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Seasonal variation in enzyme activities from agro ecosystem site at Ranchi, Jharkhand

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Abstract- Activities of dehydrogenase, urease, cellulase and phosphatase were studied from agroecosystem site at Ranchi, Jharkhand. Phosphatase activity was higher in comparison to other enzymes. Significant positive correlation was observed between organic carbon content and the enzymes except dehydrogenase. Cellulase was significantly positively correlated with the activities of dehydrogenase, urease and phosphatase.

Key words: dehydrogenase, urease, cellulase, phosphatase

INTRODUCTION

Soil enzymes are involved in various decomposition and chemical transformation in the soil and measurement of enzyme activities can give indications of the extent of specific processes in the soil and can sometimes be used as indicators of soil fertility.¹ Soil is a complex system with the Physical, chemical and biological properties of it being in dynamic equilibrium. Studies on soil enzymatic activities provide valuable information regarding the biochemical processes in soil. Soil microorganisms enzymatic reactions forms a part of respiratory pathways and are related to the variety of soil and its temperature and moisture conditions.^{2,3} Enzymes released during decomposition process play a significant role in different biogeochemical cycles.⁴

Microbial status and physico chemical conditions of the soil are indicated by the enzyme activities hence they are also used as sensors of soil degradation.^{5,6} A number of reports are available dealing with various aspects of

enzyme activities in soils around the world and its relation to other factors.⁷⁻⁹ The present study was undertaken to determine the seasonal variation in enzyme activities and the relationship of enzymes with soil properties.

MATERIALS & METHODS

The surface soil samples were collected from agro ecosystem site adjoining the Ranchi University campus, Morhabadi. The samples collected were mixed thoroughly, brought to the laboratory in polythene bags and screened through a 2mm sieve. The samples were kept at 4°C until used. Estimation of physico chemical parameters and enzyme activities were done within fortnight. Soil temperature was measured by the help of soil thermometer whereas moisture content of the soil was determined by Oven drying method. Organic carbon and nitrogen content of soil was estimated according to Walkley and Black (1934)¹⁰ and Kjeldahl method of Jackson (1973)¹¹. Soil respiration was estimated following the alkali absorption method of Witkamp (1966)¹².

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The procedure of Casida *et al.*, (1964)¹³ was followed for the assay of dehydrogenase activity in soil and urease activity was estimated following Tabatabai and Bremner (1972)⁸ with modification of Kaplan (1969)¹⁴. Phosphatase and Cellulase activity in soil were measured following Kramer and Yardei (1959)¹⁵ and Ross (1965)¹⁶ respectively.

The study area experiences three well-defined seasons. The winter season starts from October to February, the summer season starts from March to mid-June and the rainy season starts from mid-June to September.

OBSERVATION

The range of variation of different physico chemical parameters during different seasons has been presented in table 1.

Table 1- Variation in physico chemical parameters of soil during the study period.

Parameters	Range
Soil Temperature	15.6 ± 0.98 - 24.73 ± 1.23 °C
Soil moisture	9.15 ± 1.02 - 22.36 ± 1.21 %
Organic carbon content	5.98 ± 0.06 - 7.493 ± 0.14 mg C g ⁻¹
Nitrogen content	0.53 ± 0.01- 0.617 ± 0.03 mg N g ⁻¹
Soil respiration	343.3 ± 6.45 - 472.7 ± 6.83 mg CO ₂ kg ⁻¹ hr ⁻¹

The seasonal variation in dehydrogenase activity is shown in table 2 with a peak value in rainy season (10.2 ± 1.87 µg formazan g⁻¹ dry soil h⁻¹) and least in winter (6.5 ± 0.61). Dehydrogenase activity had a significant positive correlation with soil respiration (r=0.678, p<0.02) whereas, a positive correlation was obtained with soil organic carbon matter, total nitrogen content, soil temperature and soil moisture (Table 3). Dehydrogenase activity had a positive significant correlation with enzyme activities like cellulase (r=0.877, p<0.001) and urease (r=0.62, p<0.05) whereas; a positive correlation with phosphatase was obtained (Table 4). Two way analysis of variance showed a significant difference in enzyme activity between season but it was not significant between the study sites (F = 10.818; df= 2, 4; p < 0.025 and F = 3.92; df = 2, 4) (Table 5).

Variation in urease activity in different season is shown in Table 2. A maximum of 47.89 ± 4.13 µg NH₃ g⁻¹ soil h⁻¹ was obtained in rainy season with a minimum of 25.47 ± 2.32 µg NH₃ g⁻¹ soil h⁻¹ in summer. The correlation between soil moisture and urease activity revealed a significant positive correlation (r = 0.714, p < 0.01). Urease activity had a significant positive correlation with organic

matter, total nitrogen, soil moisture and soil respiration (Table 3). Urease activity had a significant positive correlation with phosphatase activity (r= 0.817, p < 0.01). Correlation between urease and other enzyme activity was positive and significant (Table 4). A significant difference in enzyme activity between season (F = 35.635; df = 2, 4; p < 0.005) but there was no significant difference in enzyme activity between the sites (F = 0.470; df = 2,4) (Table 6) was revealed by two way ANOVA.

Phosphatase activity increased from a minimum (38.96 ± 2.74 µg phenol g⁻¹ soil h⁻¹) in summer to a maximum (55.71 ± 4.89 g phenol g⁻¹ soil h⁻¹) in rainy season (Table 2). Phosphatase activity has a significant positive correlation with soil moisture content (r = 0.611, p < 0.05) (Table 3). Phosphatase activity had a positive significant correlation with organic matter (r= 0.705, p < 0.02). Phosphatase activity had a significant positive correlation with enzyme activity like urease and cellulase (r= 0.817, p < 0.01 and r= 0.809, p < 0.01) respectively (Table 4). Two way analysis of variance showed a significant difference in enzyme activity between season but it was not significant between the study sites (F = 12.60; df= 2, 4; p < 0.025 and F = 0.383; df = 2, 4) (Table 7).

Seasonal variation in cellulase activity is shown in Table 2 having a peak value of 53.12 ± 4.12 µg glucose g⁻¹ soil h⁻¹ in rainy season and least in winter 27.39 ± 2.53 µg glucose g⁻¹ soil h⁻¹. Cellulase activity had positive significant correlation with soil organic matter and soil respiration (Table 3). It also had a significant positive correlation with other enzyme activity (Table 4). A significant difference in enzyme activity between seasons (F = 31.135; df = 2; 4, p < 0.005), whereas, no significant difference was obtained between different sites (F= 0.141; df = 2, 4) (Table 8) by two-way analysis of variance.

DISCUSSION

Pati and Sahu (1998)¹⁷ observed a significant positive correlation between soil respiration and dehydrogenase activity in both normal and fluoride treated soil. A similar relationship between the two parameters was also reported by Stevenson (1959)¹⁸ whereas, Mishra and Sahoo (1997)¹⁹ did not observed a significant positive correlation between soil respiration and dehydrogenase activity. In the present study a significant positive correlation (r = 0.678, p < 0.02) was obtained between the two parameters showing agreement with previous reports of Pati and Sahu (1998)¹⁷ and Stevenson (1959)¹⁸.

Table 2- Enzyme activity in soil sample (Mean ± SEM) in different seasons.

Season	Dehydrogenase ¹	Urease ²	Phosphatase ³	Cellulase ⁴
Winter	6.5 ± 0.61	33.38 ± 2.11	44.42 ± 3.16	27.39 ± 2.53
Summer	8.5 ± 1.38	25.47 ± 2.32	38.96 ± 2.74	31.81 ± 3.9
Rainy	10.2 ± 1.87	47.89 ± 4.13	55.71 ± 4.89	53.12 ± 4.12

1. $\mu\text{g formazan g}^{-1}\text{ soil h}^{-1}$ 2. $\mu\text{g NH}_3\text{ g}^{-1}\text{ soil h}^{-1}$
 3. $\mu\text{g phenol g}^{-1}\text{ soil h}^{-1}$ 4. $\mu\text{g glucose g}^{-1}\text{ soil h}^{-1}$

Table 3- Correlation coefficient among different enzyme activity and edaphic parameters.

	Organic carbon	Nitrogen	Soil respiration	Soil moisture	Soil temperature
Dehydrogenase	0.433 ^{NS}	0.421 ^{NS}	0.678 ^{**}	0.292 ^{NS}	0.428 ^{NS}
Urease	0.666 ^{**}	0.650 ^{***}	0.725 [*]	0.714 [*]	0.294 ^{NS}
Phosphatase	0.705 ^{**}	0.382 ^{NS}	0.450 ^{NS}	0.611 ^{***}	0.284 ^{NS}
Cellulase	0.586 ^{***}	0.555 ^{****}	0.705 ^{**}	0.498 ^{****}	0.509 ^{****}

*p < 0.01, **p < 0.02, ***p < 0.05, ****p < 0.10, NS Non significant

Table 4- Correlation matrix among different soil enzyme activity.

	Dehydrogenase	Urease	Phosphatase	Cellulase
Dehydrogenase	-	0.620 ^{***}	0.538 ^{****}	0.877 [*]
Urease	-	-	0.817 ^{**}	0.876 [*]
Phosphatase	-	-	-	0.809 ^{**}
Cellulase	-	-	-	-

* p < 0.001, ** p < 0.01, *** p < 0.05, ****p < 0.10

Table 5- ANOVA test among dehydrogenase activity in soil at different sampling sites and in different seasons.

Source of variation	Sum of Square	Degree of freedom	Mean square	Variation ratio F	Significance
Between season	20.76056	2	10.38028	10.81811	p < 0.025
Between sites	7.523356	2	3.761678	3.920343	NS
Residual	3.838111	4	0.959528		

Table 6- ANOVA test among urease activity in soil at different sampling sites and in different seasons.

Source of variation	Sum of Square	Degree of freedom	Mean square	Variation ratio F	Significance
Between season	775.7646	2	387.8823	35.635	p < 0.005
Between sites	10.24427	2	5.122133	0.470	NS
Residual	43.53853	4	10.88463		

Table 7- ANOVA test among phosphatase activity in soil at different sampling sites and in different seasons.

Source of variation	Sum of Square	Degree of freedom	Mean square	Variation ratio F	Significance
Between season	437.8382	2	218.9191	12.60	p < 0.025
Between sites	13.3206	2	6.6603	0.383	NS
Residual	69.49	4	17.3725		

Table 8- ANOVA test among cellulase activity in soil at different sampling sites and in different seasons.

Source of variation	Sum of Square	Degree of freedom	Mean square	Variation ratio F	Significance
Between season	1124.896	2	562.4481	31.135	p < 0.005
Between sites	5.106467	2	2.553233	0.141	NS
Residual	72.25853	4	18.06463		

Enzyme activity can differ in soil under the same climatic conditions and even in soil with the same amount of organic matter. Besides the vegetation, temperature and soil moisture content play significant roles in regulating the soil enzyme activities. Synthesis, accumulation and activity of enzymes in heterogenous and dynamic system like soil are influenced to a great extent by various physical, physico-chemical and biological constitution of the soil.

Mishra *et al.*, (1979)¹ found a significant correlation between soil moisture, organic matter content and enzyme activity (cellulase). They also found a positive correlation of cellulase activity with total nitrogen content of the soil. In the present investigation a significant positive correlation between total nitrogen and cellulase activity ($r=0.555$, $p < 0.10$) was found whereas, cellulase activity had a positive correlation with soil moisture ($r=0.498$, $p < 0.10$) and a significant positive correlation with organic matter content ($r=0.586$, $p < 0.05$) was obtained and the findings are in conformity with those of Mishra *et al.*, (1979)¹.

Level of enzyme activity in soil and total soil respiration (carbon dioxide evolution) are considered to give some guide to the microbiological activity prevailing in the soil system. The breakdown of macromolecular constituent of plant litter involves the extra cellular enzymes secreted by microorganisms and root to the soil and the assessment of soil enzyme activity gives the fertility status of the soil. Urolytic microorganisms and root exudates secretes urease enzyme. Urea added to the soil as fertilizer is hydrolysed by the enzyme urease resulting in the release of ammonia and causing damage to germinating seedlings and young plants, nitrite toxicity and other pollution problem.²⁰ Thus research concerning to the distribution and factor affecting urease activity in soil has helped the use of urea as nitrogen fertiliser and in the management of agricultural land.

A higher organic carbon content and microbial population supplemented with congenial soil reaction (pH 6.1 to 8.0) may have contributed significantly in production of urease at elevated levels in these soils of the study area. In contrast, soils having low organic content and microbial population with strongly to moderately acidic in reaction have low urease activity.²¹ Dash *et al.*, (1981)²² observed a positive correlation of total nitrogen, carbon and specific conductance with urease activity. Organic carbon and total nitrogen content are indices of organic matter content in soil. Positive correlation of urease activity with organic

carbon, total nitrogen and specific conductance suggest that organic matter content accounted for most of the variations in soil urease activity.^{22,23} Mishra (1989)²⁴ found that urease activity was negatively correlated with moisture of the soil supporting the suggestions that the organic matter content and specific conductance were the chief determinants of urease activity. In the present investigation urease activity was positively correlated with organic matter ($r=0.666$, $p < 0.02$) and total nitrogen content ($r=0.650$, $p < 0.05$) and is in conformity with the findings of Dash *et al.*, (1981)²².

As regards levels of phosphatase in soils belonging to the three land situations, lowland exhibited a higher activity of 43.80 to 114.77 μg phenol released g^{-1} soil h^{-1} , whereas, the uplands contained the least activity of 9.56 to 32.41 μg phenol released g^{-1} soil h^{-1} . Soils of lowlands being rich in organic and inorganic adsorptive surfaces are likely to immobilize and protect the enzymes from proteolytic degradation more efficiently than upland soils.²⁵ A moderate phosphatase activity (38.93 ± 2.74 to 55.71 ± 4.89 μg phenol released g^{-1} soil h^{-1}) was recorded at the sampling sites.

Mishra and Sahoo (1997)¹⁹ recorded a positive insignificant correlation between organic carbon content and dehydrogenase activity, a similar positive correlation ($r=0.433$) between organic matter content and dehydrogenase activity in the present study is in conformity with the findings of Mishra and Sahoo (1997)¹⁹.

All the four enzymes examined excepting dehydrogenase established a highly significant and positive correlations with organic carbon of soils.²⁵ Urease activity with correlation coefficient of $r=0.62388$ has been shown to vary from 41.35 μg urea-N hydrolyzed g^{-1} h^{-1} in soils having low organic carbon (0.5%) to 45.94 and 59.82 μg urea-N hydrolyzed g^{-1} h^{-1} (0.75%) organic carbon, respectively. Likewise, phosphatase has been found to increase gradually from the lowest level of 38.09 (in low organic matter soil) to 39.59 (in medium organic matter soil) and finally to the highest activity of 58.77 μg phenol g^{-1} h^{-1} by Sharma (1993)²⁵. Cellulase, too, responded positively with increasing organic carbon resulting its highest activity of 31.212 n mole glucose g^{-1} m^{-1} with high organic carbon and the lowest of 0.587 n mole glucose g^{-1} m^{-1} in presence of low organic content.²⁵ A positive effect of organic matter on enzyme levels may be attributed partly to the better survival and growth of microorganisms due to

enhanced availability of carbon and energy source in presence of organic matter and partly to the protection of enzymes from the microbial degradation by complexing with humic substances.

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