The Journal of Animal & Plant Sciences, 22(2): 2012, Page: 457-462
ISSN: 1018-7081

TEMPORAL CHANGES IN SOIL UREASE, ALKALINE PHOSPHATASE AND DEHYDROGENASE ACTIVITY IN RAINFED WHEAT FIELD OF PAKISTAN

M. Akmal, M. S. Altaf, R. Hayat, F. U. Hassan* and M. Islam**

Department of Soil Science & SWC, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi 46300 Pakistan
*Department of Agronomy, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi 46300 Pakistan
**National Fertilizer Development Centre, Islamabad, Pakistan
Corresponding author E-mail: akmal@uaar.edu.pk

ABSTRACT

Soil enzymes play a vital role in nutrient mineralization and their activity is an excellent sensor in predicting the capacity of nutrient supply to plants. Temporal changes in three soil enzymes activity (urease, alkaline phosphatase and dehydrogenase) in rainfed wheat were studies through field experiment conducted at the research farm of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan. Wheat (*Triticum aestivum* L.) variety Chakwal-50 was grown with N and P combined fertilizer treatments as NP (100:80 kg ha^{-1}) and ½ NP (50:40 kg ha^{-1}), and CK (no fertilizer applied). Twelve experimental plots each of size 6 m × 6 m were prepared to accommodate three treatments each with four replications. The layout of the experimental plots in the field was according to Randomized Complete Block Design (RCBD). Soil samples were taken from each experimental plot at 0-15 cm depth on monthly basis from crop sowing up to the time of harvest and analyzed for soil urease, alkaline phosphatase and dehydrogenase activity, and for nitrate nitrogen and available phosphorus. The data collected were analyzed by two ways ANOVA to quantify the effect of fertilizer treatments and sampling timings on soil enzymes activity and nutrients availability. Results showed that enzymes activity increased from October (sowing time of crop) to November, followed by slight decline in December, thereafter maximum activity was observed during February and March (vigorous crop growth period) which again decreased up to May (crop maturity time). All the three enzymes showed strong positive relation with rainfall but weak relation with atmospheric temperature. Enzymes activity were positively related with P availability but negatively related with nitrate contents in the soil. It was found that the application of N and P fertilizers significantly ($P<0.05$) increased the activity of all three soil enzymes (urease, alkaline phosphatase and dehydrogenase) over control. A 75.5 % and 22.0 % higher alkaline phosphatase and dehydrogenase activity in NP treatment was found compared to that in the control, respectively.

Key words: alkaline phosphatase, dehydrogenase, rainfed soil, urease, wheat.

INTRODUCTION

Enzymes play key roles in the cycling of nutrients in nature and their activity is sensitive to agricultural practices and considered as an index of soil fertility (Nannipieri *et al*., 2002; Yao *et al*., 2006). Soil urease is an extracellular enzyme involved in the hydrolysis of urea-type substrates and its activity is important in the transformation of urea fertilizer. Soil phosphatase hydrolyzes the ester bonds binding P to C (C-O-P) in organic matter and in this process inorganic P is released from organically bound P. Singh and Walker (2006) reported that under phosphorus deficiency conditions, both plants and microorganisms release phosphatase enzymes into the soil which have the potential to mobilize the P reserve. Dehydrogenase enzyme oxidizes soil organic matter by transferring protons and electrons from substrates to acceptors. Soil dehydrogenase is considered as an indicator of overall microbial activity because it occur intracellulary in all living microbial cells and is linked with microbial oxydoreduction processes (Stepniewska and Wolinska, 2005).

The activity of an enzyme depends on its location in the soil (Nannipieri *et al*., 2002). In coarse-textured soils with low organic matter, enzymatic activity depends more on intracellular enzymes. The activity of soil enzymes is also affected by different abiotic factors such as temperature, moisture, soil pH, and oxygen content. Pavel *et al*. (2004) reported that temperature and moisture influence enzyme activities indirectly through microbial growth and substrate availability. Yang *et al*. (2008) studied the activities of soil urease, phosphatase, catalase and invertase at various growth stages of cucumber and observed higher activity during the vigorous growth stage of cucumber while lower activity during the early and late growth stages. However, Gu *et al*. (2009) investigated the temporal fluctuations in the activities of dehydrogenase and urease enzymes at different growth stages of two rice varieties and found higher activities at seedling stage than at tillering stage. It has been shown by Sardans *et al*. (2008) that soil urease activity is higher in winter than in summer.

In rainfed areas of Pakistan, crop cultivation depends on rainfall and moisture availability in the soil. As the changes in rainfall and temperature pattern may affect enzymes activity and ultimately nutrients availability to the crop, so the work regarding the seasonal changes in enzymes activity in rainfed soils of Pakistan is very important which has not been reported so far. This study was planned to record the temporal changes in urease, alkaline phosphatase and dehydrogenase activities in relation to nutrients (N&P) availability in the soil under rain-fed wheat cultivation. The relationship was also developed between soil enzymes activity and seasonal variables.

MATERIALS AND METHODS

Field experiment and soil sampling: The field experiment was conducted at research farm of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi. A composite soil sample representing the whole experimental field was taken before sowing of crop for the analysis of basic soil physical, chemical and biological properties. A sub-sample of this was air dried, ground and passed through 2.0 mm sieve for the analysis of soil texture, pH, EC, total organic carbon (TOC), nitrate nitrogen and available phosphorus, while a fresh sub-sample was passed through a 2.0 mm sieve, mixed thoroughly and prepared for determination of soil enzymes (urease, alkaline phosphatase and dehydrogenase) activity. Twelve experimental plots each of size 6 m × 6 m were prepared to accommodate three treatments with four replications. The basic layout of the experimental plots in the field was according to Randomized Complete Block Design (RCBD) in which replicates were blocks. Wheat (*Triticum aestivum* L.) variety Chakwal 50 was sown on 15th October 2009 with N and P combined fertilizer treatments as NP (100:80 kg ha^{-1}) and ½ NP (50:40 kg ha^{-1}), and CK (no fertilizer applied). Urea and DAP were used as N and P sources and their whole calculated dose were applied at the time of sowing. After sowing of crop, soil samples were taken from each experimental plot at 0-15 cm depth on monthly basis (with 30 days interval) up to crop maturity and analyzed for soil urease, alkaline phosphatase and dehydrogenase activity and also for nitrate nitrogen and available phosphorus. The data collected were analyzed by two ways ANOVA to quantify the effect of fertilizer treatments and sampling timings on soil enzymes activity and nutrients availability.

Soil physico- chemical analysis: Soil texture was analyzed with Boyoucos Hydrometer method and soil textural class was determined by using ISSS triangle. Soil pH and EC were determined by 1:1 (soil: water) suspension procedure by using soil pH meter and EC meter. Total organic carbon (TOC) was determined by acid digestion method, and NO_3-N was determined by salicylic acid method using Spectrophotometer at 410 nm wave length. Available phosphorus (P) was determined by Olsen's method and absorbance was recorded on spectrophotometer at 610 nm (Page *et al*. 1982).

Analysis of soil enzymes activity: For the analysis of soil urease activity, 5 g soil was taken in an Erlenmeyer flask (100 ml) and 2.5 ml urea solution was added. Then stopper the flask and incubated at 37 ^{o}C for 2 hours. After incubation 50 ml of KCl solution was added and shaken the flask for 30 minutes. After filtration, the filtrate was analyzed for ammonium content. The blank was performed as described above but with 2.5 ml distilled water and added the urea solution at the end of the incubation and immediately before KCl addition. For ammonium estimation 1 ml of the clear filtrate was taken into an Erlenmeyer flask (50 ml), then added 9 ml of distilled water, 5 ml of Na salicylate/ NaOH solution and 2 ml of dichloroisocyanide solution and allowed to stand at room temperature for 30 minutes and optical density was measured at 690 nm. The soil alkaline phosphatase activity was measured by taking 1 g soil in Erlenmeyer flask (50 ml) and treated with 0.25 ml of toluene, 4 ml of MUB (Modified Universal Buffer, pH of 11 for alkaline phosphatase) and 1 ml of p-nitrophenyl phosphate (PNP) solution made in the same buffer. After stopping the flask, contents were mixed and incubated for 1 hour at 37 ^{o}C. After incubation, 1 ml of $CaCl_2$ (0.5 M) and 4 ml of NaOH (0.5 M) were added. Mixed the contents and filtered the soil suspension through whatman no. 2v folded filter paper. To perform control, 1 ml of PNP solution was added after the addition of 1 ml $CaCl_2$ (0.5 M) and 4 ml of NaOH (0.5 M) and immediately before filtration of soil suspension and optical density was measured at 400 nm. Dehydrogenase activity in soil was determined by estimating the rate of production of tri-phenyl formazan (TPF) from tri-phenyl tetrazolium chloride (TTC). Briefly 5 g soil was mixed with 5 ml triphenyl tetrazolium chloride (TTC) in test tube. Contents were mixed and incubated for 24 hours at 30 ºC. The control contained only 5 ml Tris buffer (without TTC). After incubation, 40 ml acetone was added to each test tube and the tubes were shaken thoroughly and further incubated at room temperature for 2 hours in the dark. The soil suspension was then filtered and optical density of the clear supernatant was measured at 546 nm**.** Due to the light sensitivity of triphenyltetrazolium chloride (TTC) and triphenyl formazan (TPF), all procedures were performed under diffused light (Alef and Nannipieri, 1995).

Statistical analysis: Two ways ANOVA was performed to evaluate the effect of fertilizer treatments and sampling time on soil enzyme activity and nutrients (N&P) availability (Steel *et al*., 1997). Least significant difference (LSD) at $P<0.05$ was used to determine

whether the means differed significantly. Correlation coefficient was also calculated to determine the correlation of enzymes activity with nutrients availability and seasonal variables. Statistics 8.1 software was used for the statistical analysis of data.

RESULTS AND DISCUSSION

Basic soil properties and weather data: The physico-chemical analysis of soil (Table 1) depicted that soil belonged to sandy loam textural class with pH value of 7.97 and EC 0.41 dS m^{-1}. The total organic carbon in the soil was 0.6 %, while nitrate nitrogen and available P was 5.26 mg kg^{-1} and 4.63 mg kg^{-1}, respectively. The activity of urease, alkaline phosphatase and dehydrogenase enzyme was 628 mg NH_4-N kg^{-1} $2h^{-1}$, 33.2 mg PNP kg^{-1} h^{-1}, and 56.8 mg TPF kg^{-1} 24 h^{-1} , respectively. The weather data showed very little rainfall from October to January; however it increased up to 116 mm in the month of March (Fig. 1), and then again decreased from April to May period. Temperature followed the usual pattern of consistent decrease from October to January and thereafter, it increased steadily and touched maximum of 29 ^oC in May.

Dynamics of soil enzymes activity: The urease activity increased from October (crop sowing time) to November. Then declined slightly from November to December followed by again increase from December to February. The maximum urease activity was found in February which decreased toward March and little changed up to May (at harvest). Throughout crop duration maximum urease activity was observed in NP treatment followed by ½ NP treatment and least in the control (Fig. 2). Over all, NP and ½ NP treatments showed 3.0 % and 1.9 % higher urease activity over control. Soil alkaline phosphatase activity in rainfed wheat field (Fig. 3) showed significant variation among treatments throughout the crop duration. The enzyme activity increased from October to November in the fertilized plots, followed by decline in December sampling. Alkaline phosphatase activity again increased from December with maximum in March in all treatments. However, there was slight decline again from March to April and then remain almost similar up to May (the last sampling time) in fertilizer treatments. Fertilizer treatments showed 75.7 % and 31.6 % higher phosphatase activity over control. Temporal changes in soil dehydrogenase activity (Fig. 4) indicated increase in the activity from October to November followed by little decrease from November to December, thereafter it again increased from December to March and achieved the maximum value. From March to April there was little decrease in dehydrogenase activity in ½ NP and control treatments which remained unchanged up to May.

The enzymes activity showed variations among the three treatments from start up to the end of crop duration, and fertilizer treatments gave higher values of enzyme activity then control. It appeared that alkaline phosphatase activity increased by 75.7 % and 31.6 % in NP and ½ NP treatments over control. Ebhin Masto *et al*. (2006) found significant seasonal effects on soil phosphatase activity and reported sharp increase in alkaline phosphatase due to wheat cultivation compared to fallow field which indicates the strong rhizospheric effects on phosphatase activity. Yang *et al*. (2008) determined the activities of soil urease, phosphatase, catalase and invertase at various growth stages of cucumber and found higher activity during the vigorous growth stage of cucumber while lower activity during the early and late growth stages. Wen-Hui *et al*. (2007) in a long term field experiment applied different rates of inorganic fertilizers to the rice crop and found significant higher enzymes (urease, acid phosphatase, dehydrogenase and invertase) activities by the application of mineral fertilizers. On average they recorded 433 %, 120 % and 83 % increase in dehydrogenase, acid phosphatase and urease activity when the combined dose of NPK was applied. They further observed that urease activity was mainly influenced by application of N, whereas, acid phosphatase activity was mainly stimulated by application of P, demonstrating that these enzymes were mainly influenced by single nutrient elements in the experiment, while the dehydrogenase activity was influenced by both P and N applications. Gu *et al*. (2009) investigated the temporal fluctuations in the activities of dehydrogenase and urease enzymes at different growth stages of two rice varieties. According to them dehydrogenase and urease activities at seedling stage were higher than at tillering stage, however, at stem elongation and heading stage the activity was highest but decreased at maturity stage. In the present study we found significant effects of fertilizer (NP) on enzymes activity. Alkaline phosphatase activity increased by 75.7 % and 31.6 % in NP and ½ NP treatments compared to that in the control. Also dehydrogenase activity in the NP and ½ NP increased by 22.0 % and 9.2 % over that in the control. However, there was only 3.0 % and 1.9 % increase in urease activity in NP and ½ NP treatments compared to that in control. Juan *et al*. (2008) found lowest urease activity in the untreated soils which increased by 62.5 % with the application of mineral fertilizers.

Dynamics of nutrients (N & P) availability: The results of nitrate nitrogen at different intervals in wheat field treated with various levels of chemical fertilizers are shown in Fig. 5. The values of nitrate increased from October to November in all treatments, however, decreased with different rates from November up to March in various treatments. From, March to April, again little increased and then decreased from April to May. Chemical fertilizer treatments significantly increase the

values of nitrate. NP and ½ NP treatments indicated 45.5 % and 19.5 % higher value of nitrate over the control. There was a significant increase in available P contents from October to November followed by slight decrease at December sampling and then maximum value in January (Fig. 6). From January to April decrease in available P was observed and then slight change up to May. Fertilizer treatment showed significantly higher value of available P over control. There was 37.4 % and 26.2 % higher P contents in NP and ½ NP treatments compared to that in the control.

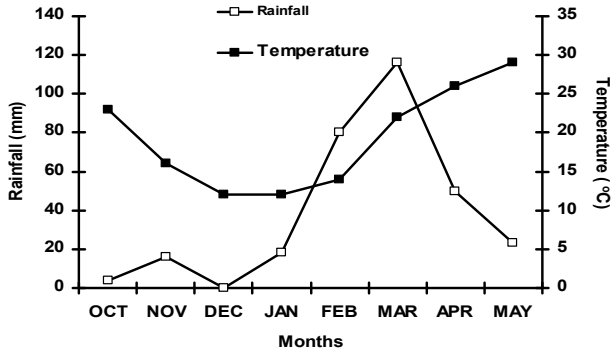

Fig. 1. Changes in rainfall and temperature for the experimental area during crop period.

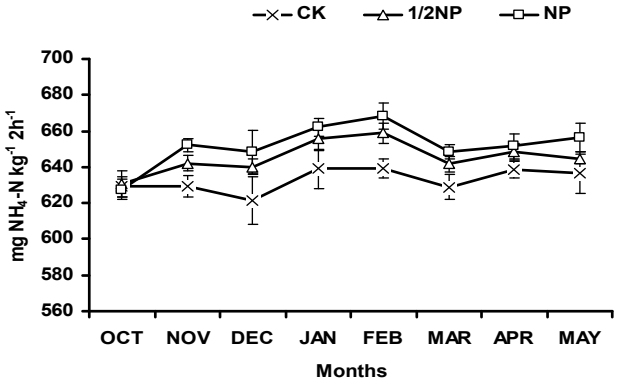

Fig. 2. Temporal changes in soil urease activity (mg NH_4-N kg^{-1} $2h^{-1}$) in rainfed wheat field.

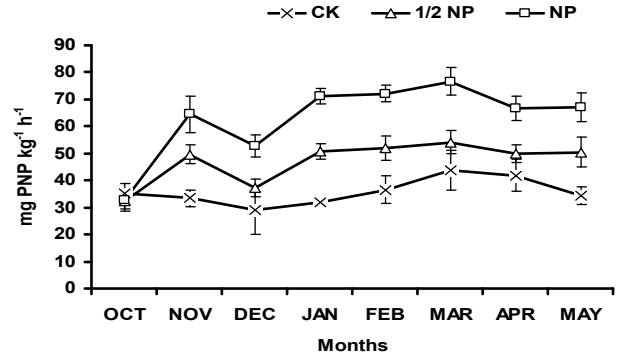

Fig. 3. Temporal changes in soil alkaline phosphatase activity (mg PNP kg^{-1} h^{-1}) in rainfed wheat field.

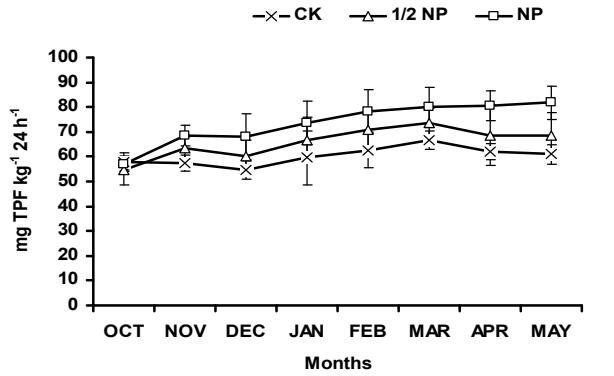

Fig. 4. Temporal changes in soil dehydrogenase activity (mg TPF kg^{-1} $24h^{-1}$) in rainfed wheat field.

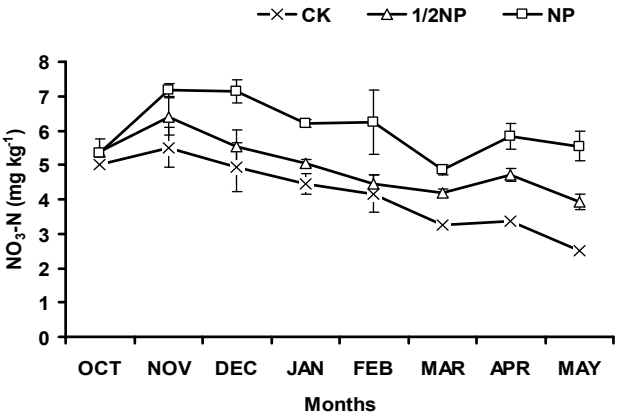

Fig. 5. Dynamics of nitrate nitrogen in rainfed wheat field.

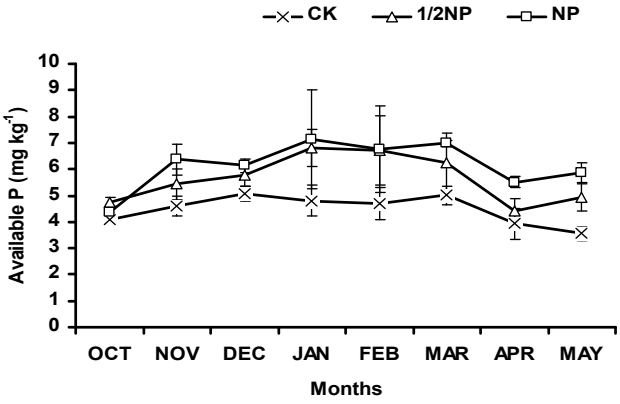

Fig. 6. Dynamics of available phosphorus in rainfed wheat field.

Correlation analysis: Correlation results of soil enzymes activity (urease, alkaline phosphatase and dehydrogenase) with nutrients (N and P) availability and climatic variables (rainfall and temperature) are given in Table 2. The results showed that all the three soil enzymes activity have negative relation with nitrate contents but positive relation with P availability in the soil. However, this positive or negative relation was not very strong, except

for dehydrogenase which showed strong negative relation (-0.713) with nitrate values in the soil. A positive relationship of enzymes activity with rainfall was observed, however, atmospheric temperature showed poor negative correlation with urease activity and positive correlation with dehydrogenase activity in the soil. In present study all three enzymes showed positive correlation with rainfall. Increased moisture due to rainfall enhanced the activity of soil enzymes, specially phosphatase and dehydrogenase in this study. Similar have been reported by Sardans and Penuclas (2005), they observed strong positive correlation between activity of both acidic and alkaline phosphatase with soil water availability. Frey *et al*. (1999) reported that temperature and moisture influenced enzyme activities indirectly through increasing microbial growth and substrate availability. In present study a poor negative relationship of urease with atmospheric temperature, while a positive relation of dehydrogenase with temperature was observed, however, the activity of alkaline phosphatase was not well related with temperature. Sardans *et al.* (2008) observed the seasonal changes in soil urease activity and found higher urease activity in winter when the soil temperatures were low than in summer. Tripathi *et al.* (2007) found higher soil dehydrogenase activity in the warm season than in the cool season, but the hydrolytic enzyme activities of soil were similar in summer and winter season. In our study, highest activities of soil enzymes was observed during February and March sampling. In these two months there was higher rainfall and also vigorous crop growth, which increased enzymes activity up to their maximum values, however, enzymes activity decreased toward maturity.

Table 1. Basic properties of soil used in the study.

Soil Properties	Units	Values
Textural class		Sandy loam
pH		7.97
EC	dS m^{-1}	0.41
Total organic carbon	%	0.6
Nitrate Nitrogen	mg kg^{-1}	5.26
Available P	mg kg^{-1}	4.63
Urease activity	mg NH_4-N kg^{-1} $2h^{-1}$	628
Alkaline phosphatase activity	mg PNP kg^{-1} h^{-1}	33.2
Dehydrogenase activity	mg TPF kg^{-1} 24 h^{-1}	56.8

Table 2. Relationship of soil enzymes activity with nutrients availability and climatic variables.

	Nitrate Nitrogen (mg kg^{-1})	Available Phosphorus (mg kg^{-1})	Rainfall (mm)	Atmospheric Temperature (^oC)
Urease (mg NH_4-N kg^{-1} $2h^{-1}$)	-0.237	0.431	0.355	-0.240
Alkaline Phosphatase (mg PNP kg^{-1} h^{-1})	-0.496	0.399	0.762	0.096
Dehydrogenase (mg TPF kg^{-1} 24 h^{-1})	-0.713	0.258	0.793	0.276

Conclusion: Rainfall or soil moisture effect soil enzymes activity more than the atmospheric temperature in the rainfed region. Enzymes activity is higher at the middle crop growth than early and later stages. The urease, alkaline phosphatase and dehydrogenase activity in the rainfed soil can be increased by the application of nitrogenous and phosphatic chemical fertilizers. The information generated in this study may be utilized for maintaining soil health and crop management in the rainfed region.

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