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Urease Activity and Its Kinetics in Selected Benchmark Soils of Indo-Gangetic Plains, India

Ashutosh Kumar · Alok K. Srivastava · Kulandaivelu Velmourougane · Gurjant Singh Sidhu · S. K. Mahapatra · R. S. Singh · A. K. Sahoo · K. Das · T. H. Das · S. K. Reza · T. Bhattacharyya · D. Sarkar · A. K. Sharma

Abstract A study was undertaken in the established benchmark (BM) soil series in different agro-ecological sub-regions of Indo-Gangetic Plains (IGPs), India with an objective to assess the urease activity and its kinetics at different soil depths. The urease activity declined with increase in soil depth in all the selected BM soils of IGP. The mean urea hydrolysis in the surface horizon (0–30 cm; 18.2 μg NH_4^+/h) was 2.6-folds higher than the sub-surface horizon (121–150 cm; 7.01 μg NH_4^+/h). The enzyme velocity (V_{max}) and enzyme efficiency (K_m/V_{max}) of urease hydrolysis were at par in surface and sub-surface horizons. The average K_m value of urease enzyme in surface and sub-surface horizons were 4.53 and 3.96 mM, respectively. The coefficient of variation, K_m for surface horizons showed higher variability and low affinity of soil urease towards substrate urea than the sub-surface horizon. Negative Pearson's correlation coefficient was recorded between urease activity and soil depth ($R = -0.86$), while significant positive correlation was observed between urease activity with organic carbon ($R = 0.81$) and nitrogen ($R = 0.81$).

Keywords Soil urease · Enzyme kinetics · Benchmark soils · Soil depth

A. Kumar · A. K. Srivastava (✉) · A. K. Sharma
National Bureau of Agriculturally Important Microorganisms, Mau 275101, UP, India
e-mail: aloksrivastva@gmail.com

K. Velmourougane
Central Institute for Cotton Research, Nagpur, Maharashtra, India

G. S. Sidhu
National Bureau of Soil Survey and Land Use of Planning, Regional Centre, New Delhi, India

S. K. Mahapatra · T. H. Das · S. K. Reza · T. Bhattacharyya · D. Sarkar
National Bureau of Soil Survey and Land Use of Planning, Nagpur, Maharashtra, India

R. S. Singh
National Bureau of Soil Survey and Land Use of Planning, Regional Centre, Udaipur, India

A. K. Sahoo · K. Das
National Bureau of Soil Survey and Land Use of Planning, Regional Centre, Kolkata, India

Introduction

In recent times there has been an increased interest in developing various techniques of evaluating soil health. Soil biological activities have been suggested as one of the important indicators of soil quality. Among the indigenous soil components, soil enzymes have been suggested as one of the potential biological indicators of soil quality because of their relationship to soil biology, ease of measurement, and rapid response to changes in soil management [1]. Among various soil enzymes, urease (urea amidohydrolase EC 3.5.1.5: catalyzing the hydrolysis of urea to CO_2 and NH_3) is very widely distributed in nature, and has been detected in plants, animals, and microorganisms [2–4].

Urease plays an important role in the efficient use of urea fertilizer in soil and the changes in urease activity can be used as an indirect indicator of the variation in the pool of potentially available N in a soil [5]. Urease activity influences the optimum use of urea fertilizer, N volatilization, N leaching and environmental pollution related to N [6, 7]. Extreme high and low urease activities cause environmental pollution pertaining to nitrogen as low urease activity in soil causes loss of additional urea through

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leaching; whereas, high urease activity in agricultural field causes rapid decomposition of excess urea and sequentially, loss of ammonia by volatilization [8, 9].

Indo-Gangetic Plain (IGP) is the largest fertile plain of southeast Asia covering nearly 13 % of the total geographical area of the India. Rice–wheat cropping system is the major and prominent crop of IGP and covers about 50 % of the total food grains to feed up to 40 % of the population of the country [10]. Each soil horizon has a characteristic pattern of enzyme activity attributable to their catalytic activities [11]. Extracellular soil enzymes, secreted by soil microbes, are sensitive to the change of soil environment as their activities were influenced by land uses and agricultural practices [12].

Rice–wheat system demands higher nitrogen for higher yield, to compensate the demand of N, urea is widely used as the source of nitrogen in the IGP regions. The excess use of chemical fertilizers on cropping regions of IGP can affect soil health and crop fertility. There are no comprehensive reports available on soil enzyme activity and its kinetics in IGP region which can guide to formulate any agricultural strategy with special consideration of urea decomposition rate in surface as well as in subsurface soil. To fill this knowledge gap, soil surveys were conducted in selected benchmark (BM) soil of IGP to access the urease activity and kinetics with relation to different soil depths. The information generated through this study will be highly useful for the assessment and development of strategies for efficient nitrogen management as well as monitoring indicator of soil health in IGP soil.

Material and Methods

Soil Survey and Sampling

The soil samples were collected from the representative BM sites from IGP, India. IGP covers specific bio-climatic systems in agro-ecological regions (AERs). The sampling area includes five districts located in four states namely Karnal from Haryana, Etah and Chandauli from Uttar Pradesh, Ludhiana from Punjab and Udhamsingh Nagar from Uttrakhand (Fig. 1). Information collected with respect to the BM coordinates, rainfall, soil types, cropping intensity, nutrient status are presented in Table 1. Two representative pedons i.e., one under low management, LM, which is characterized by low application of major nutrients and manures, absence of agricultural residue management and soil moisture conservation practices and the other under high management, HM, having the characteristic of higher application of NPK, regular application of animal waste and agricultural residues, and adoption of soil moisture conservation techniques, were collected.

Assay of Urease Activity and Its Kinetics in Soil

Urease activity in selected BM soils (air-dried, crushed and sieved through a 2-mm mesh screen) of IGP was assayed by the modified method of Tabatabai [4]. Five ml of urea (2 %) was added to the soil samples. The residual urea was measured after incubation at 37 °C for 6 h. After centrifugation, to determine the remaining urea, an aliquot of the extract containing up to 2 ml of urea solution was pipetted and made up to 5 ml with 2 M KCl–phenylmercuric acetate. Thereafter, 5 ml of colouring reagent consisting of 2 % diacetylmonooxime and 0.5 % thiosemicarbazide in ratio of 6.7 % each in distilled water along with acid reagent was added. This solution was placed in a boiling water bath for 30 min and then cooled for next 15 min. The volume was made to 20 ml with water for measurement with a spectrophotometer (Shimadzu UV1200; Shimadzu, Tokyo) at 520 nm with blank (water instead of urea solution). The mean urease activity was calculated for urea hydrolyzed and expressed as µg NH_4^+/g dry soil/h.

Kinetic parameters of soil urease enzymes such as K_m and V_{max} for selected BM soils were calculated by Lineweaver–Burk equation which depends upon the properties of substrate concentration, the linear transformation of Michaelis–Menten equation as under.

$$\frac{1}{V} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}},$$

where V stands for enzyme reaction velocity, $[S]$ is the concentration of substrate (mM), K_m is the Michaelis constant (mM), and V_{max} is the maximum enzyme reaction velocity (mM/h). K_m indicates the affinity of urease to its specific substrate urea, and gives the substrate concentration at which the reaction rate reaches half of its maximum value (V_{max}/2).

All the analyses were carried out in triplicates and the statistical interpretations were performed by SPSS 16.0V software.

Results and Discussion

Impact of Soil Depth and Management Systems on Urease Activity

Average soil urease activity in the surface and sub-surface soils was recorded to be fluctuating between 7 and 18 µg NH_4^+/g/h. Among the BM spots of HM, highest rate of urease activity in surface soil was recorded (µg NH_4^+/g/h) in Karnal (22.73) followed by Udhamsingh Nagar (21.89), Etah (17.31), Chandauli (17.02) and Ludhiana (15.39); whereas in bottom most layer (121–150 cm), highest urease activity was recorded in Ludhiana (10.68 µg NH_4^+/g/h) followed by

Fig. 1 Benchmark location of examined soil series
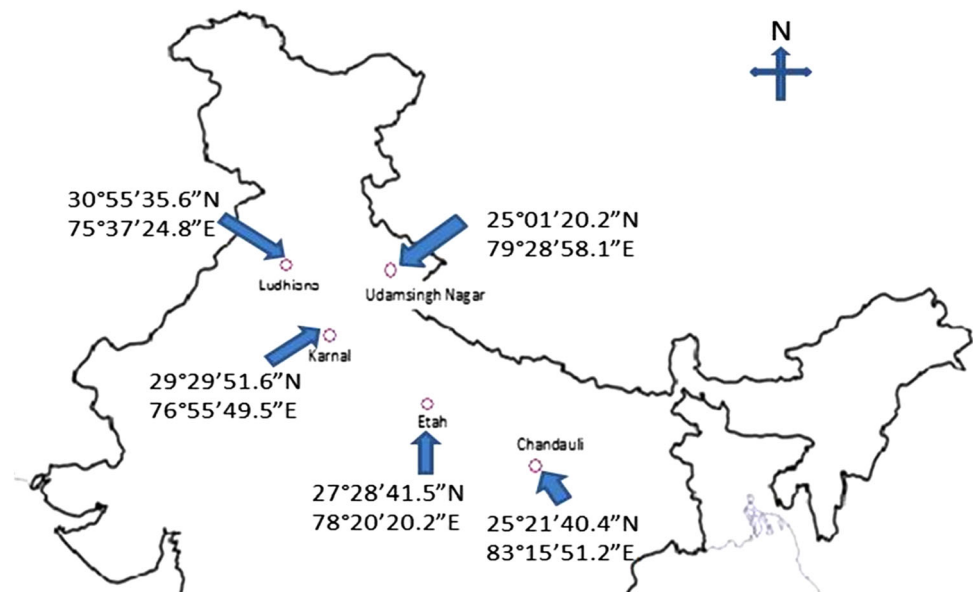
Karnal (9.51), Chandauli (6.3), Etah (5.83), and Udhamsingh Nagar (5.53; Fig. 2). In LM, higher urease activity was observed in Udhamsingh Nagar (21.88), lagged by Chandauli (16.67), Karnal (16.07), Etah (15.76), and Ludhiana (15.44) in surface soil; whereas at 121–150 cm sub-surface higher urease activity was observed in Ludhiana (9.1) followed by Udhamsingh Nagar (8.8), Chandauli (6.3), Karnal (5.46), and Etah (2.48) with an exception of Ludhiana region showing exceptionally high urease activity in all the depths (Fig. 2).

In the heterogeneous system such as soil, urease was rapidly adsorbed to humic and clay matrixes as well as influenced by physio-chemical properties of the soil. The increased urease activity in the surface soil as compared to the sub-surface soil might be due to some possible factors as variations in soil nutrients and substrates (availability of higher urea substrate), organic carbon, higher adsorbed enzyme with soil, lesser enzyme inhibitor [5, 13, 14] biological (soil microflora) and chemical properties of soil [15, 16], pH, temperature, moisture, metal distribution and soil amendments [17–21].

While positive correlation was observed between the urease activity and organic carbon ($R = 0.81$) and nitrogen ($R = 0.81$), urease activity showed negative correlation ($R = -0.86$) with soil depth under both management system. The co-efficient of variation (CV) of urease activity was found to be lesser in the surface horizon (16 %) as compared to the sub-surface horizon (35.3 %; Fig. 3). Li et al. [22] had reported that urease activity was closely related to soil nutrient conditions and recommended urease enzyme as an important parameter for estimating the soil quality index.

Kinetics of Soil Urease Enzyme

Substrate Affinity (K_m)

The mean urease substrate affinity in IGP was found to be 4.53–3.97 mM for surface and sub-surface soil, respectively. Under HM, BM soils of Udhamsingh Nagar showed highest K_m value (6.66) in the surface horizon followed by the soils of Chandauli (6.25), Ludhiana (3.53), Etah (3.44) and Karnal (3.36) whereas decreasing trend of K_m in the sub-surface horizons were reported as Udhamsingh Nagar (5.55), Etah (4.35), Chandauli (3.98), Ludhiana (3.53) and Karnal (3.33). Likewise in LM, Udhamsingh Nagar BM showed peak K_m (7.14) followed by BM of Ludhiana (4.79), Karnal (3.49), Etah (3.44), and Chandauli (3.13) in surface horizon; while in sub surface pedons, K_m ranges from Udhamsingh Nagar (4.54), Chandauli (4.26), Etah (4.16), Karnal (3.56) to Ludhiana (2.50; Fig. 4).

In IGP, urea fertilizer was applied at higher rate in rice–wheat cropping sequence since green revolution; hence a high affinity enzyme is not required to scavenge the substrate (urea). Higher K_m value in surface soils may be attributed to the formation of inhibitor–urease complex and higher substrate availability; thereby decreasing the affinity of the soil urease enzyme to its substrate. Juan et al. [15] reported that coarse properties of soil or presence of enzyme inhibitor in soil causes the conformational change in the enzyme making its active sites less accessible to the substrate and reduction of effective active site covered by humus also. Variation in K_m is highly influenced by temperature, substrate properties, pH and ionic strength [23]. Present study revealed that K_m value in surface soil was higher than sub-surface soils, which indicates

Table 1 Characteristics features of selected benchmark spots IGP of India

BM spots	Latitude and longitude	Soil series	States	AER	Rainfall (mm)	Humidity (%)	Cropping intensity (%)	Urea input (kg/ha)	pH	Organic carbon (mg/kg)	Macro nutrients (mg/kg soil)		
											N	P	K
Karnal$_1$	29°29′51.6″ N 76°55′49.5″ E	Zaifa Viran	Haryana	Sub-humid (Dry)	617	Min. 48 Max. 60	120	150	8.42	0.90	275	62	434
Karnal$_2$	29°29′51.6″ N 76°55′49.5″ E	Zaifa Viran	Haryana	Sub-humid (Dry)	617	Min. 48 Max. 60	120	150	8.26	0.30	75	75	283
Ludhiana$_1$	29°29′51.6″ N 76°55′49.5″ E	Fatehpur	Punjab	Sub-humid (Dry)	689	Min. 48 Max. 68	120	177	7.18	0.58	138	70	60
Ludhiana$_2$	29°29′51.6″ N 76°55′49.5″ E	Fatehpur	Punjab	Sub-humid (Dry)	689	Max. 68 Min. 48	120	177	7.38	0.35	112	38	84
Etah$_1$	29°29′51.6″ N 76°55′49.5″ E	Saket	Uttar Pradesh	Sub-humid (Dry)	896	Min. 42 Max. 65	145	115	8.76	0.77	113	30	365
Etah$_2$	29°29′51.6″ N 76°55′49.5″ E	Saket	Uttar Pradesh	Sub-humid (Dry)	896	Min. 42 Max. 65	145	115	8.13	0.31	75	50	400
Chandauli$_1$	29°29′51.6″ N 76°55′49.5″ E	Itwa	Uttar Pradesh	Sub-humid (Moist)	1,200	Min. 39 Max. 61	145	115	8.50	0.60	151	28	141
Chandauli$_2$	29°29′51.6″ N 76°55′49.5″ E	Itwa	Uttar Pradesh	Sub-humid (Moist)	1,200	Min. 39 Max. 61	145	115	8.50	0.27	75	20	220
Udhamsingh Nagar$_1$	29°29′51.6″ N 76°55′49.5″ E	Haldi	Uttrakhand	Sub-humid (Moist)	1,667	Min. 50 Max. 76	90	94.2	7.31	1.26	188	25	112
Udhamsingh Nagar$_2$	29°29′51.6″ N 76°55′49.5″ E	Haldi	Uttrakhand	Sub-humid (Moist)	1,667	Min. 50 Max. 76	90	94.2	7.12	1.18	151	31	75

Temperature: minimum = 4 ± 2 °C, maximum = 35 ± 2 °C

Fig. 2 Soil urease activity in horizons of different soil series of Indo-Gangetic plain (1 = high management and 2 = low management)
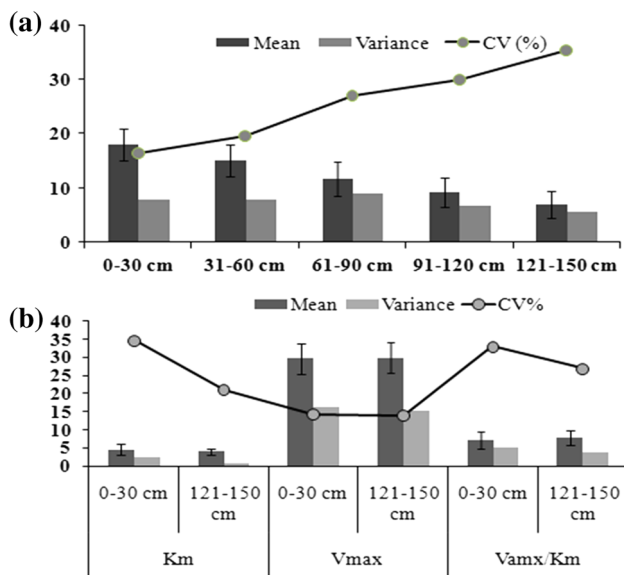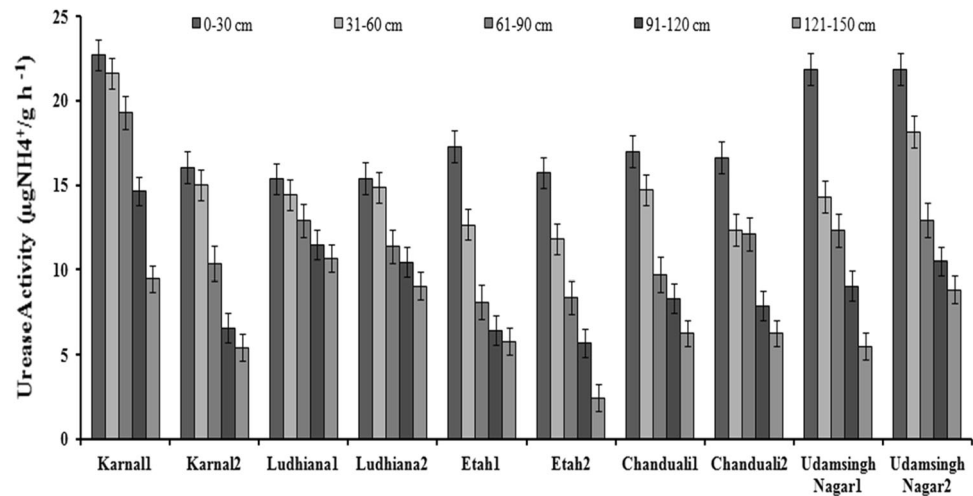

Fig. 3 Statistical characteristics of soil urease activity (**a**) and its kinetic behavior (**b**) across soil depth

low affinity for urea substrate in IGP. The reason might be due to the application of higher urea fertilizer. Almost at par magnitude in K_m of subsurface BM spot soil of IGP reveals similar abundance of urea through leaching. In surface soil, presence of higher soil organic carbon and variations in charge distribution of the enzyme active site generated by complex formation can prevent advancement of soil urease activities from interacting with substrate [24–26]. Coefficient of Variance for K_m was recorded to be 34.6 and 21.0 % in surface and sub-surface soil layer which reflects higher degree of variability in enzyme–substrate affinity in surface soil (Fig. 3).

Enzyme Velocity (V_{max})

The mean V_{max} for the selected BMs of IGP in surface (29.7 mM/h) and sub surface soil (29.9 mM/h) were at par (Fig. 3) and remain unaffected by management practices in

each soil series indicating similar inhibitory effect on soil urease activity. The range of enzyme velocity for surface soil under HM/LM existed between 25.64–37.59/23.95–34.36 mM/h for Udhamsingh Nagar–Chandauli/Ludhiana–Chandauli, whereas, 23.8–37.04/23.64–32.25 mM/h at Ludhiana–Chandauli/Ludhiana–Etah and Karnal in the sub-surface horizons (Fig. 4). Comparable enzyme velocity indicates about similar rate of saturation of substrate–enzyme complex. AER of IGP has 35 °C as maximum average annual temperature; this might be the prominent reason for high enzyme kinetics. Gioacchini et al. [21], Zhou [27] and Nannipieri et al. [28] have reported that high temperature can provide required activation energy to the enzyme, resulting in high enzyme kinetics. Present studies also confirmed that high enzyme velocity up to a certain limit in all examined benchmarked spot of IGP soil series is consequence of high temperature.

Catalytic Efficiency (V_{max}/K_m)

The ratio between V_{max} and K_m (V_{max}/K_m) has been considered as an index of the catalytic capacity of an enzyme through enzymatic reactions. More specifically number of molecules of substrate that can be processed by one molecule of enzyme in one unit of time. The mean of enzyme efficiency between surface and sub-surface horizons was 7.23 and 7.19. Karnal and Udhamsingh Nagar had highest and lowest enzyme catalytic efficiency under both managements (Fig. 3). In surface and sub-surface horizons, enzyme efficiency showed its fluctuation from 9.44 (Chandauli) to 4.62 (Udhamsingh Nagar) and 9.58 (Ludhiana) to 6.12 (Udhamsingh Nagar) under HM and LM soils, respectively (Fig. 4). The CV for catalytic efficiencies in surface horizon and sub surface horizon was calculated to be 32.9 and 26.8 %, respectively indicating more varying performance of soil urease in surface soil than subsurface soil (Fig. 3).

Fig. 4 Urease kinetics in surface and sub-surface horizons of IGP K_m, V_{max}/K_m and V_{max}
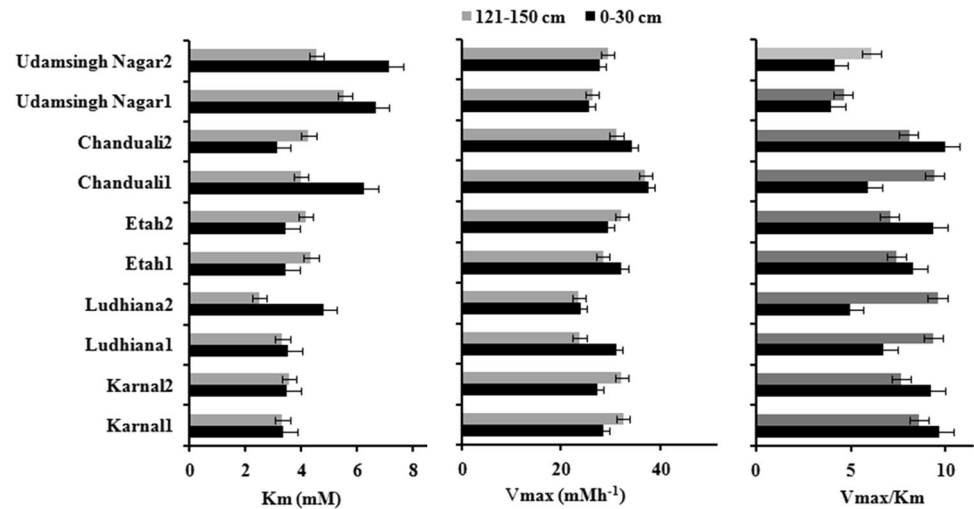
The catalytic efficiency was higher in HM due to higher value of enzyme kinetic variables as compared to LM practice soils. Previous researchers demonstrated that the significant changes in microbial communities with soil depth are responsible for fluctuation in enzymatic action and soil metabolism [29–33].

Conclusion

Soil urease activity in IGP is highly variable. It decreases exponentially from surface to sub-surface. Comparatively two–three times higher urease activity was observed in surface horizon as compared to sub-surface horizon irrespective of soil types and management systems. Urease kinetics (K_m and V_{max} value) was found to be similar throughout the sub-surface soils of selected BM spots of IGP. The high K_m value clearly revealed the low affinity of urease enzyme towards urea substrate and higher urea application in the IGP. More soil survey and analysis in the region not only lead to better understanding of soil health and monitoring of IGP soil but is also helpful in development for N management strategies.

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