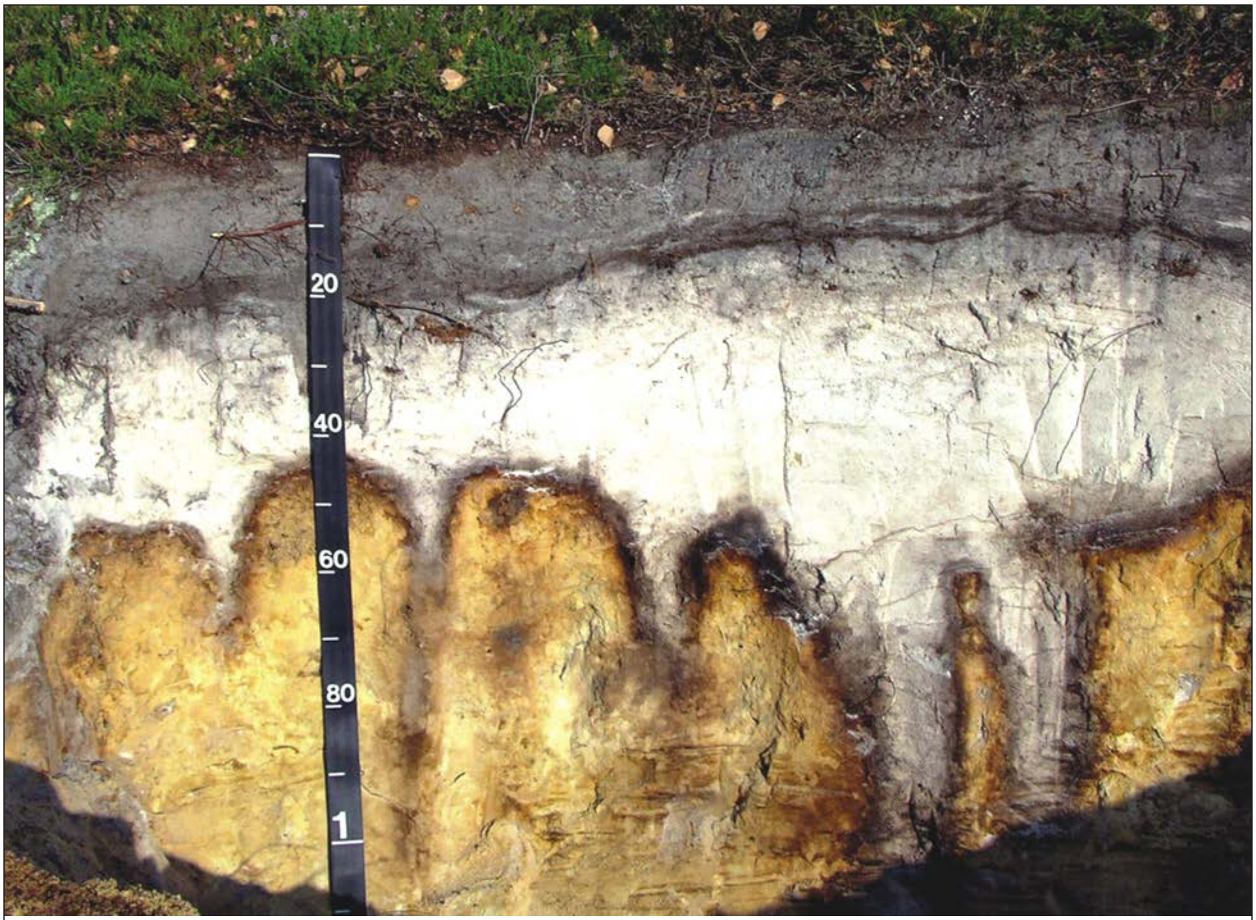

International Journal of
Soil Science

ISSN 1816-4978

Academic
Journals Inc.

www.academicjournals.com

International Journal of Soil Science 6 (4): 238-248, 2011
ISSN 1816-4978 / DOI: 10.3923/ijss.2011.238.248

Enzymatic Activities and Microbial Population in Agric-soils of Almora District of Central Himalaya as Influenced by Altitudes

[1]B.R. Maurya, [1]Vimal Singh and [2]P.P. Dhyani
[1]Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, India
[2]GB Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora, India

Corresponding Author: Vimal Singh, Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, India

ABSTRACT

Abundance of various kind of microorganisms and their activity in soil is a presumptive of fertility status of that soil. Hence, the present study was designed to assess the impact of altitudes on activity of two important soil health bioindicators in agricultural soils of Almora district of Central Himalaya. Seventy two soil samples were collected from agricultural lands at different altitudes of Almora, Uttarakhand, India and were assayed for their electro-chemical properties microbial colony forming unit (cfu) and microbial activities such as dehydrogenase and phosphatase following the standard procedures. It was recorded that soil was acidic in range with nitrogen, phosphorous and potassium in middle to high range. Also their content in soil increased at high altitude as compared to low altitude. Dehydrogenase and phosphatase activities varied with altitudes and these were high at higher altitudes. Dehydrogenase possessed a significant and positive correlation with nitrogen ($r = 0.83$) and phosphorous ($r = 0.64$) while phosphatase showed significant and positive correlation with organic carbon ($r = 0.66$). It was concluded that activity of dehydrogenase and phosphatase in soil would be an effective tool to assess the soil health.

Key words: Dehydrogenase, phosphatase, microbial colony forming unit, altitude, microbial activity

INTRODUCTION

Agriculture is the main activity of the central himalaya that depends on the natural forest vegetation cover for its sustainability. Various kinds of microbes which inhabit in the soils in the area are responsible for pedogenic processes of soil development by influencing the organic matter decomposition and mobilisation of nutrients. They play an important role in the cycling of nutrients in nature and creating a complimentary medium for biological reactions (Heritage *et al*., 1999; Oke and Ologun, 2005). Environmental conditions strongly influence soil ecosystem, changing its physical and physicochemical status and as a consequence, regulating the size of microbial world and their activities (Aira *et al*., 2007). The determination of properties of soil which are sensitive to changes caused by agricultural practices may help to monitor the changes in its sustainability (Mirani *et al*., 2002; Abdalla and Langer, 2009). This is especially true for the agricultural management and recovery of soil and to assist into the establishment of policies for the use of land.

Due to the close relation of the soil enzyme activities with the nutrient cycle and rapid response to both natural and anthropogenic factors, they have been suggested as suitable indicators of soil quality (Drijber *et al*., 2000; Calderon *et al*., 2000; Colombo *et al*., 2002; Nannipieri *et al*., 2002;

Winding *et al.*, 2005). Enzymatic activities of soil are also often used as an index of microbial activity in the soils as well as their fertility (Garcia and Hernandez, 2000).

Enzymes participate in numerous biochemical processes occurring in the soil (Trasar-Capeda *et al.*, 2000). Dehydrogenase enzymes are considered to exist in soils as integral parts of intact cells. They do not accumulate extracellularly in the soil. Dehydrogenase activity in soils provides correlative information on the biological activity and microbial populations in the soil (Wlodarczyk, 2000). Dehydrogenases conduct a broad range of oxidative activities that are responsible for degradation, i.e., dehydrogenation of organic matter by transferring hydrogen and electrons from substrates to acceptors (Margesin *et al.*, 2000; Gundi *et al.*, 2005).

One of the largest constraints to agricultural production worldwide is low phosphorus availability in soil particularly in areas where inorganic fertilizers are often unavailable. consequently, for designing sustainable agricultural systems much emphasis has been placed on the addition of organic residues to soil as a means of enhancing organic P reserves or for stimulating inorganic P release from previously unavailable soil reserves (Ryan *et al.*, 2001; Toor *et al.*, 2006).

The existence of a large reservoir of organic phosphorous in Himalayan soils that cannot be utilized by plants emphasize the role of microorganisms in converting the organic phosphorous to inorganic phosphorous. Bacteria, fungi and actinomycetes make the bound element in remains of the vegetation and in soil organic matter available to succeeding generation of plants. They release enzyme phosphatase which cleaves phosphorous from the more frequently encountered organic substrates by hydrolyzing the organically bound phosphorous (Dyhrman and Palenik, 2003; Kizilkaya *et al.*, 2007; Sharma, 2011; Gangasuresh *et al.*, 2010).

The objective of this investigation was to assess the activities of two main bioindicators i.e., dehydrogenase and phosphatase in agricultural soils of Almora district of Central Himalaya in relation to altitude. Also attempt has been made to correlate the enzymatic activities with the fertility factors i.e., organic carbon, nitrogen, phosphorous and potassium.

MATERIALS AND METHODS

Site description and soil sampling: Soil samples were collected from Almora district of Uttarakhand, India which varies from 3000 to 8450 feet above sea level. The average temperature is 24°C varying with the altitude with a dry period normally spanning from March to June, a rainy season from July to September and a winter season from October to February. The area is well populated having a population of 6,30,567 with agriculture as major activity dispersed along the slope. The main crops are wheat, rice, barley, manduwa, gahat, rajma, black soyabean. The agriculture of the area depends mainly on rain for irrigation of the field. But in some area there is availability of the canals and seeping water. The soils belong to Inceptisols, Entisols, Mollisols and Alfisols in different regions (Singh and Singh, 1992).

Soil collection: A total of seventy two soil samples were collected during October, 2009 from sites around Almora, Uttarakhand, India before tilling the soil (Fig. 1). The samples were collected following the standard soil sampling procedure and then transported to the laboratory at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi in isothermic bags. Soil samples were divided in two parts. One part of the soil samples was air dried and processed for chemical analysis. Another part of the soil sample was stored at 4°C for biological analysis.

Fig. 1: Almora district of Uttarakhand, India. *Sampling site

Chemical analysis: Soil samples were analyzed for pH in a soil solution of ratio 1:2.5 wt:vol with a glass electrode (Jackson, 1973); oxidizable SOC (Walkley and Black, 1934); Kjeldahl N by Semi-Autoanalyser (Model 2200), available P following the Olsen *et al.* (1954) method and available K by 1 N NH4OAc using a flame photometer (Jackson, 1973).

Enzymatic assay: Soil enzymes, dehydrogenase and phosphatase activities were assessed in the collected soil samples. Dehydrogenase activity was assayed by following the colorimetric method as described by Casida *et al.* (1964) using the substrate 2,3,5-triphenyltetrazolium chloride (TTC) and phosphatase activity by the method of Tabatabai and Bremner (1969) using substrate p-nitrophenyl phosphate.

Microbial population count: For enumerating the microbial population of soil serial dilution and plating technique as described by Rolf and Bakken (1987) was used. The result was expressed as cfu g^{-1} soil.

Statistical analysis: Values of a particular characteristic of soil obtained from analysis of soil samples of different locations at same altitudes were pooled together. Analyses were made in three replications. Data were analyzed statistically to draw conclusion of significance by using the method as described by Panse and Sukhatme (1967). The test of significance was carried out at 5% level of significance by referring to 'F' table values.

RESULTS

The fertility factor of the soil is influenced by the condition prevailing in the area. Analysis of the soil samples of Almora district showed that the agricultural soil is acidic in nature. The pH of the soil increases with altitude with slight decrease around 4000-5500 feet of altitude (Fig. 2). The result showed that the agricultural soils were high in percent organic carbon content. The organic carbon content in the surveyed area was high and it was maximum at 6500-7000 feet of altitude (Fig. 2). The influence of altitude on mean content of total nitrogen, available phosphorous and available potassium in agricultural soils of Almora district is presented in Fig. 3.

Total soil nitrogen, available phosphorous and available potassium content of the agricultural soil were also influenced by altitudes. N, P and K were maximum at an altitude of 6500-7000 feet. With increasing altitude the nutrient content in agricultural soil of almora district varied greatly.

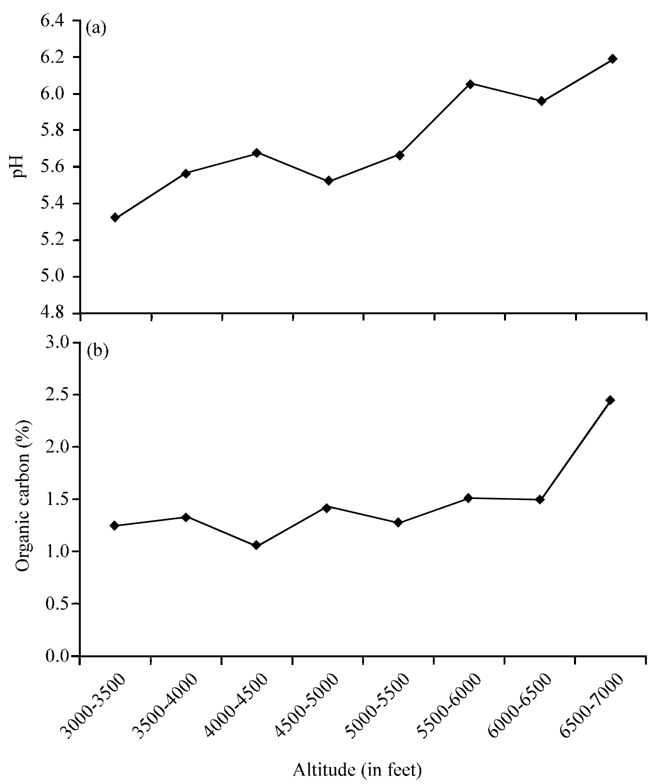

Fig. 2(a-b): Influence of altitudes on (a) pH and (b) percent organic carbon in soils of Almora

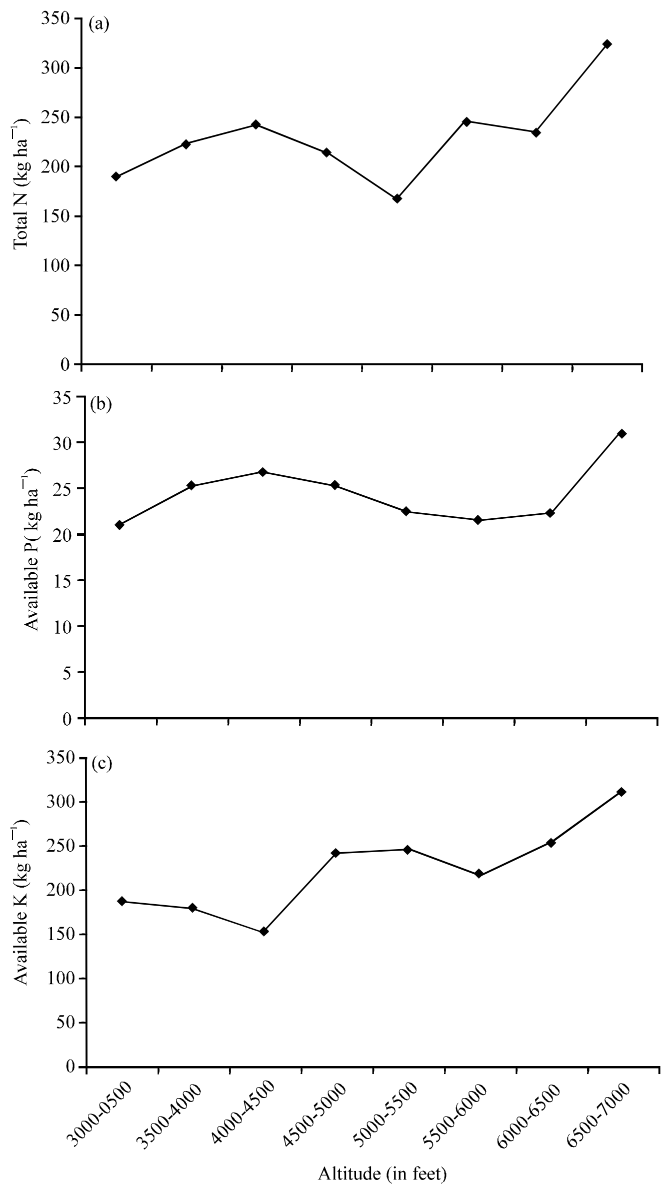

Fig. 3(a-c): Influence of altitudes on nutrient and content (a) N, (b) P and (C) K (kg ha^{-1}) in soils of Almora

Nitrogen and phosphorous content initially increased upto 4000-4500 feet of altitude and there after decreased and once again it increased. While for potassium it first decreased and then increased with increase of altitudes.

Microbial population in the agricultural soil of Almora is presented in Table 1. In the soil bacterial population was maximum followed by actinomycetes and fungal population. Analysis of the data revealed that bacterial population increased with increase of the altitudes. In contrast to bacterial population, actinomycetal population decreased as we move to the higher altitudes. Fungal population in agricultural soils of Almora was high at higher altitudes. Maximum population of bacteria (59×10^6 cfu g^{-1} soil), fungi (16×10^3 cfu g^{-1} soil) and actinomycetes (30×10^5 cfu g^{-1} soil) were observed at 6500-7000 feet, 5500-6000 feet and 3000-3500 feet, respectively.

Table 1: Effect of altitudes on soil microbial population (cfu g^{-1} soil) in agricultural soils of Almora district

Altitude	Bacteria ($\times10^6$)	Fungi ($\times10^3$)	Actinomycetes ($\times10^5$)
3000-3500	26	11	30
3500-4000	30	8	25
4000-4500	32	9	21
4500-5000	43	9	14
5000-5500	34	12	29
5500-6000	34	16	21
6000-6500	43	11	17
6500-7000	59	13	18
CD (p = 0.05)	6.68	NS	7.022
SEM±	2.73		2.866

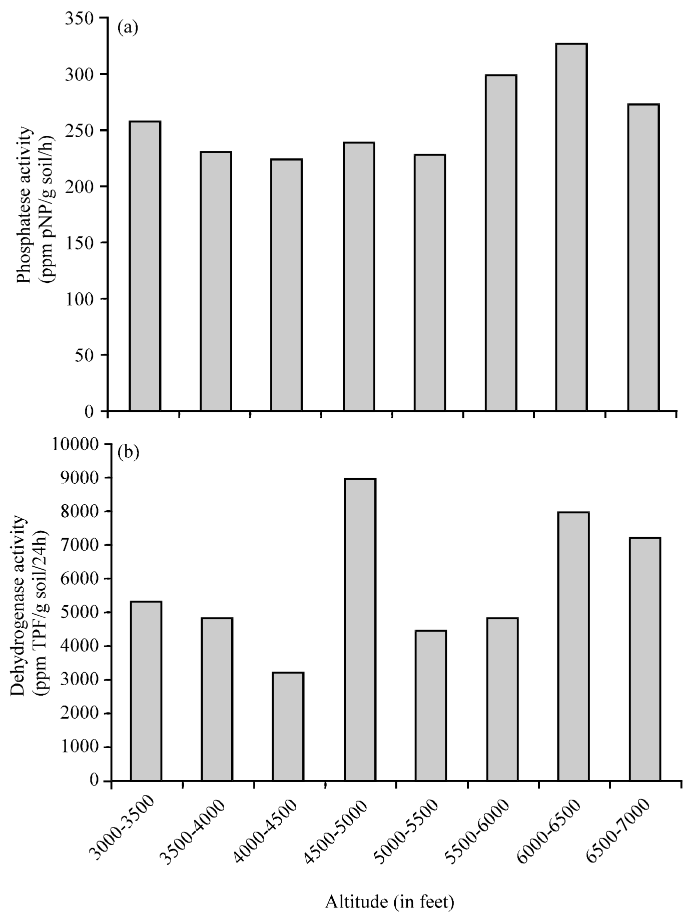

Fig. 4(a-b): Effect of altitudes on (a) dehydrogense and (b) phosphatase activity in agricultrule soils of Almora district

Enzymatic activities in agricultural soils of Almora varied widely along the altitudes. Influence of altitudes on the activities of the enzymes dehydrogenase and phosphatase is presented in Fig. 4. Maximum dehydrogenase activity was recorded at an altitude of 4500-5000 feet of height where as corresponding values for the phosphatase activity was noted at 6000-6500 feet of height. Statistical analysis of the data revealed that dehydrogenase activity was significantly influenced

Table 2: Simple correlations coefficients of different soil health properties

	pH	Organic C	N	P	K	Dehydrogenase	Phosphatase
pH	1.00						
Organic C	0.63	1.00					
N	0.77*	0.54	1.00				
P	0.38	0.25	0.76*	1.00			
K	0.64*	0.88**	0.46	0.36	1.00		
Dehydrogenase	0.57*	0.09	0.83**	0.64*	0.02	1.00	
Phosphatase	0.63	0.66	0.36	-0.26	0.43	0.07	1.00

*Significant at p = 0.05, **Significant at p = 0.01

by the altitudes. An overview of the graph reveals that phosphatase activity did not show much variation at lower altitudes but after 5000-5500 feet of altitude it was enhanced greatly.

Statistical analysis of the data using ANOVA in MS Excel revealed that organic C possess a positive and significant correlations with potassium (r = 0.88) (Table 2). Soil pH showed positive correlation with mineralisable N (r = 0.77) and potassium content (r = 0.64). The available phosphorous content of the soil was found to possess positive correlation with soil N content (r = 0.76). Enzymatic activity dehydrogenas possess significant and positive correlation with mineralisable nitrogen (r = 0.83) and available phosphorous (r = 0.64). Phosphatase enzyme activity possess positive and insignificant correlation with all the parameters except available phosphorous content with which it possess negative correlation (r = -0.26).

DISCUSSION

From the agricultural point of view the health of the soil is very important. The organic carbon content of the soil is essential for the maintenance of environment necessary for microbial flora to flourish which in turn provides nutrients to the crop plants. Agricultural soil of Almora district is very rich in organic carbon. The main reason behind the high content of organic matter in soil is due to the plant wastes added to the soil to regain the soil potential to sustain the crop. Natural leaf litters too add the organic matter content in the soil. Culturable soil at the high altitude has more carbon as compared to the soils at low altitude. High percent organic carbon at high altitude in Almora district might be attributed to the greater precipitation at the higher altitude and associated greater amounts of plant biomass and a thicker soil crust. This might also be due to the conversion of forest in culturable land at high altitude while frequent agricultural practices at low altitude lead to the loss of carbon matter from soil. This is in accordance to Abera and Belache (2011). Lower average annual temperature, retarding decomposition might also result in a greater amount of C sequestration in soil at the higher elevations.

Although the soil nitrogen content varied, it increased with increasing altitudes with some variation. The nitrogen content like organic carbon too is above normal range which may be a function of longer residence time of the nitrogen in the litter and decreasing soil N losses. In parallel to nitrogen, phosphorous and potassium also increased with altitude with some fluctuation. It has been observed that farmers at these altitudes depend on more organic matters for the carbon source. Addition of organic matter has been found to be good agricultural management to increase the content of organic carbon. The higher organic matter depicts the high carbon content. As a result the addition of organic matter contributed to the soil N, P and K which caused positive correlation with content of percent organic C. It is true as the content of organic matter in the soil has lead to the increment of mineralisable nitrogen, phosphorous and potassium in the soil. Similar results with positive correlation of organic carbon with nitrogen, phosphorous and potassium has also been reported by other researchers like Rezende *et al.* (2004), Verma *et al.* (2010) and Onweremadu (2007).

Waring and Schlesinger (1985) reported that microorganisms can mineralize up to 80% of dead plant materials. With the increasing awareness of the soil microbial activities many studies have been conducted in this regard. Temperature, pH, soil water content, land use changes and nutrient availability have been identified as key variables controlling the soil microbial activities. These facts are in evident to the experimental findings of Anderson and Nilsson (2001), Allison *et al.* (2008), Ye *et al.* (2009), Kang *et al.* (2003), Jia *et al.* (2006) and Enowashu *et al.* (2009).

Soil enzymes either extracellular or intracellular are a good indicator of soil microbial activities. Soil microbes contribute to plant growth in various ways. Microbial population in the agricultural soils of Almora district varies with altitude. This variation in microbial population may be attributed to the soil condition prevailing in the area due to land usage. Dehydrogenase and phosphatase are synthesized by soil microorganisms in response to the presence of suitable substrate.

Herman and Maier (2009) stated soil dehydrogenase activity is a good indicator of overall microbial activity in soil. It can serve as a good indicator of soil condition. According to Rao *et al.* (2003), soil dehydrogenase activity responds to changes in soil quality related to previous land usage. Soil dehydrogenase activity reflects the workings of a group of intracellular enzymes that are present in living soil microbes. When a soil sample is air-dried the dehydrogenase activity may increase (Rao *et al.*, 2003) or decrease (Ross and McNeilly, 1972) depending on the makeup of the soil itself or the particular soil horizon.

Dehydrogenases possess a high correlation with the nitrogen and phosphorous which could be due to the fact that dehydrogenase is an intracellular enzyme associated mainly with the microbial respiratory process. The higher dehydrogenase activity was likely due to C input in the soil in the form of organic manure or may be due to root mass that enhanced microbial activity. The reason is being supported by the work of Subrahmanyam *et al.* (2011) who demonstrated that dehydrogenase activity is sensitive to soil organic matter.

Low phosphorus availability in soil remains one of the constraints to agricultural production worldwide particularly in areas where inorganic fertilizers are unavailable. The element phosphorus is essential for microbial and plant growth. It is generally accepted that plants and microorganisms utilize only inorganic P, thus the mineralization of the organic P fraction can be an important factor in plant nutrition. Labile organic P compounds are mineralized in soils by enzymes, collectively called phosphatases that catalyze the hydrolysis of esters and anhydrides. In our results of the altitudinal effect on phosphatase activity revealed that phosphatase activity was greater at high altitudes as compared to the lower altitude. Statistical analysis revealed an inverse relationship between phosphatase activity and phosphorous content of soil which is in contradiction to the result of Kizilkaya *et al.* (2007). This may also be due to the presence of high organic matter content in the soil as a result of shifting cultivation and addition of huge amount of organic matter and leaf litter from the trees. It may therefore be assumed that the observed increase of phosphatase activities at higher altitudes may be influenced by the microbial population and the use of organic matter in soils.

CONCLUSION

Nitrogen, phosphorous and potassium content in the soil of Almora district increased with altitude with some fluctuation. Activities of both the enzyme under study i.e. Dehydrogenases and phosphatases were greater at high altitudes as compared to the lower altitude. It may be assumed that the observed increase of phosphatase activities at higher altitudes may be influenced by the microbial population and the use of organic matter in soils. While going for selecting a biological indicator for such soil types, the influence of the altitude must be kept in mind.

The present work concludes that the influence of the altitude must be kept in mind while going for selecting a biological indicator for such soil types.

ACKNOWLEDGMENTS

Authors thank GB Pant Institute of Himalayan Environment and Development (GBPIHED) for funding the research work. We also acknowledge Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Science, BHU, Varanasi for providing the facilities for the research to be conducted.

REFERENCES

Abdalla, M.A. and U. Langer, 2009. Soil enzymes activities in irrigated and rain-fed vertisols of the semi-arid tropics of Sudan. Int. J. Soil Sci., 4: 67-79.

Abera, Y. and T. Belachew, 2011. Land use effects on soil organic carbon and nitrogen in some soils of bale, Southeastern Ethiopia. Trop. Subtrop. Agroecosyst., Vol. 14.

Aira, M., F. Monroy and J. Dominguez, 2007. *Eisenia fetida* (Oligochaeta: Lumbricidae) modifies the structure and physiological capabilities of microbial communities improving carbon mineralization during vermicomposting of pig manure. Microbial. Ecol., 54: 662-671.

Allison, S.D., C.I. Czimczik and K.K. Treseder, 2008. Microbial activity and soil respiration under nitrogen addition in Alas-kan boreal forest. Global Change Biol., 14: 1156-1168.

Anderson, S. and S.I. Nilsson, 2001. Influence of pH and temperature on microbial activity, substrate availability of soil-solution bacteria and leaching of dissolved organic carbon in a mor humus. Soil Biol. Biochem., 33: 1181-1191.

Calderon, F.J., L.E. Jackson, K.M. Scow and D.E. Rolston, 2000. Microbial responses to simulated tillage in cultivated and uncultivated soils. Soil Biol. Biochem., 32: 1545-1559.

Casida, L.E., D.A. Klein and T. Santro, 1964. Soil dehydrogenase activity. Soil Sci., 98: 371-376.

Colombo C., G. Palumbo, F. Sannino and L. Gianfreda, 2002. Chemical and biochemical indicators of managed agricultural soils. 17th World Congress of Soil Science, Aug. 14-20 Datanet India Pvt. Ltd., Delhi. 1740-1740.

Drijber, R.A., J.W. Doran, A.M. Parkhurst and D.J. Lyon, 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biol. Biochem., 32: 1419-1430.

Dyhrman S.T. and B. Palenik, 2003. Characterization of ectoenzyme activity and phosphate-regulated proteins in the coccolithophorid *Emiliania huxleyi*. J. Plankton Res., 25: 1215-1225.

Enowashu E., C. Poll, N. Lamersdorf and E. Kandeler, 2009. Micro-bial biomass and enzyme activities under reduced nitrogen deposition in a spruce forest soil. Applied Soil Ecol., 43: 11-21.

Gangasuresh, P., V. Muthuselvi, E. Muthulakshmi, S. Muthumari and G. Maniammal, 2010. Synergistic efficiency of phosphate solubilizer associated with nitrogen fixer on the growth of soybean (*Glycine max*). Int. J. Biol. Technol., 1: 124-130.

Garcia, C. and M.T. Hernandez, 2000. Research and Perspectives of Soil Enzymology in Spain. CEBAS-CSIC, Murcia, Spain.

Gundi, V.A.K.B., G. Narasimha and B. R. Reddy, 2005. Interaction effects of insecticides on microbial populations and dehydrogenase activity in a black clay soil. J. Environ. Sci. Health, 40: 69-283.

Heritage, J., G. Evans and R.A. Killington, 1999. The Microbiology of Soil and of Nutrient Cycling: Microbiology in Action. Cambridge University Press, Cambridge, ISBN: 9780521621113.

Herman, D.C. and R.M. Maier, 2009. Physiological methods, In: Environmental microbiology. Maier, R.M., I.L. Pepper and C.P. Gerba (Eds.). Academic, San Diego, USA., pp: 235-267..

Jackson, M.L., 1973. Soil Chemical Analysis. 1st Edn., Prentice Hall of India Pvt. Ltd., New Delhi, India.

Jia, B., G. Zhou, Y. Wang, F. Wang and X. Wang, 2006. Effects of temperature and soil water-content on soil respiration of grazed and ungrazed *Leymus chinensis* steppes, inner Mongolia J. Arid Environ., 67: 60-76.

Kang, S.Y., S. Doh, D. Lee, D. Lee, V.L. Jin and J.S. Kimball, 2003. Topographic and climatic controls on soil respiration in six temperate mixed-hardwood forest slopes, Korea. Global Change Biol., 9: 1427-1437.

Kizilkaya, R., F. Bayrakli and A. Surucu, 2007. Relationship between phosphatase activity and phosphorus fractions in agricultural soils. Int. J. Soil Sci., 2: 107-118.

Margesin, R., A. Zimmerbauer and F. Schinner, 2000. Monitoring of bioremediation by soil biological activities. Chemosphere, 40: 339-346.

Mirani, Z.D., M.A. Narejo and F.C. Oad, 2002. Sustainable agriculture endeavors: Perceptions of farmers and extension agents. J. Applied Sci., 2: 27-28.

Nannipieri, P.B., E. Kandler and P. Ruggiero, 2002. Enzyme Activity and Microbial and Biochemical Processes in Soil. In: Enzymes in the Environment: Activity, Ecology and Applications, Burns, R.G. and R.P. Dick (Eds.). Marcel Dekker Inc., New York, pp: 1-33.

Oke, D.O. and I.T. Ologun, 2005. Effects of mulch from four *Agroforestry* species on the moisture content, temperature and microbial population in a humid tropical soil. J. Biological Sci., 5: 326-329.

Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean, 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. National Agricultural Society, USA., pp: 939.

Onweremadu, E.U., 2007. Availability of selected soil nutrients in relation to land use and landscape position. Int. J. Soil Sci., 2: 128-134.

Panse, V.G. and P.V. Sukhatme, 1967. Statistical Methods for Agricultural Workers. 2nd Edn., ICAR Publication, New Delhi, India, pp: 44.

Rao, M.A., F. Sannino, G. Nocerino, E. Puglisi and L. Gianfreda, 2003. Effect of air-drying treatment on enzymatic activities of soils affected by anthropogenic activities. Biol. Fertil. Soil, 38: 327-332.

Rezende, L.A., L.C. Assis and E. Nahas, 2004. Carbon, nitrogen and phosphorus mineralization in two soils amended with distillery yeast. Bioresour. Technol., 94: 159-167.

Rolf, A.O. and L. R. Bakken, 1987. Viability of Soil Bacteria: Optimization of plate-counting technique and comparison between total counts and plate counts within different size groups. Microb Ecol., 13: 59-74.

Ross, D.J. and B.A. McNeilly, 1972. Effects of storage on oxygen uptakes and dehydrogenase activities of beech forest litter and soil. N. Z. J. Sci., 15: 453-462.

Ryan, P.R., E. Delhaize and D.L. Jones, 2001. Function and mechanism of organic anion exudation from plant roots. Annu. Rev. Plant Physiol. Plant Mol. Biol., 52: 527-560.

Sharma, K., 2011. Inorganic phosphate solubilization by fungi isolated from agriculture soil. J. Phytol., 3: 11-12.

Singh, J.S. and S.P. Singh, 1992. Forests of Himalaya: Structure, Functioning and Impact of Man. Gyanodaya Prakashan, India.

Subrahmanyam, G., G. Archana and L.S. Chamyal, 2011. Soil microbial activity and its relation to soil indigenous properties in semi-arid alluvial and estuarine soils of mahi river basin, Western India. Int. J. Soil Sci., 10.3923/ijss.2011

Tabatabai, M.A. and J.M. Bremner, 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem., 1: 301-307.

Toor, G.S., S. Hunger, J.D. Peak, J.T. Sims and D.L. Sparks, 2006. Advances in the characterization of phosphorus in organic wastes: Environmental and agronomic applications. Adv. Agro., 89: 1-72.

Trasar-Capeda C., M.C. Lieros, S. Seoane and F. Gil-Sotres, 2000.. Limitations of soil enzymes as indicators of soil pollution. Soil Biol. Biochem., 32: 1867-1875.

Verma, B.C., S.P. Datta, R.K. Rattan and A.K. Singh, 2010. Monitoring changes in soil organic carbon pools, nitrogen, phosphorus and sulfur under different agricultural management practices in the tropics. Environ. Monitoring Assessment, 171: 579-593.

Walkley, A. and I.A. Black, 1934. An examination of the degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci., 37: 29-38.

Waring, R.H. and W.H. Schlesinger, 1985. Forest Ecosystems: Concepts and Management. Academic Press, New York.

Winding A., K. Hund-Rinke and M. Rutgers, 2005. The use of microorganisms in ecological soil classification and assessment concept. Ecotoxicol. Environ. Saf., 62: 230-248.

Wlodarczyk, T., 2000. Some of aspects of dehydrogenase activity in soils. Int. Agrophysics, 14: 365-376.

Ye, R., A.L. Wright, K. Inglett, Y. Wang, A.V. Ogram and K.R. Reddy, 2009. Land-use effects on soil nutrient cycling and microbial community dynamics in the Everglades Agricultural Area, Florida. Commun. Soil Sci. Plant Anal., 40: 2725-2742.