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Changes in Enzyme Activity During the Decomposition of Plant Residues in Soil

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Abstract: An incubation experiment was conducted under laboratory conditions to study the changes in some soil enzymes during the decomposition of plant residues. Soil samples amended with powdered plant material of wheat, maize and sesbania were incubated for 8 weeks at moisture content of 60% of the maximum water holding capacity and 22-26 °C for 8 weeks. At 0, 2, 4 and 8 weeks of incubation, portions of soil were analyzed for total C and activity of different enzymes. Maximum dehydrogenase and invertase activity was observed for sesbania and minimum in unamended soil, however there was no consistent trends with incubation intervals. Cellulase activity was not affected strongly by organic amendments, however it increased with time in amended than unamended soils. The differences between different treatments were non significant for urease.

Key words: Dehydrogenase, cellulase, invertase, organic matter, urease

Introduction

Plant residues added to the soil are transformed into CO_2, microbial material and relatively stable humus components (Shields *et al.*, 1973). The rapidity with which these transformations occur depends not only on the environmental conditions, but also on the chemistry of the residues. Decomposition of plant residues results in the production of CO_2, microbial biomass and metabolites, and stable humus compounds. Being a microbially mediated process, decomposition of plant residues is accompanied by the changes in enzymes responsible for most of these transformations. These changes are commensurate with the chemical makeup of the plant residues. Soil enzymes are undoubtedly one of the main components participating in and assuring the correct sequence of all the biochemical routes in soil biogeochemical cycles (Ladd, 1985). Hence, it is pertinent to study the activity of some soil enzymes during the decomposition of plant residues differing in chemical composition. It has also been proposed that measurement of changes in soil enzyme activities may provide a useful index of changes in soil quality (Visser and Parkinson, 1992).

The idea of using microbial indicators of soil fertility was introduced and established by Waksman (1922). The rationale being their central role in the cycling of C and N (Visser and Parkinson, 1992) and their sensitivity to changes in the soil environment (Brookes, 1995). Nannipieri *et al.* (1978) reported the criteria for measurement of microbial growth and activity in soil. These and other studies suggest a significant bearing of crop residue management on soil enzymes (Svensson and Pell, 2001). This is attributed mainly to the difference in microbial communities supported by the residues (Parmelee *et al.*, 1989).

The diversity and population of soil microorganisms and the enzymes produced will depend mainly on the chemical composition of the plant residues. Hence, chemistry of the plant residues not only determines their own fate but regulates the microbial activity as well. In turn, microbial activity is responsible for the release and plant availability of essential nutrient elements like nitrogen (N) and phosphorus (P). Plant residues rich in easily decomposable C and mineralizable N (for example leguminous plant residues) are generally more useful for application to agricultural soils. Those rich in lignins are expected to make higher contributions to stable organic matter fraction (humus). Objective of the present work was to study the changes in some important enzymes during decomposition in soil of plant residues different in chemical composition. Four enzymes involved in transformation of C and N were studied i.e., cellulase, dehydrogenase, invertase, and urease.

Materials and Methods

Soil: The sandy-loam soil used in the studies was collected from experimental fields of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. This was obtained by digging surface soil to a depth of 15 cm. Air-dried and sieved (< 2 mm) soil had the following physico-chemical characteristics: organic C, 0.6%; total N, 0.09%; NH_4^+-N, 4.2 μg g^{-1} soil; NO_3^-+ NO_2^- -N, 67.9 μg g^{-1} soil; pH (1:2.5, soil:water suspension), 7.4; EC, 0.8 dSm^{-1}; water holding capacity, 25%; sand, 60%; silt, 21%; clay, 19%.

Plant residues: Finely powdered straw of wheat, maize, and sesbania were used for incorporation in potted soil. Sub-samples of plant material were analyzed for total carbon (C), total nitrogen (N), 2N H_2SO_4 hydrolyzable C and hydrolyzable N (Table 1). The three types of residues generally differed in most of the characteristics. However, wheat material was entirely different from the other two especially in N concentration, which among themselves were similar in several respects including C and N content and hydrolyzable N.

Incubation experiment: Six-kg portions of air-dried and sieved soil were placed in 64 plastic pots. Soil in sets of 16 pots each was amended as follows: I) no amendment (control), ii) wheat straw at 0.5%, iii) maize straw at 0.5%, and iv) sesbania straw at 0.5%. Moisture content of the soil was adjusted to 15% (w/w) with tap water and maintained at this level throughout the incubation by making up the weight loss. Incubation was carried out at 22-26 °C for 0, 2, 4 or 8 weeks and managed in a way that all the samples were obtained on the same date. The soil samples were kept at 4 °C before being analyzed for total C and enzymes like cellulase, dehydrogenase, invertase, and urease.

Analytical methods: For the determination of pH, ECe, texture, and water holding capacity, methods (Anonymous, 1954) were used. Total N and mineral N (NH_4^+-N and NO_3^-+ NO_2^--N) were determined by micro-Kjeldahl method (Bremner and Mulvaney, 1982; Keeney and Nelson, 1982). Plant residues were also subjected to hydrolysis in order to determine the proportion of easily mineralizable C and N. One gram portions of the plant material were refluxed in 50 ml of 2N H_2SO_4 for 1hr; the contents of the reflux flask filtered through a scintered funnel and the filtrate was analyzed for total C and N.

Dehydrogenase activity: Method described by Friedel *et al.* (1994) was followed for the determination of dehydrogenase activity of the incubated soil samples. In the 3 g moist soil, one ml of glucose solution (30 mg L^{-1}) and 0.5 ml of 3% solution of 2,3,5 triphenyl tetrazolium chloride (TTC) was added and volume was made to 5 ml with 0.1M Tris buffer (2.42 g of Trizma dissolved in 200 ml of distilled water; pH 7.6-7.8). A control was also kept containing glucose solution and tris buffer but without the substrate. After mixing thoroughly, samples were incubated at 37°C for 24 hours followed by centrifugation. The supernatant was transferred to a flask containing 10 ml of acetone. After shaking for ½ hr, the samples were centrifuged at 4000 rpm for 10 minutes and absorbance was noted at 485 nm on spectrophotometer (Spectronic-21, Bausch and Lomb). A correction factor (cf) was calculated by dividing different concentrations (ppm) of 1,3,5-triphenyl tetrazolium formazan (TPF) with absorbance. Unit of activity was µg formazan formed g^{-1} soil $24hr^{-1}$ and calculated by using the formula:

$$\mu g \text{ formazan formed } g^{-1} \text{ soil } 24 \text{ hr}^{-1} = \frac{\text{cf. x 100 x volume made x O.D.}}{\text{Weight of moist soil x \% dry matter}}$$

Urease activity: Method described by Pancholy and Rice (1973) was used for the determination of urease activity in soil. Briefly, 0.5 ml toluene was mixed with 5 g moist soil. After 15 minutes, 10 ml of phosphate buffer (17.85 g KH_2PO_4 per 500 ml added to 500 ml solution of K_2HPO_4 containing 20.66 g of the salt, pH 7.6) and 10 ml of 10% urea solution was added. The reactants were incubated at 30°C for 24 hrs, followed by shaking for 15 minutes with 15 ml of 1N KCl solution. After filtration, 5 ml of the filtrate were analyzed for NH_4-N by steam distillation according to Kjeldahl method (described later).

Cellulase activity: Method described by Pancholy and Rice (1973) was followed. Toluene treated samples (as described for urease) were mixed with 10 ml of 0.5 M acetate buffer (solution of 15.72 ml acetic acid and 34.02 g of CH_3COONa in 1 L flask containing deionized water, pH 5.9) and 10 ml of 1% carboxymethyl cellulose (CMC). In control the substrate CMC was not added. After incubation at 30°C for 24 hrs, samples were centrifuged at 4000 rpm for 20 minutes. The supernatant was filtered and aliquots were analyzed for reducing sugar using DNS method of Gascoigne and Gascoigne (1958).

Invertase activity: The method described by Ross (1966) was followed and protocol was similar as for cellulase except that substrate was 10 ml of 5% sucrose solution. Reducing sugars were measured as the end product.

Determination of reducing sugars: To 5 ml of filtrate, equal amount of DNS solution (3,5, dinitrosalicylic acid) were added and left in boiling water for 5 minutes, the samples were quickly cooled and after centrifugation at 4000 rpm for 10 minutes, absorbance was noted at 550 nm on spectrophotometer. Treating different concentrations of glucose with DNS solution also drew standard curve and concentrations of unknown were determined by using the curve. Unit of activity was defined as mg glucose released g^{-1} soil 24 hr^{-1}. Dissolving 128 g sodium potassium tartarate, 10 g sodium hydroxide, 10 g DNS, 2 g phenol, and 0.5 g sodium sulphate in water and making the volume to 1 L to prepare DNS reagent.

Statistical analysis: The data were subjected to analysis of variance (ANOVA) in completely randomized design with factorial arrangements of treatments. Standard deviation was determined using MS-EXCEL software.

Results and Discussion

Plant residues used in this study differed significantly in their chemical characteristics (Table 1). Carbon content of the residues varied between 35 and 39%, the maximum being in wheat while maize and sesbania showed almost similar values. As expected, wheat straw had low N content of 0.49% resulting in a wider C/N ratio i.e., 80.0. Maximum N concentration of 2.56% was determined in sesbania resulting in a narrow C/N ratio of 13.88. Maize, which is a non-legume, also had characteristics fairly similar to that of sesbania because it was harvested at a relatively tender stage i.e., 6 weeks after germination. It had 2.09% N and a C/N ratio of 16.84, which can be considered optimum for a net mineralization of N. In wheat, 30.15% of the total C was in acid hydrolyzable forms, while in maize and sesbania this fraction was 42%. Compared with C, much lower amounts of residue N were hydrolyzable being 3.03, 12.82 and 19.2 mg g^{-1} material of wheat, maize and sesbania, respectively. In terms of percentage, however, 62.09, 61.27 and 75.13% of the total N was hydrolyzable in wheat, maize and sesbania, respectively. Higher proportion of hydrolyzable N in sesbania and other legumes may indeed be the reason for a rapid mineralization of their N as reported by other workers (Haider and Azam, 1982; Azam *et al.*, 1985; Azam *et al.*, 1993). The C/N ratio of the hydrolysate was lowest (7.78) in case of sesbania and highest in case of wheat (38.85), with maize showing a value of 11.55 (Table 1). The hydrolyzable fraction may also be considered as that more susceptible to microbial transformations. A narrow C/N ratio of the hydrolyzate will help in a net mineralization of N, while wider C/N ratio could lead a net immobilization of N and thus its restricted availability to plants. Immobilization and remineralization of N is reported as governed by the labile C component of the plant residues (Haider and Azam, 1982; Azam *et al.*, 1985; Azam *et al.*, 1993; Fox *et al.*, 1990).

Activity of different enzymes was assayed at 0, 2, 4, and 8 weeks of incubation of soil with wheat, maize and sesbania (Table 2). Dehydrogenase is a universal enzyme that belongs to the group of oxidoreductase enzymes, produced by all organisms and linked with respiratory processes (Bolton *et al.*, 1985). It transfers the hydrogen from hydrogen-containing compounds to another hydrogen carrier. Dehydrogenase activity is a measure of the intensity of microbial metabolism in soil and thus the microbial activity of soil (Page *et al.*, 1982). Therefore, level of enzyme using the end product as a measure can be used as an index of total microbial activity (Skujins, 1976). Indirectly, this may also indicate the availability of C and energy source for the microorganisms. In present studies, significant differences were observed in differently amended soil. Averaged over all the 4 sampling intervals, maximum dehydrogenase activity was observed for sesbania and minimum in unamended soil. No consistent trends in dehydrogenase activity with time of incubation were observed. The three types of residues had a variable effect on dehydrogenase activity suggesting that even a similarity in chemical composition may not induce similar changes in microbial functions. Nature of plant residues is reported to affect the enzyme activity differently. 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 amendment. However, the enzyme activity remained unchanged during extended period of incubation. In some other studies, dehydrogenase activity is reported to decrease with time (Azam and Malik, 1985; Lodhi *et al.*, 2000).

Invertase is another enzyme indicative of overall microbial activity. It is widely distributed in plants and animals and in certain microorganisms. This enzyme is involved in the breakdown of sucrose into glucose and fructose. In the present study, production and activity of the enzyme was more in amended compared with unamended soil and was the highest in soil amended with sesbania. However, the differences in the treatments were not that wide as observed for dehydrogenase. On an average, maize and sesbania showed similar activity over the incubation period (data not tabulated).

Cellulase enzyme is restricted to the organisms capable of using cellulose as a C source and is mostly restricted to fungi (Gascoigne and Gascoigne, 1958). This is an extracellular enzyme and converts cellulose into glucose, cellobiose and higher molecular weight oligosaccharides (Deng and Tabatabai, 1994). In the present studies, cellulase activity was not affected strongly by organic amendment. However, it increased with the time of incubation in amended but not in unamended soil.

Soil urease has attracted a great deal of attention due to the increasing use of urea as a fertilizer. Almost similar results were obtained for urease, which is a more specific enzyme, involved in the hydrolysis of urea. This enzyme is produced by both microorganisms and plant roots and helps in the transformation

Table 1: Chemical characteristics of plant residues

Parameters	Plant material		
	Wheat	Maize	Sesbania
Total C, mg g^{-1}	390.71	352.36	354.66
% C	39.07	35.24	35.47
Total N, mg g^{-1}	4.88	20.92	25.56
% N	0.49	2.09	2.56
C/N ratio	80.00	16.84	13.88
Hydrolyzable C, mg g^{-1}	117.80	148.05	149.43
Hydrolyzable C, % of total C	30.15	42.02	42.13
Hydrolyzable N, mg g^{-1}	3.03	12.82	19.20
Hydrolyzable N, % of total N	62.09	61.27	75.13
C/N ratio of hydrolysate	38.85	11.55	7.78

Table 2: Activity of some soil enzymes following incubation for different time periods

Soil amendments	Weeks of incubation			
	0	2	4	8
Dehydrogenase activity, μg Formazan g^{-1} soil $24hr^{-1}$				
Nil	52.77d*	52.63c	53.18d	56.84d
Wheat	197.27a	115.57b	98.50c	91.63b
Maize	119.19c	118.09b	123.49b	78.71c
Sesbania	170.12b	146.60a	294.02a	252.01a
Invertase activity, mg reducing sugars released g^{-1}soil 24 hr^{-1}				
Nil	1.64d	1.54c	1.78c	1.54c
Wheat	2.47c	1.74b	2.07b	1.69b
Maize	4.08a	2.26a	2.02b	1.97a
Sesbania	3.78b	2.41a	2.27a	1.86ab
Cellulase activity, μg reducing sugars released g^{-1}soil 24 hr^{-1}				
Nil	30.4a	37.6a	35.2c	30.4c
Wheat	23.2b	20.8b	31.2d	27.2d
Maize	12.8c	15.2bc	47.2b	38.4a
Sesbania	14.4c	13.6c	59.2a	33.6b
Urease activity, NH_4-N released g^{-1} soil 24 hr^{-1}				
Nil	0.265a	0.298a	0.328a	0.311a
Wheat	0.269a	0.307a	0.321a	0.317a
Maize	0.265a	0.292a	0.286b	0.300a
Sesbania	0.235b	0.292a	0.296b	0.302a

*, figures in a column (set of 4 values each) sharing a similar letter are not significantly different from each other.

of urea N into forms amenable to plant uptake i.e. NH_4 which is subsequently nitrified by nitrifying microorganisms. The differences in urease activity between different treatments were not significant suggesting that organic matter amendment had no particular influence on the production and activity of the enzyme. In some other studies, however, an increase in urease activity due to organic amendment has been reported (Moreno *et al.,* 1999), while negligible effect was reported in some other studies (Azam and Malik, 1985). Complexation with humus compounds is reported to stabilize urease (Nannipieri *et al.,* 1996) resulting in a fairly stable enzyme activity during prolonged incubations. In addition, organic matter is reported to stabilize the urease enzyme and protect it from degradation (Zhengping *et al.,* 1991). Because of such reasons, urease maintained a certain stable level irrespective of the treatments in the present study.

In summary, the results of present studies indicated a relatively rapid oxidation of C from soil amended with wheat; cellulase activity was higher in this case during early incubation period. Organic amendment caused an increase in the activity of dehydrogenase and invertase, while urease was almost unaffected. The three types of plant residues had a variable effect on enzyme activity. These results suggested that the nature of added organic matter will have a significant bearing on the microbial functions and cycling of C and nutrient elements. Hence, chemical composition of plant residues needs to be considered while planning the organic matter additions to the soil.

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