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Assessment of Dehydrogenase and Phosphatase Activities under Cotton-Wheat and Rice-Wheat Cropping Systems in Sangat Block of Bathinda District, Punjab

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Soils and their functions are critical for ensuring the provision of various ecosystem services. Soil enzymes activities were considered as an essential component of soil health, which can change due to anthropogenic activities, cropping practices and intensive land-use management systems. This study was conducted to estimate the changes in soil enzymatic activity under cotton-wheat and rice-wheat cropping systems in south-western Punjab, India. The soils under cotton-wheat system had higher dehydrogenase activity (DHA) of 49.17 µg TPF g^{-1} h^{-1} at 0-15 cm depth and 45.56 µg TPF g^{-1} h^{-1} at 15-30 cm depth compared with rice-wheat cropping system (47.81 µg TPF g^{-1} h^{-1} at 0-15 cm depth and 43.95 µg TPF g^{-1} h^{-1} at 15-30 cm depth). However, acid phosphatase (101.1-93.1 µg PNP g^{-1} soil h^{-1}) and alkaline phosphatase (118.4-107.2 µg PNP g^{-1} soil h^{-1}) activity was higher under rice-wheat cropping system than cotton-wheat cropping system (114.3-102.8 and 83.8-78.4 µg PNP g^{-1} soil h^{-1}, respectively). Therefore, soil enzyme activities can be considered indicators of changes in restoration-induced soil quality and sensitivity to environmental factors and restoration practices.

Key words: Dehydrogenase, phosphatases, cotton-wheat, rice-wheat, arid soils

Soil enzymes play a vital role in the catalysis of those reactions that are necessary for organic matter decomposition and cycling of nutrients. Moreover, enzymes are also involved in energy transfer, crop productivity and environmental quality (Tabatabai 1994). Soil enzymes are of microbial and plant origin and their activities show the activity of intracellular and extracellular enzymes and bound enzymes to clay and organic matters. They may correlate well with nutrient availability (Asmar *et al*. 1994). These activities are important to determine soil quality under different usages, anthropogenic and non-anthropogenic destruction and different types of habitats (Grierson and Adams 2000; Sinsabaugh *et al*. 2002). Microbiological and biochemical status of soil is considered as a sensitive indicator of soil ecological stress (Ruf *et al*. 2003). Soil enzymes have been suggested as potential indicators of soil quality among microbiological and biochemical factors due to biological nature and rapid response to changes in soil management when compared to other biological properties (Asmar 1994). Soil enzymes can also be used as potential indicators of nutrient cycling processes and fertility management (Fliebach *et al*. 2007). Among the soil enzymes, dehydrogenase activity (DHA) has been recognized as an important indicator of the oxidative metabolism in soils and thus of the metabolic activity (Watts *et al.* 2010), because being exclusively intracellular, it is linked to viable cells. Dehydrogenase activity is one of the most adequate, important and one of the most sensitive bioindicator of soil fertility (Wolinska and Stepniewska 2012). Its activity depends on the same factors, which influence soil microorganism's abundance and activity (Wolinska and Stepniewska 2012). Soil phosphomonoesterase (acid and alkaline phosphatase) activities play an important role in catalyzing the hydrolysis of P-ester bonds binding phosphorus (P) to carbon (C) in organic matter, thereby releasing inorganic P which are assimilable by plants (Pascual *et al*. 2002). Soil microorganisms participate in the processes that are crucial for long-term sustainability of agricultural systems (Nannipieri *et al*. 2003).

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Cropping systems that return high levels of green manure/crop residues significantly increase the activities of several enzymes (Bolton *et al.* 1985). Angers *et al.* (1997) reported greater levels of alkaline phosphatase activity in rotations including barley-red clover compared to monoculture systems, and that soils under no-till with a corn-oats-alfalfa rotation contained the largest alkaline phosphatase activity. Crop rotations under different cropping systems can provide higher input and diversity of organic materials to the soil and generally contain higher enzyme activities than under monoculture. The enzyme activities are sensitive to the positive effects of crop rotations compared to monoculture (Klose *et al*. 1999). Crop rotations change the soil habitat due to their difference in extract nutrients, depth of roots, amount of residue, which remain in soil and difference in their components (Balota *et al.* 2004)**.** Similarly**,** different crops in cropping system can produce various residues and root exudates to boost soil microbial diversity and activity, and increase soil microbial activities (Li *et al.* 2019). The cropping intensity of Bathinda district was significantly increased mainly due to increased in rice area (Panigrahy *et al.* 2004). However, in Sangat block of Bathinda district the coverage of cotton-wheat cropping system is higher as compared to other blocks along with rice-wheat cropping system due to medium to unfit underground irrigation water quality and undulated topography of the block. The objectives of this research were to determine the effects of cropping systems on soil microbial community and soil enzyme activities under different cropping system adopted in the region. Therefore, the present study was undertaken to assess the dehydrogenase and phosphatase activities under both cropping system in Sangat Block of Bathinda District, Punjab.

Material and Methods

Study area and its climate

Bathinda district of Punjab is lying between 29°30′ and 32°32′ N latitude, 73°55′ and 76°55′ E longitudes. The climate of the area is characterized by a large seasonal variation as well as fluctuations both in monthly rainfall and temperature. The district falls in the semi-arid region of Punjab having annual average rainfall of < 500 mm. The soil sampling sites (villages) of Sangat block (30°211′ N latitude and 74°945′ E longitude) is situated in Bathinda district. The soils of the block were non-saline, loamy sand to sandy clay loam with alkaline in reaction and low in soil organic carbon (Yadav 2020). The soil samples under cotton-wheat and rice-wheat cropping systems were collected from the sites, wherein these cropping systems were practices for a long period, and most of the farmers were used recommended doses of fertilizers as per Punjab Agricultural University for each crops. Soil samples were collected from surface (0-15 cm) and sub-surface (15-30 cm) layers under cotton-wheat and rice-wheat fields from different villages (Table 1) of Sangat block in Bathinda district, Punjab. Altogether 100 samples were collected from each system from two depths collectively leading to 400 samples.

Processing and soil analysis

The study was conducted in soil and water testing laboratory, PAU, Regional Research Station, Bathinda, Punjab. All the soil samples collected were stored in refrigerator and were sieved through 2-mm sieve for biological analysis. Dehydrogenase activity in soil was determined, using the reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC) (3%) method (Klein *et al*. 1971). Soil sample (1.0 g) was placed in a screw cap test tube and 0.5 mL of 1% glucose was

Table 1. Soil sampling sites under cotton-wheat and rice-wheat cropping systems

Cotton-wheat cropping system		Rice- wheat cropping system	
1. Guru Saniwala (5)	10. Sangat (6)	1. Gehri Buttar (5)	10. Phallar (6)
2. Mehta (5)	11. Koth Guru (7)	2. Sangat (5)	11. Ruldhu Singh Wala (9)
3. Shergarh (5)	12. Mohlan (10)	3. Koth Guru (9)	12. Jai Singh Wala (5)
4. Bhagwangarh (5)	13. Jassi Baghi (3)	4. Mohlan (5)	13. Ghudda (5)
5. Malwala (5)	14. Paka Kala (5)	5. Jassi Bagwali (7)	14. Bajak (5)
6. Dunewala (5)	15. Paka Khurd (8)	6. Machana (6)	15. Nanadgarh (6)
7. Machhana (5)	16. Sakhu (5)	7. Paka Kala (6)	16. Chak Attatrsingh Wala (4)
8. Gurthari (5)	17. Phallar (6)	8. Paka Khurd (7)	17. KalJharani (5)
9. Gehri Buttar (5)	18. Ruldhu Singh Wala (5)	9. Sakhu (5)	

*Figures in parentheses denotes the number of samples collected from each village

added with addition of 0.2 mL 3% TTC along with one tube without soil as a control. The tubes were incubated for 24 h at 37 °C. After incubation, 10 mL methanol was added to each tube, shaken, incubated for 2 h in refrigerator and the colour intensity was recorded at 485 nm using a spectrophotometer. The DHA was expressed as µg triphenyl formazane (TPF) produced g^{-1} dry soil h^{-1} at 37 °C. Acid and alkaline phosphatase activity in soil was determined by Tabatabai and Bremner (1969). Soil sample (1.0 g) was taken in 15 mL capacity screw cap test tube and 0.2 mL of toluene was added, then 4 mL of p-nitrophenyl phosphate solution was prepared in acetate (pH 5.4 for assay acid phosphatase) and borax-NaOH buffer (pH 9.2 for assay of alkaline phosphatase) was added and the tube was swirled for few seconds to mix the contents. The test tube was covered with screw cap and then placed in incubator at 35 °C. After 1 h of incubation, caps were removed and 0.5 *M* $CaCl_2$ and 4 mL of 0.5 *M* NaOH was added, and then the soil suspension was filtered through Whatman no. 42 filter paper. The yellow colour intensity of the filtrate was measured by a spectrophotometer at 420 nm wavelength. Acid and alkaline phosphatase activities were expressed as µg ρ-nitro phenol produced g^{-1} dry soil h^{-1}. The data generated was analysed for each soil property for each cropping system using Microsoft-Excel.

Results and Discussion

Dehydrogenase activity

The data on DHA under cotton-wheat cropping system represented in fig. 1 revealed that DHA activity ranged from 32.5 to 67.4 µg TPF g^{-1} h^{-1} at 0-15 cm depth and 29.8-58.6 µg TPF g^{-1} h^{-1} at 15-30 cm thus resulting into higher mean DHA value at surface layer (49.1 µg TPF g^{-1} h^{-1}) than sub-surface layer (45.5 µg TPF g^{-1} h^{-1}). Higher DHA activity was reported in Gurusar Saniwala (53.2-67.4 µg TPF g^{-1} h^{-1}) and (47.4-58.6 µg TPF g^{-1} h^{-1}) and lowest DHA activity was observed in Koth Guru (32.5-50.1 µg TPF g^{-1} h^{-1}) and (29.8-48.9 µg TPF g^{-1} h^{-1}) at 0-15 cm and 15-30 cm depth, respectively.

The data on DHA under rice-wheat cropping system (Fig. 2) ranged from 30.4 to 65.4 µg TPF g^{-1} h^{-1} at 0-15 cm depth and 29.5 to 56.4 µg TPF g^{-1} h^{-1} at 15-30 cm depth thus resulting into higher mean DHA value at surface soil (47.8 µg TPF g^{-1} h^{-1}) than sub-surface soil (43.9 µg TPF g^{-1} h^{-1}). Higher DHA activity was reported in Gehri Buttar (43.5-65.4 µg TPF g^{-1} h^{-1}) and (41.3-56.4 µg TPF g^{-1} h^{-1}) and lowest DHA activity was observed in Mohlan (35.2-39.2 µg TPF g^{-1} h^{-1}) and (33.4-35.2 µg TPF g^{-1} h^{-1}) at 0-15 cm and 15-30 cm depth, respectively.

Higher DHA activity was observed in surface soil than sub-surface soil in both the systems which may be attributed to the greater availability of organic carbon, nutrients and stimulated microbial activity in the surface soil. The DHA in soil depends on the content of soluble organic carbon (Zaman *et al.* 2002) and the increased organic matter in the surface soil horizon enhanced the soil enzyme activities. Furczak and Joniec (2007) showed that stimulation of DHA was accompanied by an increase in the number of the microbial groups and improvement in other living conditions (aeration and moisture). Significant correlations between soil organic matter and enzyme

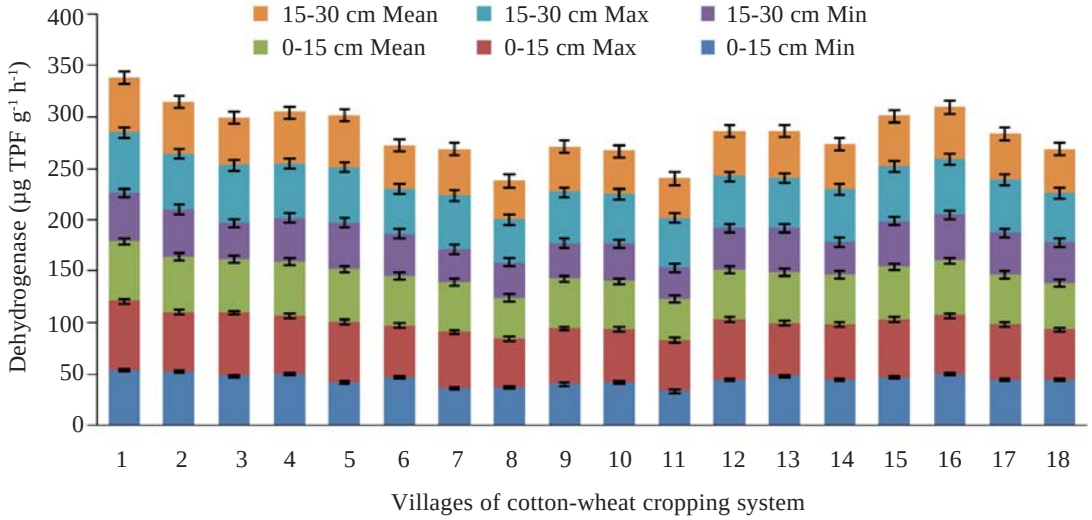

Fig. 1. Dehydrogenase activity under cotton-wheat cropping system. Bars represent the standard error of mean

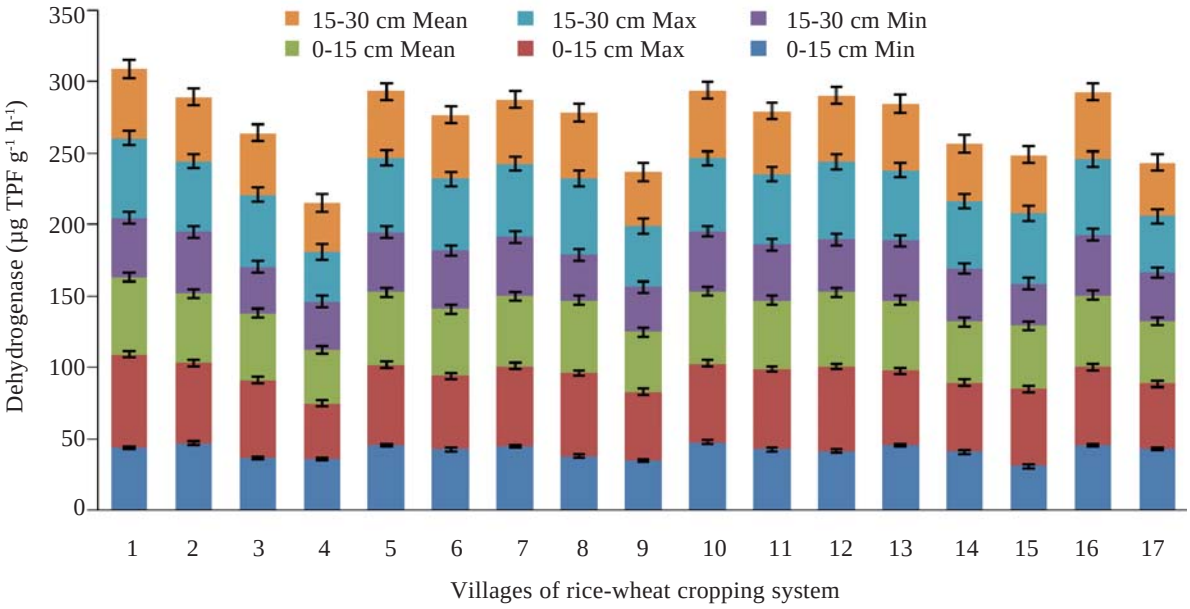

Fig. 2. Dehydrogenase activity under rice-wheat cropping system. Bars represent the standard error of mean

activities was also observed by Roldan *et al.* (2005). Similarly, the plant rhizosphere release large quantity of metabolites from living root hairs or fibrous root systems may create a niche that influences microbial activity in surface soils and increased DHA in upper surface as compared to subsurface soil layers. Many researchers assessed the diversity of microbial populations in the rhizosphere of different plant species, including clover and ryegrass (Stamenov *et al.* 2012) and maize (Hajnal-Jafari 2010). Jat *et al.* (2020) observed that in sub-surface soil, DHA activity was lower in all stages of crop growth irrespective of scenarios, because microbial activity is decreased with depth.

Acid and Alkaline phosphatase activity

The data on acid and alkaline phosphatase under cotton-wheat cropping system represented in Fig. 3 revealed that acid phosphatase activity ranged from 65.7 to 97.4 µg PNP g^{-1} soil h^{-1} at 0-15 cm depth and 56.4 to 90.0 µg PNP g^{-1} soil h^{-1} at 15-30 cm depth thus resulting into higher mean acid phosphatase value at surface layer (83.7 µg PNP g^{-1} soil h^{-1}) than sub-surface layer (78.4 µg PNP g^{-1} soil h^{-1}). Higher mean value for acid phosphatase was reported in Jassi Baghi (87.4 µg PNP g^{-1} soil h^{-1}) and village Sangat (82.6 µg PNP g^{-1} soil h^{-1}) at 0-15 cm and 15-30 cm depth, respectively. Similarly, alkaline phosphatase activity ranged from 98.4 to 143.5 µg PNP g^{-1} soil h^{-1} at 0-15 cm depth and 82.4 to 132.2 µg PNP g^{-1} soil h^{-1} at 15-30 cm depth indicating higher mean activity of alkaline phosphatase at surface layer (114.2 µg PNP g^{-1} soil h^{-1}) than sub-surface layer (102.7 µg PNP g^{-1} soil h^{-1}). Higher mean value for alkaline phosphatase was reported in Paka Kala with 123.4 µg PNP g^{-1} soil h^{-1} and 113.0 µg PNP g^{-1} soil h^{-1} at 0-15 cm and 15-30 cm depth, respectively.

The data on acid phosphatase and alkaline phosphatase under rice-wheat cropping system (Fig. 4) ranged from 65.5 to 143.5 µg PNP g^{-1} soil h^{-1} at 0-15 cm depth and 56.7 to 132.4 µg PNP g^{-1} soil h^{-1} at 15-30 cm depth with higher mean acid phosphatase activity at surface layer (101.1 µg PNP g^{-1} soil h^{-1}) than sub-surface layer (93.1 µg PNP g^{-1} soil h^{-1}). Among the villages, higher mean value for acid phosphatase was reported in Jai Singh Wala (122.6 µg PNP g^{-1} soil h^{-1}) and in Nanadgarh (110.1 µg PNP g^{-1} soil h^{-1}) at 0-15 cm and 15-30 cm depth respectively. Similarly alkaline phosphatase activity ranged from 102.4 to 166.4 µg PNP g^{-1} soil h^{-1} at 0-15 cm depth and 89.5 to 143.6 µg PNP g^{-1} soil h^{-1} at 15-30 cm depth with higher mean alkaline phosphatase activity at surface soil (118.4 µg PNP g^{-1} soil h^{-1}) than sub-surface soil (107.2 µg PNP g^{-1} soil h^{-1}). Higher mean value for alkaline phosphatase was reported in Bajak (138.3 µg PNP g^{-1} soil h^{-1}) and in Nanadgarh (125.0 µg PNP g^{-1} soil h^{-1}) at 0-15 and 15-30 cm depth, respectively.

Like DHA, the phosphatase activity was also higher in surface soil than sub-surface soil. The higher phosphatase activity in the surface soils could be attributed to the greater demand for P from soil by

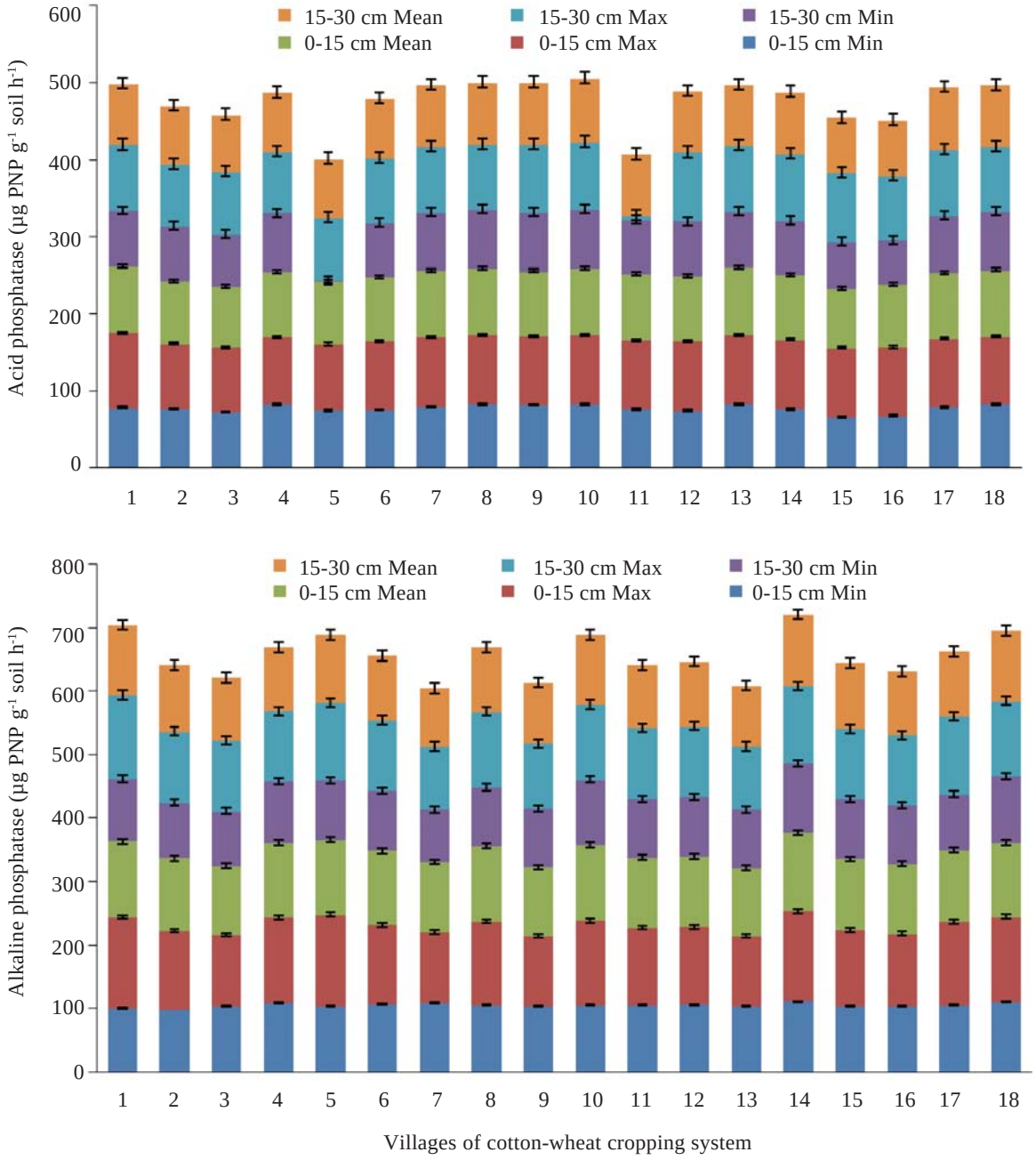

Fig. 3. Acid and alkaline phosphatase under cotton-wheat cropping system. Bars represent the standard error of mean

the crops for its growth and symbiotic functioning (Mitran *et al.* 2018). These enzymes (acid and alkaline phosphatases) are housed in the roots of plants and soil microbes (Dakora and Phillips 2002). Acid phosphatase enzymes are located in root exudates and in some instances in the rhizospheric soil of plants roots (Duff *et al.* 1994). On the other hand, alkaline phosphatases are formed mainly by soil microorganisms (Tabatabai 1994). Deng and Tabatabai (1997) observed that phosphatases activities decreased with increasing soil depth. They thought this decrease may be associated with the decrease in organic carbon content. Most enzyme activities in the surface soil were higher than deep soil. This may be because there were more soil microorganism and plant residues in the surface soil, which were the main parts of soil enzymes. The alkaline phosphatase activity was higher in soils of all these villages than acid phosphatase activity under both cropping system, because of alkaline pH in these soils. The alkaline

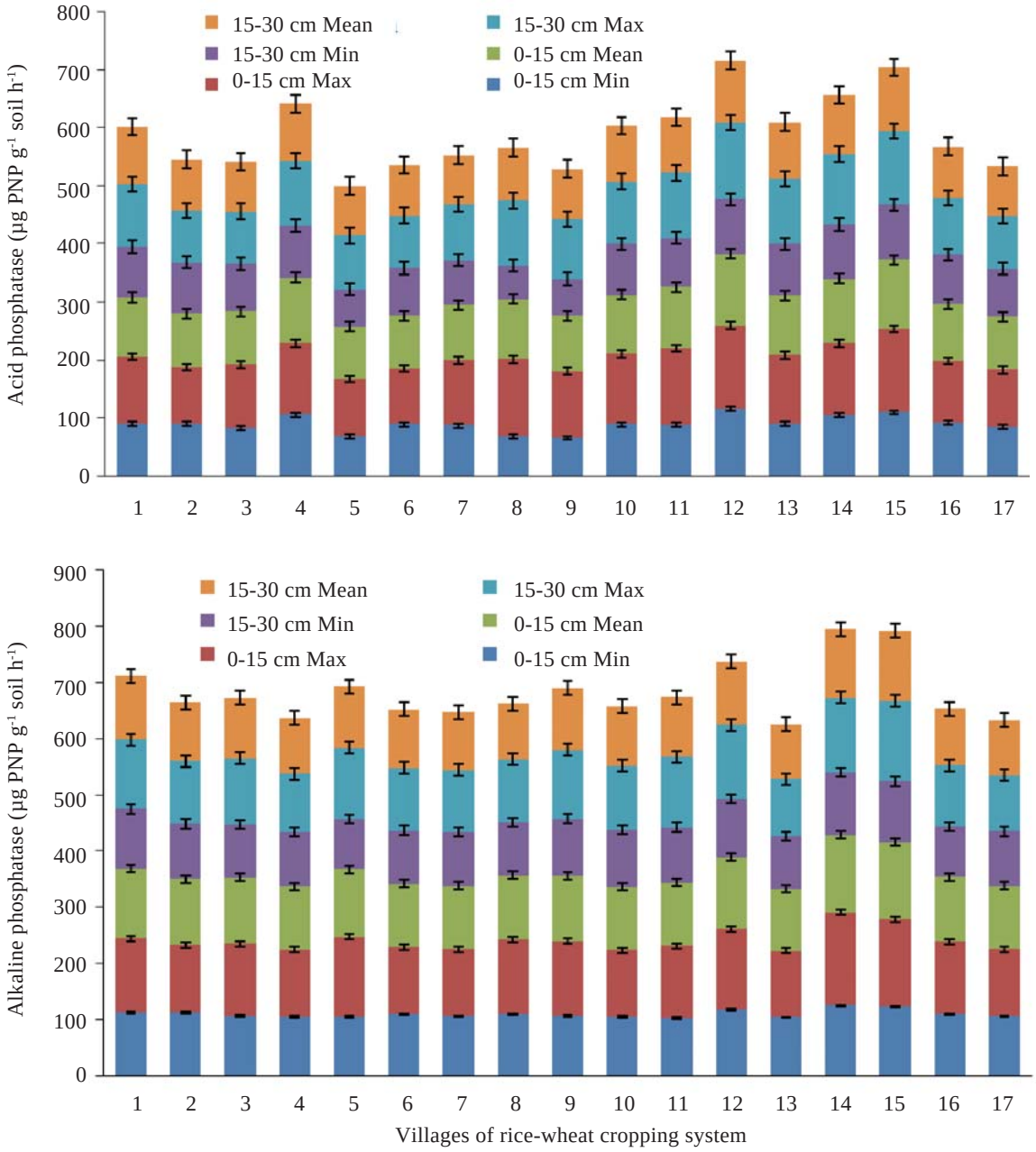

Fig. 4. Acid and alkaline phosphatase under rice-wheat cropping system. Bars represent the standard error of mean

phosphatases are secreted by microorganisms such as bacteria, fungi and earthworms (Hebrien and Neal 1990) and function catalytically above pÇ 7. In our study, the soil pH ranged from 8.0 to 9.5 and 8.0 to 9.3 in surface and 8.1 to 9.7 and 8.1 to 9.4 in sub-surface under cotton-wheat and rice-wheat cropping system, respectively. Dick *et al.* (2000) assured that phosphatases have been often closely correlated with soil pH. The pH of the soil solution exerts a strong control of the soil enzyme activities (Chhonkar *et al.* 2007).

Comparison of soil biological properties among cotton-wheat and rice-wheat cropping sequences

Table 2 reveals that dehydrogenase activity was maximum under cotton-wheat cropping system at 0-15 cm depth (49.17 µg TPF g^{-1} day^{-1}) and at 15-30 cm depth (45.56 µg TPF g^{-1} day^{-1}) than rice-wheat cropping system (47.81 µg TPF g^{-1} day^{-1} at 0-15 cm depth and 43.95 µg TPF g^{-1} day^{-1} at 15-30 cm depth). This may be due to proper moisture and soil aeration suitable for active microbial population under cotton-wheat cropping system as compared to rice-wheat

Table 2. Comparison of soil biological properties among cotton-wheat and rice-wheat cropping sequences

Soil parameters	Cotton-wheat cropping system				Rice-wheat cropping system			
	0-15 cm		15-30 cm		0-15 cm		15-30 cm	
	Range	Mean ± SEM	Range	Mean± SEM	Range	Mean± SEM	Range	Mean ± SEM
DHA	32.5-67.4	49.2±0.6	29.8-58.6	45.6±0.61	30.4-65.4	47.81±0.6	29.5-56.4	43.9±0.6
Acid phosphatase	65.7-97.4	83.8±0.6	56.4-90.0	78.4±0.65	65.5-143.5	101.14±0.5	56.7-132.4	93.1±0.9
Alkaline phosphatase	98.4-143.5	114.3±0.9	82.4-132.2	102.8±.9	102.4-166.4	118.46±1.1	89.5-143.6	107.2±0.7

cropping system. Brzeziñska *et al.* (1998) indicated that the major factor determining soil DHA was its aeration status. Furthermore, Furczak and Joniec (2007) showed that stimulation of DHA was accompanied by an increase in the number of the microbial groups and improvement in other living conditions like aeration and moisture. Alkaline phosphatase activity was maximum under rice-wheat cropping system (118.4 µg PNP g^{-1} soil h^{-1} at 0-15 cm depth and 107.2 µg PNP g^{-1} soil h^{-1} at 15-30 cm depth) than cotton-wheat cropping system (114.2 µg PNP g^{-1} soil h^{-1} at 0-15 cm depth and 102.7 µg PNP g^{-1} soil h^{-1} at 15-30 cm depth). Acid phosphatase activity was also maximum under rice-wheat cropping system (101.4 µg PNP g^{-1} soil h^{-1}) at 0-15 cm depth and (93.1 µg PNP g^{-1} soil h^{-1}) at 15-30 cm depth than cotton-wheat cropping system (83.7 µg PNP g^{-1} soil h^{-1} at 0-15 cm depth and 78.4 µg PNP g^{-1} soil h^{-1} at 15-30 cm depth). Acid phosphatase activity was higher under rice-wheat cropping system compared to cotton-wheat cropping system which may be due to low pH of soil under rice-wheat cropping system compared to cotton-wheat cropping system. Soil pH is an important factor that drives prokaryotic microbial community composition (Lauber *et al.* 2009). The application of mineral fertilizer significantly decreases the soil pH (Luo *et al.* 2015). The continuous application of mineral fertilizers decreases soil pH, due to the release of hydrogen ions by oxidation of ammonium, which is commonly used as a nitrogenous fertilizer in rice cultivation (Kumar *et al.* 2017). Similarly, in rice field soils are highly irrigated and remain submerged for a long period, therefore anaerobic microbial population may be higher compared to cotton field soil. Although, decomposition of soil organic matter is decreased under anaerobic conditions simulated during rice (Kögel-Knabner *et al.* 2010), however alternate wet and dry spells characterized by rice-wheat rotation stimulate microbial activity and increased phosphatse activity in soil (Denef *et al.* 2001) by microbes as well as by fibrous root system of rice.

Conclusions

Our results suggest that the different soil enzymatic activity and their connections provide different biological activity and appear to be highly sensitive under contrasting cropping systems in a south-western Punjab. Soil dehydrogenase activity was 3.4 per cent higher in cotton-wheat than rice-wheat cropping system. Acid and alkaline phosphatse activity was 19.8 per cent and 4.8 per cent higher in rice-wheat than cotton-wheat cropping system. The biological activities were observed to be higher in surface than sub–surface soil layer. Understandings such changes to variation in resource utilization patterns in soil food webs, under diverse cropping systems contribute to variation in biological activity and nutrient availability.

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