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Assessment of Soil Enzyme Activities Based on Soil Samples from the Beas River Bed, India Using Multivariate Techniques

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ABSTRACT

This study was aimed at assessing soil enzyme activities in the Beas River bed for the pre-monsoon, post-monsoon and winter seasons. Soil samples were collected in triplicates from four sites for each season and analysed for 21 soil characteristics. The soil enzymes assessed were urease, catalase, polyphenol oxidase (PPO) and invertase. The hypothesis tested was that the enzyme activities are determined by soil characteristics and other environmental variables. Data were analysed using analysis of variance, multiple comparison test, cluster analysis, principal component analysis, stepwise multiple linear regression analysis and artificial neural networks. It was concluded from the study that maximum soil urease, PPO and invertase activities occurred during the winter season. There were two factors underlying the enzyme activities: factor-1 for urease and catalase, and factor-2 for PPO and invertase. Urease activity was increased to a maximum by the phosphate content of the soil, an important component of animal excreta. Nickel and Cu are the prosthetic groups of urease and PPO which contributed a maximum to the activities of the respective enzymes.

Keywords: River Beas, soil enzyme activities, multivariate techniques, artificial neural networks

INTRODUCTION

Soil micro flora plays a significant role in the decomposition and mineralisation of organic matter by producing enzymes (Burns, 1982). In the soil subsystems, biochemical functions are carried out by soil enzymes (Burns, 1983; Sinsabangh *et al.*, 1991). Because of their involvement in the cycling of N, C and P, soil enzymes are considered as bio-indicators of soil fertility (Schoenholtz *et al.*, 2000). Soil enzymes are formed from plant residues both as extracellular and intracellular enzymes (Burns, 1986; Mobley *et al.*, 1989). Soil enzyme activities increase when there is an increase in content of organic matter. The higher activities of enzymes correspond to larger microbial communities and greater stability of enzymes adsorbed on the humic materials (Marinari and Antisari, 2010). Enzyme activities act as biomarkers to assess the quality of soil based on their sensitivity

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to soil management practices, nutrient cycling, organic matter decomposition and bioremediation activities. Invertase enzyme is important because it releases sugars used by microorganisms (Shi *et al*., 2008; Rahmansyah and Sudiana, 2010). Gu *et al.* (2009) studied the soil enzymes activities of urease, invertase and polyphenol oxidase (PPO) from China. Mondal *et al*., (2015) studied the seasonal variation of soil enzyme activities such as urease and invertase in fluoride stressed areas of West Bengal, India. Zhang *et al.,* (2015) studied urease, invertase and PPO activities from Gurbantunggut desert, Xinjiang. The present study was aimed at assessing soil enzyme activities in the river bed of the River Beas for pre-monsoon, post-monsoon and winter seasons respectively.

MATERIALS AND METHODS

Study Area

The River Beas originates in central Himachal Pradesh, India, 32.21′N lat., 77°05′E at an altitude of 2050 m above sea level, and merges with the river Sutlej at Harike, Punjab, after traversing a distance of about 470 km. Soil samples were collected from the river bed of the River Beas between the towns of Beas and Harike over a stretch of 63 km at the following sites (Figure 1):

1. Beas (31.510' N and 75.305' E)
2. Kishanpura (31.409' N and 75.189' E)
3. Goindwal Sahib (31.376' N and 75.162' E)
4. Harike (31.150' N and 74.951' E)

Soil Sampling and Enzyme Activities

Landsat (TM) data were obtained from the United States Geological Survey (USGS) (http://glovis.usgs.gov/). Map of the study area was prepared by using Erdas Imagine '11' and Arc GIS 9.3 software. Soil samples were collected on 7th June 2013 (pre-monsoon season), 14th October 2013 (post-monsoon season) and 26th February 26, 2014 (winter season)in triplicates at a depth of 0-5 cm and stored at 4^{o}C in a refrigerator. All the soil samples were ground and sieved with a 0.6-mm sieve in order to remove any effect of particle size before analysis. Standard methods for soil analysis were followed as described earlier (Kumar *et al*., 2015).

Catalase activity was measured following the method of Guan *et al.*, (1986). To 2 g of soil, 40 ml of distilled water and 5 ml of 0.3% H_2O_2 were added. The mixture was shaken at 25^{o}C for 20 min. Then, 5 ml of 1.5 M H_2SO_4 was added and the contents were titrated with 0.1 M $KMnO_4$.

The activities of urease and PPO were estimated by following the method of Guan (1986). For urease, 5 g of moist soil was incubated at 37^{o}C for 2 h in 20 ml of borate buffer. After incubation, 50 ml of 1 M KCl solution was added and the mixture was shaken for 30 min. Absorbance was determined using the uv-visible spectrophotometer at 690 nm.

For PPO activity, to 5 g of soil sample, 10 ml of distilled water, 6 ml of 0.1% ascorbic acid, and 10 ml of 0.02 M catechol were added. Then, the soil

suspension was incubated in a water bath for 2 min at 30°C. Subsequently 3 ml of 10% phosphoric acid was added and the filtrate was titrated with 0.005 M iodine.

Invertase activity was measured by using the method of Guan *et al*. (1986). To 5 g of dried soil, 0.2 ml of methylbenzene was added and allowed to stand for 15 min. Then, 15 ml of 18% sucrose solution and 5 ml of phosphate buffer were added, and incubated for 24 h at 37°C. After incubation, 3 ml of 3, 5-dinitrosalicyclic acid solution and 5 ml of deionized water were added to 0.5 ml of the filtrate. All tubes were placed in the boiling water bath for 5 min and then cooled to room temperature. Finally the solution was diluted to 50 ml, and absorbance was determined using the uv-visible spectrophotometer at 508 nm.

Statistical Analysis

Data was statistically analysed by using one way ANOVA, cluster analysis (CA), principal component analysis (PCA), factor analysis (FA), stepwise multiple linear regression analysis (SMLR) and artificial neural network analysis (ANN). MS-Excel-2007, PAST, Minitab-14, Statistica-12 and self-coded software were used for the analysis.

RESULTS AND DISCUSSION

Table 1 shows the soil characteristics of the sampled soils from the river bed of the Beas for different seasons. Table 2 summarises the statistics of the enzyme activities in the soil samples collected from the river beds of Beas for different seasons. Differences in soil enzyme activities were found to be significant as given in Table 2. Urease, PPO and invertase activities were found to be at a maximum during the winter season. Activity of catalase is sensitive to biological factors and is closely related with major soil nutrient elements (Asmar *et al*.,

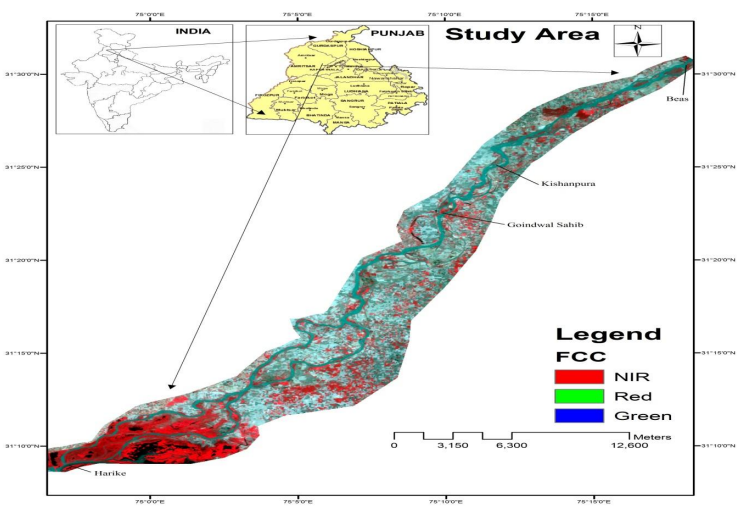

Figure 1: Study area

TABLE 1
Chemical characteristic of soils from pre-monsoon, post-monsoon and winter seasons of Beas river bed.

Characteristics	Pre-monsoon season Mean±SD	Post-monsoon season Mean±SD	Winter season Mean±SD	F-Ratio *(p<0.05)	HSD (p<0.05)
pH	8.26±0.096	8.16±0.074	6.65±0.09	406.50*	0.176
Conductivity (µS/cm)	281.00±72.88	357.85±121.44	156.65±26.63	5.95*	164.34
WHC (%)	35.36±2.76	39.30±4.95	38.78±6.82	0.69	ns
H (%)	0.32±0.07	0.31±0.08	1.05±0.68	4.45*	0.794
C (%)	0.16±0.02	0.17±0.02	0.15±0.02	0.25	ns
N (%)	0.084±0.02	0.094±0.01	0.135±0.04	3.21	ns
P (mg/g)	0.013±0.010	0.006±0.005	0.093±0.043	13.97*	0.051
Na (mg/g)	4.19±1.23	1.91±0.26	4.39±1.12	7.91*	1.93
K (mg/g)	2.70±0.42	1.75±0.23	2.13±0.70	3.77	ns
Ca (mg/g)	15.80±1.11	17.20±3.18	11.85±2.18	5.71*	4.58
Mg (mg/g)	4.61±1.01	5.73±2.99	3.09±1.01	1.91	ns
Fe (mg/g)	37.48±12.42	28.67±2.94	30.29±6.62	1.273	ns
Zn (mg/g)	0.031±0.006	0.030±0.006	0.031±0.002	0.033	ns
Mn (mg/g)	1.20±0.39	0.985±0.22	0.385±0.11	9.93*	0.531
Ni (mg/g)	0.142±0.11	0.34±0.32	0.074±0.056	1.91	ns
Cr (mg/g)	0.023±0.004	0.022±0.003	0.022±0.004	0.088	ns
Cu (mg/g)	0.020±0.007	0.016±0.004	0.016±0.003	0.981	ns

ns = not significant and '*'= significant at $p < 0.05$.

TABLE 2
Soil enzyme activities of Beas river bed for pre-monsoon, post-monsoon and winter seasons.

Characteristics	Pre-monsoon season Mean±SD	Post-monsoon season Mean±SD	Winter Season Mean±SD	F-Ratio *(p<0.05)	HSD (p<0.05)
Urease mg $N\text{-}NH_4^+$ 100 g^{-1} soil 24 h^{-1}	7.34^{ab}±0.94	7.09^{b}±0.96	11.51^{a}±3.32	3.99*	4.31
Catalase 0.1 M $KMnO_4$ g^{-1} soil	0.154^{b}±0.028	0.25^{a}±0.036	0.167^{b}±0.023	18.22*	0.042
PPO 0.005 M I_2 g^{-1} soil h^{-1}	22.25^{ab}±2.18	20.35^{b}±3.52	25.40^{a}±1.97	5.91*	3.44
Invertase glucose mg g^{-1} soil 24 h^{-1}	2.82^{c}±0.67	3.75^{a}±0.52	4.37^{a}±2.23	8.33*	0.82

Values with same superscript or no superscript in the same row imply that the values are not significantly different from each other at $p > 0.05$.

1992; Rodriguez-kabana and Truelove, 1982). Mondal *et al.* (2015) reported the range of invertase (activity to be 0.41 to 3.97 glucose mg g^{-1} soil 24 h^{-1} in fluoride stressed areas of Birbhum district, West Bengal. Trasar-Cepeda *et al*., (2008) also studied urease activity (in agricultural and forest soils from Spain and found it to range from1.9 to 17.7 µmol NH_3 g^{-1} h^{-1}. Our results showed slight variations from their work.

Cluster analysis was applied to the enzyme activities for different seasons (Figure 2). The enzyme activities were similar during the pre-monsoon and post-monsoon seasons, but different during the winter season. The difference in soil enzyme activities may be attributed to differences in temperature during the seasons, winter being the coldest month. PCA was also applied to the enzyme activities (Table 3). The first two components of PCA explained more than 99% of the total variance for the pre-monsoon (86.32% and 12.97%), post-monsoon (93.59% and 6.02%) and winter seasons (96.07% and 3.91%), respectively. In factor analysis, two factors were mainly responsible for soil enzyme activities (Figure 3). Factor-1 accounted for 39% of the total variance and had negative loading on urease, but positive loading on catalase, with communalities 0.551 and 0.869. This factor indicates the fertility of the soil. Invertase and PPO had

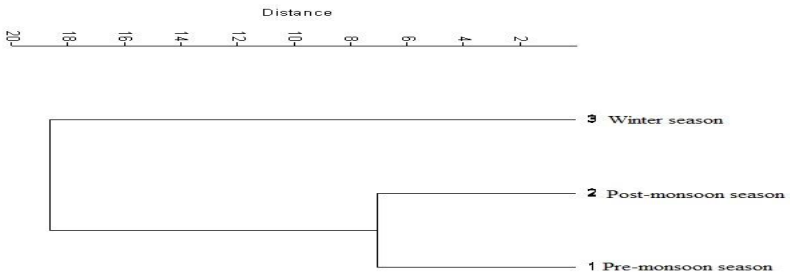

Figure 2: Cluster analysis of enzyme activities for different seasons

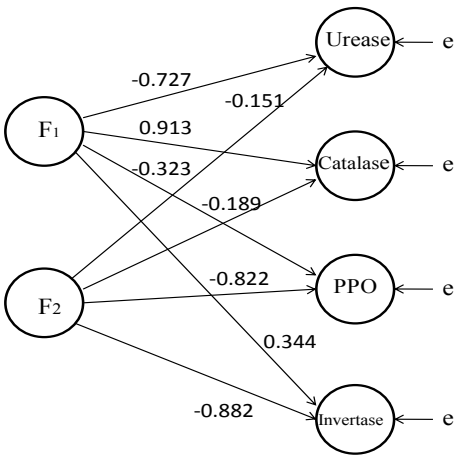

Figure 3: Factor analysis of soil enzymes in the different seasons

TABLE 3
Percent variance explained of soil enzyme activities for different seasons from Beas river bed.

Seasons	Principal component		
	PC1	PC2	PC1+PC2
Pre-monsoon season	86.32%	12.97%	99.29%
Post-monsoon season	93.59%	6.02%	99.61%
Winter season	96.07%	3.91%	99.98%

negative loadings on factor-2 which accounted for 37% of the total variance with communalities 0.896 and 0.780. This factor accounts for decomposition of soil matter.

In stepwise MLR analysis (Table 4), 83.6% of urease activity was accounted for by pH and P, and in metal analysis, 99.8% of urease activity was accounted for by Na, K, Ca, Fe, Zn, Mn and Ni. Urease activity is largely dependent on P, and P is the most important component of animal and human excreta. Ni is the prosthetic group of urease, and Ni contributes maximum to urease activity. Dependence of catalase activity on conductivity and C was explained to the extent of 38.9% of the variation, and in metal analysis, 74.8% of catalase activity was accounted for by Na and Ni. Conductivity explained maximum variability in catalase activity, and in metal analysis, Ni was found to contribute maximum to the catalase activity. For PPO activity, 48% was accounted for by C and N, and in metal analysis 79.6% of the PPO activity was explained by Ca, Fe and Cu. N contributed maximum to the PPO activity. Cu is the prosthetic group of PPO, and contributed maximum to the activity of PPO. Dependence of invertase activity on conductivity, C and N was explained to the extent of 40.9%. In metal analysis, 88.5% of invertase activity was accounted for by Ca and Ni. Nitrogen explained the maximum variability in invertase activity, and in metal analysis, maximum variability was explained by Ni. Nayak *et al.,* (2007) reported that the activities of soil enzymes are enhanced to different degrees by organic manure incorporation and noted significant and positive relationships of the enzyme activities with C and N. Sucrose, the substrate of soil invertase, is partially responsible for the breakdown of plant litter in the soil (Frankenberger and Johanson,1983). Urease enzyme is responsible for the hydrolysis of urea into NH_3 and CO_2. Urease activity indicates the N supply to the plants. Variations in the activity of urease enzyme are due to variations in the physcio-chemical characteristics of the soil, organic matter and N accumulation, considered as substrates for soil urease. PPO has a very important function in the cycling of aromatic compounds. Soil catalase is considered to be a potential indicator of aerobic microbial activity and has been related to the number of micro-organisms and soil fertility (Trasar-Cepeda *et al*., 1999). Heavy metals inhibit enzyme reactions by forming complexes

TABLE 4
Stepwise multiple regression of soil enzyme activities with soil characteristics

Characteristics	Equation	R^2
	Physico-chemical characteristics	
Urease activity (mg N-NH_4^+ 100 g^{-1} soil 24 h^{-1}) =	-29.36 + 4.3 pH + 132 P (mg/g)	0.836 ($p<0.001$)
Catalase activity (0.1 M $KMnO_4$ g^{-1} soil) =	0.178 + 0.0003 conductivity (µS/cm) – 0.42 C (%)	0.389 ($p<0.05$)
PPO activity (0.005 M I_2 g^{-1} soil h^{-1}) =	32.85 – 100 C (%) + 62 N (%)	0.480 ($p<0.05$)
Invertase activity (glucose mg g^{-1} soil 24 h^{-1}) =	7.39 + 0.019 conductivity (µS/cm) – 96 C (%) + 68 N (%)	0.409 ($p<0.05$)
	Metal analysis	
Urease activity (mg N-NH_4^+ 100 g^{-1} soil 24 h^{-1}) =	12.93 + 1.17 Na (mg g^{-1}) – 6.71 K (mg g^{-1}) – 27.24 Ca (mg g^{-1}) – 0.25 Fe (mg g^{-1}) + 592 Zn (mg g^{-1}) + 5.03 Mn (mg g^{-1}) – 16.30 Ni (mg g^{-1})	0.998 ($p<0.001$)
Catalase activity (0.1 M $KMnO_4$ g^{-1} soil) =	0.23 – 0.018 Na (mg g^{-1}) + 0.13 Ni (mg g^{-1})	0.748 ($p<0.001$)
PPO activity (0.005 M I_2 g^{-1} soil h^{-1}) =	37.71 – 48.50 Ca (mg g^{-1}) – 0.227 Fe + 368 Cu (mg g^{-1})	0.796 ($p<0.001$)
Invertase activity (glucose mg g^{-1} soil 24 h^{-1}) =	5.54 – 11.1 Ca (mg g^{-1}) + 9.8 Ni (mg g^{-1})	0.885 ($p<0.001$)

Relevance of dependent variable on the basis of β-regression coefficients						
	β_1	β_2	β_3	β_4	β_5	β_6
Urease activity	pH (0.83)	P (1.55)				
Catalase activity	Cond. (0.66)	C (-0.20)				
PPO activity	C (-0.77)	N (0.68)				
Invertase activity	Cond. (0.92)	C (-1.02)	N (1.04)			
	Metal analysis					
Urease activity	K (-1.01)	Ni (0.86)	Zn (0.74)	Mn(0.54)	Fe(-0.53)	Na(0.43)
Catalase activity	Ni (0.53)	Na (-0.51)				
PPO activity	Ca (-0.84)	Fe (-0.59)	Cu (0.56)			
Invertase activity	Ni (0.87)	Ca (-0.26)				

with their substrates or blocking functional groups of the enzymes (Speir *et al*., 1995). A negative relationship of metals, i.e., Fe, Na, K and Ca with enzyme activities, indicates that microbes secreting these enzymes are sensitive to the metal concentration in the soil. Compared to the pre-monsoon and post-monsoon seasons, enzyme activities were found to be higher during the winter season. This may be due to high nitrogen and phosphorus contents in the soil, and slow litter decomposition during this season. Boerner *et al.,* (2005) and Mukhopadhyay and Joy (2010) studied the variations in soil enzyme activities with respect to seasons. High soil organic content was responsible for higher enzyme activities in the upper layer of soil (Hu *et al.*, 2005). ANN models fitted well with the observed and the simulated data (Figures 4 a, b, c and d). The correlations between target and output values from ANN for catalase, urease, PPO and invertase were highly

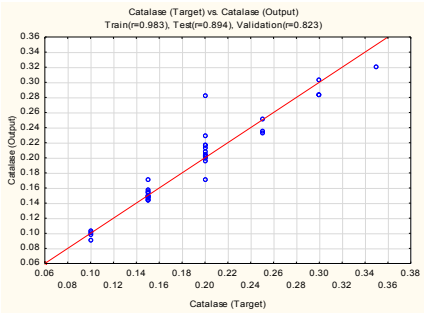

Figure 4(a): Correlation between target and output catalase enzyme using ANN model

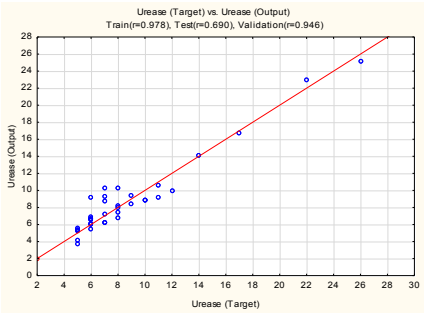

Figure 4(b): Correlation between target and output urease enzyme using ANN model

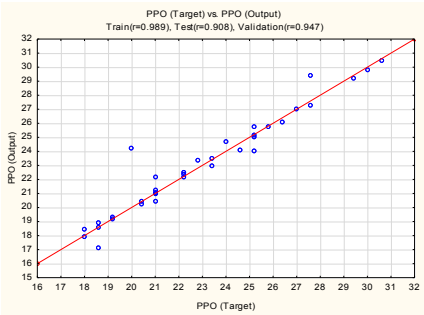

Figure 4(c): Correlation between target and output PPO enzyme using ANN model

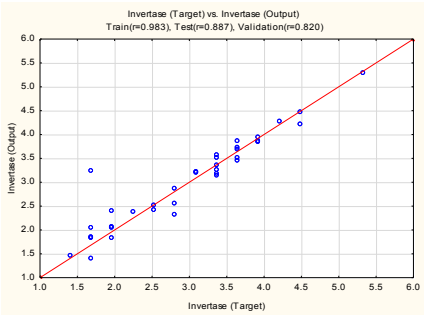

Figure 4(d): Correlation between target and output invertase enzyme using ANN model

significant, implying that ANN can simulate enzyme activities based on soil characteristics.

CONCLUSION

From the present study, it was established that maximum activities of enzymes occur in the winter season. Cluster analysis revealed that enzyme activities were similar during the pre-monsoon and post-monsoon seasons. Factor analysis showed that factor-1 influences the fertility of the soil, whereas factor-2 is responsible for the decomposition of soil organic matter. In SMLR analysis, phosphorus explained maximum variability in urease activity and it to be noted that P is the most important component of human and animal excreta. Nickel is the prosthetic group of urease and contributes maximum to the activity of urease. Similarly Cu is the prosthetic group of polyphenol oxidase enzyme which contributes to the activity of this enzyme.

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