC/g of soil. With increase in soil incubation period cellulase activity was improved by one fold up to 14th day and declined at further intervals in both soil samples. For instance, the enzyme activity in test soil (litter) at 0 day interval was 170µg of glucose liberated/g of soil where as 630µg of glucose/g at 14th day interval and decreased to 320µg/g. Higher levels of enzyme activity were observed in the test soil than control at all incubations. For instance the cellulase activity was 170µg/g in test soil were as 140µg/g in control soil at initial day. Similar pattern was noticed at remaining intervals. A slight variation in the cellulase activity was observed at all incubation days in the absence of substrate (CMC) in both soils. For instance, the cellulase activity was 120µg of glucose/g of soil at 0 day where as 180 µg/ g at 14th day interval and reduced to 150µg/g in the test sample. Same trend was observed in control soil (Fig.1). Higher cellulase activity was observed in test soil than the control at all incubations. For in- stance the enzyme activity was 120µg/g in test against 110µg/g in control sample. Analogous trend was observed at remaining soil incubation days. Higher cellulase activity was observed in the litter soil in the present study could be attributed to the presence of high organic content and microbial population. (Table 2)

According to Joshi et al., (1995) cellulase activity was greatly increased in soils treated with cellulose as a substrate. Narasimha et al., (1999) made similar observations in soils discharged with effluents of cotton ginning industry stimulated the soil cellulase activity. In contrast, cellulase activity was greater in mineral fertilized soil than in organically amended soil (Supradip Saha et al., 2008).

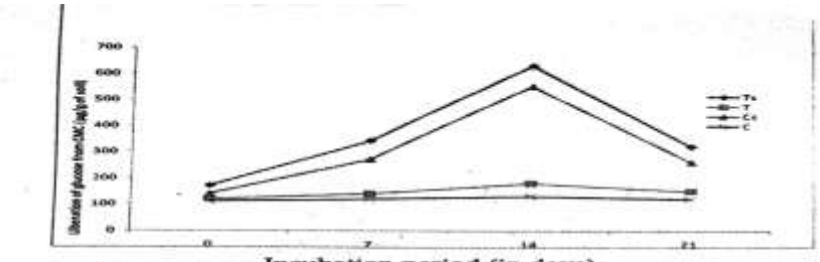

Fig. 2 Cellulase activity in forest litter and control soils

2 . Protease activity

Protease enzymes are widely distributed among the soils exhibiting a wide range of activities and properties (Ladd and Butler, 1972). These enzymes are involved in the initial hydrolysis of protein com- pounds of organic nitrogen to simple amino acids. Protease can hydrolyze not only added proteins but also native soil proteins and peptides. Soil samples

with/without litter composition were incubated with 60% water holding capacity at room temperature. After incubation two soil samples were supplemented with/without 1% sodium caseinate in order to determine enzyme activity in litter/control soils. Protease activity was determined in terms of tyrosine equivalents formed in trichloro acetic acid soluble fraction during 6 hours at 30°C. Protease activity in terms of formation of tyrosine from casein remained steady over a period of first 14 days and then onwards slightly declined in further intervals of measurement made in the present study (Fig. 2).

At initial day the enzyme activity was 160µg of tyrosine/g in test soil; it was increased to 420µg/gat 14th day of incubation and reduced to 160µg/g at 21st day. Similar trend was observed in control soil. Higher protease activity was recorded in test sample than control at all incubations. For instance at initial day the test sample exhibited 160µg/g against 100µg/g in control soil. Same trend was continued at the rest of incubation days. The protease activity in both soil samples without supplementation of substrate also shows analogoustrend. With increase in incubation days the enzyme activity increased up to 14th day and declined at further incubation. For instance, at 0 day interval the protease activity was 60µg/g in the test, it was in- creased to 290µg/g at 14th day and declined to 110µg/g at 21st day of incubation. Similar trend was observed in control soil. Increase in the protease ac- tivity was recorded in test soil compared to control at all incubation days (Fig.2). For instance, at initial day incubation, the casein hydrolyzing enzyme ac- tivity was 60µg/g in test soil where as 30µg/gin control soil. Identical trend was noticed at remaining days.

Increased proteolytic activity in litter soil may be due to availability of suitable substrates (Casein), decrease in soil pH and increased proteolytic micro- organisms in soil. Soil protease activity was correlated with number of micro flora as reported earlier by Narasimha et al., (1999). Similarly, soils treated with tomato processing waste (Sarade and Joseph Richard, 1994), effluents of cotton ginning mills (Narasimha, 1997) paper mill (Chinnaiah et al., 2002), improved soil protease activity than control. The rates of protease activity were higher with the organic amendments than in mineral fertilizer amended and unamended soils (Supradip saha et al., 2008). In contrast, soil polluted with organic matter (Ladd and Butler, 1969), cement dust from cement industries (Shanthi, 1993), waste water treatment plant discharge (Montuelle and Volat, 1998; Zdenek Filip et al., 2000), herbicides (Pahwa and Bajaj, 1999), insecticides (Omar and Abd-Alla, 2000) ceased the soil protease activity.

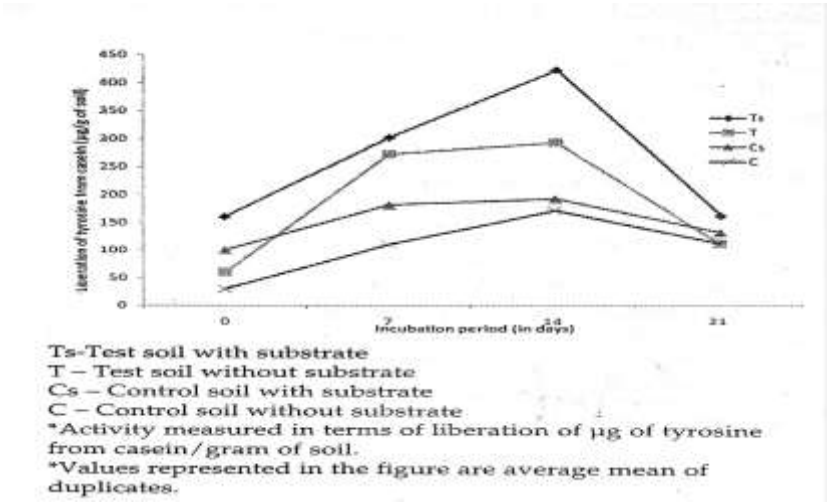

Fig. 3. Protease activity in forest litter and control soils

3 . Dehydrogenase activity

Soil dehydrogenase activity measured in terms of formation of formazan from triphenyl tetrazolium chloride was also chosen as good index of microbial activity in soil. The soil dehydrogenase system is due to rather wide group of soil enzymes which transfer electrons to available acceptors. Its activity appears to be more dependent on metabolic state of microbial population of the soil rather than activity of specific free enzymes. The dehydrogenase activ- ity was maximum at 14th day interval and there on- wards decline in both samples with/without sub strate. For instance at 0 day incubation, the dehydrogenase activity was 118ugof formazan /g of soil, it was increased to 239µg/g at 14th day and later declined to 158µg/g at 21st day. The control soil also exhibited same trend. Higher dehydrogenase activity was recorded in test sample in comparison to control. For instance, at initial day of incubation, the enzyme activity was 118 µg/g in test sample where as 8.05 µg/g was recorded in control. Similar trend was followed at remaining incubation days (Fig. 3). The dehydrogenase activity was maximum at 14th day interval in the absence of substrate in both the soil samples. For instance, the enzyme activity was noted as 5.3µg/g at 0

day 2.68µg/g control soil. Soil samples with litter always in the test soil against recorded significantly higher dehydrogenase activ- ity than control soil samples. But no regular pattern of increments in dehydrogenase activity in test sample over control soil was observed. Similarly, addition of organic materials, composts and low metal sludges has been found to increase soil dehydrogenase activity (Chander and Brookes, 1991 b; Giusquiani et al., 1994). The enzyme activity was higher in the rhizosphere soils than in non-rhizosphere sludge amended soils. However, the addition of sludges or composts at the higher rates re- versed this effect causing a decrease in dehydrogenase activity (Reddy et al., 1987). Dehydrogenase enzyme is high in soils polluted with pulp and pa- per mill effluents (McCarthy et al., 1994) but low in soils polluted with flyash (Pitchel and Hayes, 1990). Moreno et al., (1999) and Masciandaro et al., (2000) studied dehydrogenase activity under the influence of organic matter and reported an increase following the organic matter amendement. Amendment of sesbania straw to the soil improved dehydrogenase activity than wheat straw, maize straw amended soils and unamended soil (Sajjad et al., 2002). The dehydrogenase activity had ranges of 170.67 to 221.66 µg TPF/g in pinus densiflora and Quercus mongolica forest soils that showed lower values than in Kawngneung site (Lee et al., 1998). Higher dehydrogenase activity of 106.28 nmol/g/2hr was recorded in oak oriented forest area where as less (66.37 nmol/g/2hr) in pine oriented forest area(prashant, 2010).

Soils discharged with effluent waste water from pulp and paper mills exhibited relatively higher dehydrogenase activity than soil without corresponding irrigation (Kannan and Oblisami, 1990). Increase in soil dehydrogenase activity was attributed to low pH and high organic content in the effluents. Reddy and Faza (1989) compared dehydrogenase activity in soil amended with/without industrial sludge. The activity was more in soils with- out sludge than in soils amended with sludge.

4. Conclusion

Vegetation plays an important role in soil formation (Chapman and Reiss, 1992). Forest ecosystem contributes allot of organic matter in the form of leaves, twigs, branches, reproductive parts, fruits etc., which after decomposition results in the formation of organic matter and release of nutrients (Tandel *et al.,* 2009). Forest trees help improving soil fertility through biological nitrogen fixation, phosphorus solubilization and decomposition of organic matter in their rhizosphere and non rhizosphere zone. These processes play an important role in plant nutrition and maintaining soil fertility (Prasad and Mertia, 2005). Various soils exhibits enzyme activities but differ based on the respective crops and are related to microbial biomass, therefore changes in enzymes and microbial activities could alter the availability of nutrients for plants uptake (Dick *et al.,* 1988 a) and these changes are potential sensitive indicators of soil quality.

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