Journal of Medicinal Plants Research Vol. 6(14), pp. 2714-2720, 16 April, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.707
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Seasonal variation of urease and alkaline phosphatase activity in natural and artificial habitats of hazel

Moraghebi F.[1]*, Matinizadeh M.[2], Khanjani S. B.[3], Teimouri M.[2] and Afdideh F.[4]

[1]Department of Biology, Shahre Ray Branch, Islamic Azad University, Tehran, Iran.
[2]Research Institute of Forests and Raneland, The Forest Branch, Tehran, Iran.
[3]Agriculture and Natural Resources Research Center of Gillan, Pylmbra. Iran.
[4]Young Researchers Club, Shahre Rey Branch, Islamic Azad University, Tehran. Iran.

Accepted 6 October, 2011

The aim of this research was to study the activities of urease and alkaline phosphatase in two natural hazel habitats (Makesh and Fandoghlo) and compare their activities in an artificial habitat (Alborz). Soil sampling was done in spring and summer. The activity of urease and alkaline phosphatase was evaluated by enzyme- substrate reaction. The urease and alkaline phosphatase activity was more in spring in three studied habitats. In addition, results indicated the more enzyme activity in Fandoghlo than their activity in Makesh habitat in both season. In other hand, there was a positive relationship between available phosphorous and nitrogen percentage with urease and alkaline phosphatase activity in Fandoghlo habitats.

Key words: Alkaline phosphatase, hazel, season, soil, urease.

INTRODUCTION

The hazels (*Corylus*) are a genus of deciduous trees and large shrubs native to the temperate northern hemisphere. The genus is usually placed in the birch family Betulaceae, they have simple, rounded leaves with double-serrate margins. The flowers are produced very early in spring before the leaves, and are monoecious. The seeds are nuts 1 to 2.5 cm long and 1 to 2 cm in diameter, surrounded by an involucre (husk) which is partly to fully enclosed. Hazels (*Corylus sp*) grow in north of Iran. Iran produces approximately 2% of world hazelnut. Soil is a living system that influences the ecosystem balance and there are a lot of biological and biochemical processes occurring in soil especially in micro level. Enzymes are critical in these processes and release nutrients in different cycles and make elements available for plants. Then assessment of some enzymes can be used as an important indicator to determine biological potential of soil and ecosystem (Gil-Sotres et al., 2005). Most of natural ecosystems have been damaged during the last decade because of over harvesting, industrial development and agricultural activities. There are some regional and international programs to protect from natural ecosystems and rehabilitation of damaged areas. Understanding basic information on different aspects of natural ecosystems as soil quality and function is necessary to succeed in these programs. It is difficult to monitor the long term effects of anthropogenic activities on forest soil. Forest soils are complicated in point of biological, chemical and biological and there are a lot of challenges in determination of soil quality parameters (Staddon et al., 1998).

Monitoring of these effects by the use of trees' growth or soil organic matter is not satisfactory, because the changes observed for these factors are very slow (Dick, 1994 et al; Turco et al,, 1994). Then it has been suggested to use microbial related parameters in soil quality assessment. Soil enzymes activities, CO_2 emission and microbial biomass are sensitive to environmental changes of soil and can be used to monitor of anthropogenic activities effects on soil (Turco,

*Corresponding author. E-mail: f.moraghebi@iausr.ac.ir, moraghebi@yahoo.com.

1994 et al; Kennedy and Papendic, 1995). Biological and biochemical properties of soil respond rapidly to environmental stresses. Microbial activities are important in degradation of organic matter and release inorganic matters. Enzymes are critical in these responses (Parkinson and Visser and Parkinson, 1992; Gil-Sotres et al., 2005). Measurements of soil enzyme activity have been used extensively for assessment of different process occurring in nutrient cycles in soils (Nannipieri et al., 1990; Tabatabai and Dick, 2002). The activity of more than 100 enzymes has been determined in soil (Tabatabai and Dick, 2000). Soil enzyme activities are sensitive to deterioration effects of human and environment and their activity can be used as a tool to assess soil response to management practices and environmental stresses (Dick et al., 1988; Nannipieri et al., 1990), since they are highly sensitive to external factors. Measurement of soil enzymatic activity in comparison with other soil properties is cheaper and easier. Soil enzymes have microbial and plant origin and their activity show the activity of intracellular enzymes, extracellular and bound enzymes to clay and organic matters. The aim of the study was to study urease alkaline phosphatase activities in two natural hazel habitats (Makesh and Fandoghlo) and compare their activities in an artificial habitat (Alborz) in spring and autumn.

MATERIALS AND METHODS

Site description

The study was conducted on two natural and one artificial hazel habitats.

The Fandoghlo habitat

This natural habitat locates in 45 km distance from Ardabil, Iran. The habitat is next to Fandoghlo village and its height from sea level is 1450. Its climate according to Dumbarton is very humid and extremely cold. There are 3 dry months according to rain-temperature curve. The texture of soil is loam-silt with 6.8 acidity.

Makesh habitat

This natural habitat has 35 km distance from Talesh, Iran. Its height is 1400 to 1500 m above sea level. Its climate according to Dumbarton is very humid and extremely cold and there is no dry season. The soil has mainly loam texture loam-silt with pH 6.9.

Alborz habitat

The hazel seeds were collected about 8 years from mentioned natural habitats and planted in Alborz Research Center. This research center with 80 ha area locates in 15 km far from of Karaj, Iran. The mean of annual rain, minimum and maximum annual temperatures are 25 mm, 21.7 and 41°C, respectively, and its climate is semi dry. The soil has mainly loam texture loam and loam-clay silt with pH 8.2.

Soil sampling and analysis

Soil samples were taken randomly from natural and artificial habitats. Soil samples were collected in spring (May) and summer (September). Samples were placed in tightly sealed plastic bags and transferred immediately to the laboratory at 4°C. The soil samples were passed through a 2 mm sieve and divided into two fractions: one fraction for the determination of physical and chemical factors, which were kept at room temperature and the other fraction for measuring of soil enzymes activities which was stored at 4°C.

Chemical properties determination

Chemical analyses were done on air-dried and sieved (2 mm) soil samples. Soil pH and EC was measured with a glass electrode in 1:2.5 soil/water suspension. Total soil N was determined by Kjeldahl digestion (Bremmer and Mulvaney, 1982), and the organic-C was resolute by dichromate digestion (Walkley and Black, 1934). Olsen's bicarbonate extractable P (PO_4^{3-}) was also measured (Olsen et al., 1954).

Enzyme activities

Alkaline phosphatase activity

The activity of alkaline phosphatase (EC 3.1.3.2) was determined based on the method of (Ohlinger, 1996). The reaction mixtures consisted of 1.0 g soil, 1.0 ml PNP (disodium ρ- nitrophenyl phosphate 0.115 MM) and 2.0 ml MA buffer (maleate buffer1 0.1 M, pH 11).

The reaction mixtures were incubated at 37°C for 1 h. After incubation, the reaction was stopped by adding 1.0 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH. Concentration of p- nitrophenol (NP) produced in the assays of phosphotase activities was calculated from a *p*-NP calibration curve after subtracting the absorbance of the control at 400 nm wavelength using a UV-VIS spectrophotometer Bausch @Lambda (spectronic 21). Two analytical replicates and one control were analyzed for each soil sample. Soil moisture content was determined from the loss in weight after drying at 105°C for 24 h. Enzymes activities are expressed as microgram (µg) ρ-nitro phenol per gram (g) soil per hour at 37°C.

Urease assay

By using enzyme- substrate reaction, urease release ammonium from its buffered substrate urea at 37°C. This product is extracted by KCl.

The reaction between ammonium and salycilate sodium create a color product that its absorbance is measured at 690 nm by a spectrophotometer (Ohlinger, 1996). The enzyme activity expressed as µg nitrogen g^{-1} h^{-2}.

RESULTS

Table 1 shows the several chemical properties of soil. As shown in Table 1, the amount of available P was 56.8, 23.21 and 32.42 in Makesh, Fandoghlo and Alborz habitats, respectively. The nitrogen percentage was 0.43, 0.17 and 0.25 in Makesh and Fandoghlo and Alborz habitats, respectively.

Table 1. Some chemical characters of soil in studied sites.

Parameter	C (%)	Organic matter (%)	K (ppm)	N (%)	Ca	CEC (ppm)	P (ppm)	Mg
Makesh	3.9	6.7	989	0.43	26.4	270	56.8	6.4
Fandighlo	3.71	4.64	279.5	0.17	30.4	300	23.21	18.4
ÓAlborz	3.65	5.2	340	0.25	32.5	14.5	32.42	290

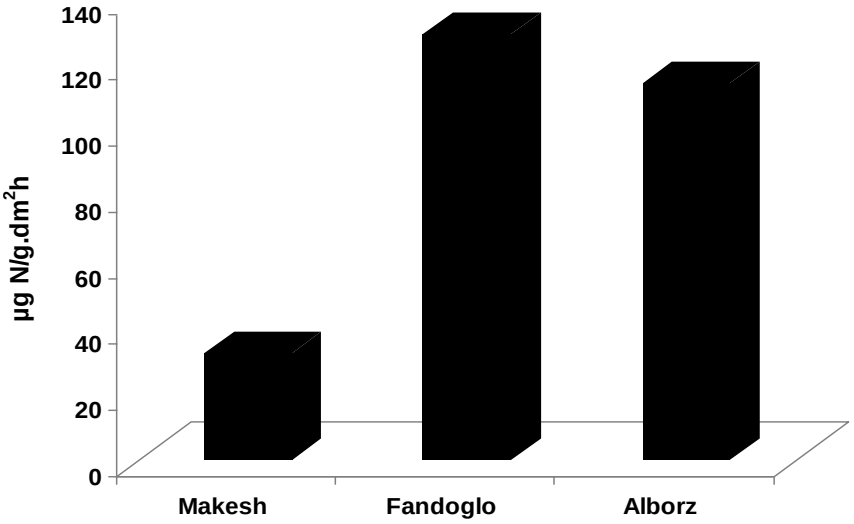

Figure 1. Urease activity in three Makesh, Fandoghlo and Alborz habitats in spring.

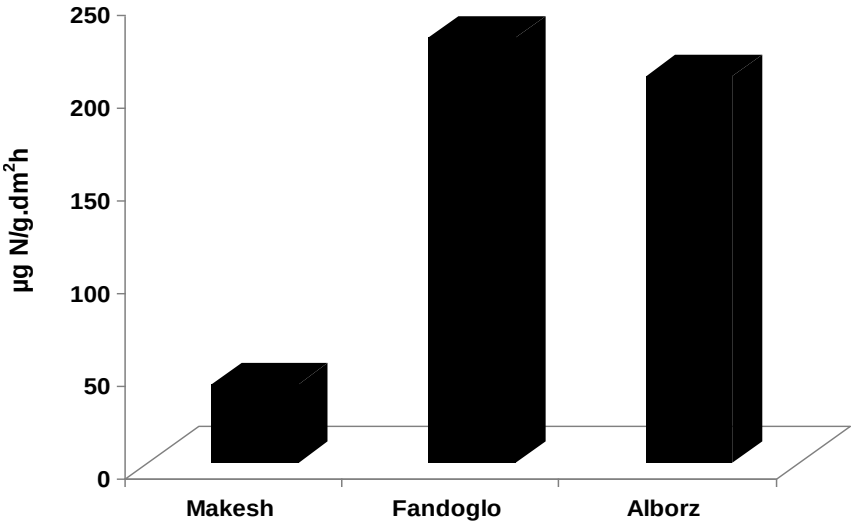

Figure 2. Urease activity in three Makesh, Fandoghlo and Alborz habitats in summer.

Variation of soil enzymatic activities in dependence of sampling time

It is evident from Figures 1 and 2 that urease activity changed with sampling time in natural habitats as well as artificial habitat. Urease activity ranged from 32.05 (±5.28) µg N g^{-1} h^{-2} in spring to 41.92 (±6.53) µg N g^{-1} h^{-2} in summer in Makesh habitat (Figures 1 and 2). This enzyme activity varied from 128.81(±8.29) µg N g^{-1} h^{-2} in spring to 229.04 (±11.38) µg N g^{-1} h^{-2} in summer in

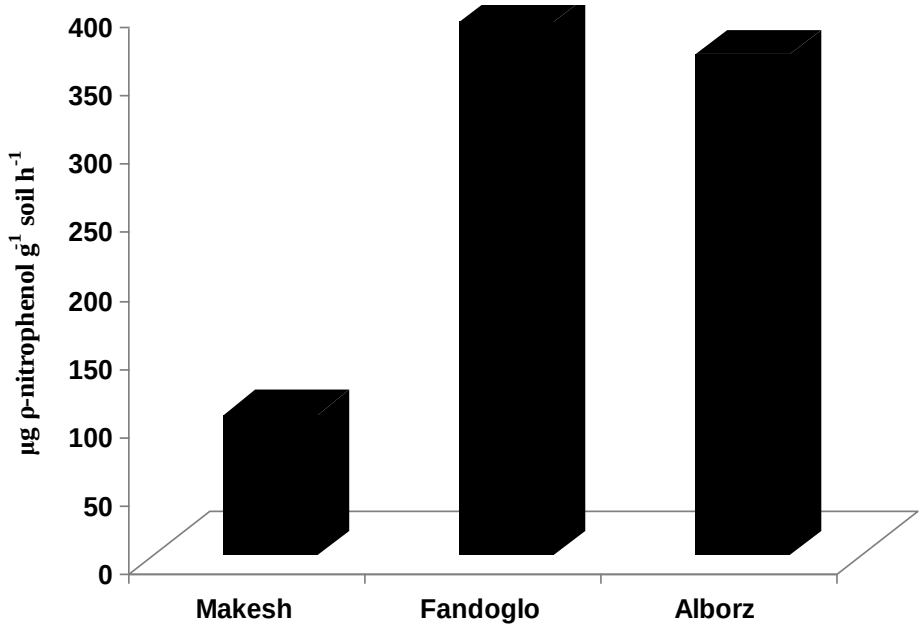

Figure 3. Alkaline phosphatase activity in three Makesh, Fandoghlo and Alborz habitats in spring.

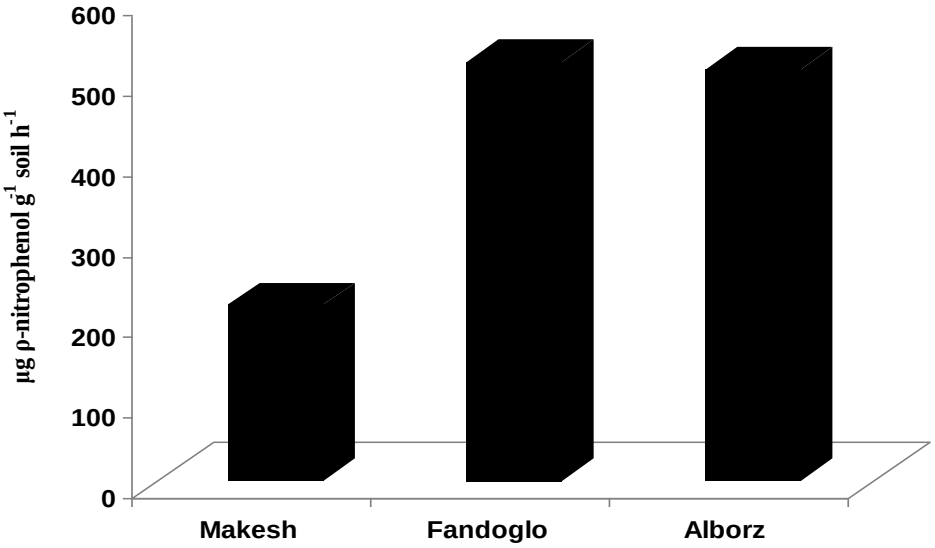

Figure 4. Alkaline phosphatase activity in three Makesh, Fandoghlo and Alborz habitats in summer.

Fandoghlo habitat (Figures 1 and 2). Urease activity ranged from 113.82 (±11.18) µg N g^{-1} h^{-2} in spring to 208.35 (±16.29) µg N g^{-1} h^{-2} in summer in Alborz habitat (Figures 1 and 2). There was a significant difference between two Fandoghlo and Makesh habitats in both seasons in urease activity. Alkaline phosphatase showed variation as urease variation based on sampling time. As shown in Figures 3 and 4, alkaline phosphatase activity changed from 102.29 (±8.11) µg ρ-nitrophenol g^{-1} soil h^{-1} in spring to 218.76 (±11.52) µg ρ-nitrophenol g^{-1} soil h^{-1} in summer in Makesh habitat. This enzyme activity varied from 389.98 (±23.18) µg ρ-nitrophenol g^{-1} soil h^{-1} in spring to 520.35 (±21.38) µg ρ-nitrophenol g^{-1} soil h^{-1} in summer in Fandoghlo habitat (Figures 3 and 4). Alkaline phosphatase activity ranged from 365.20 (±11.18) µg ρ-nitrophenol g^{-1} soil h^{-1} in spring to 510.17 (±16.29) µg ρ-

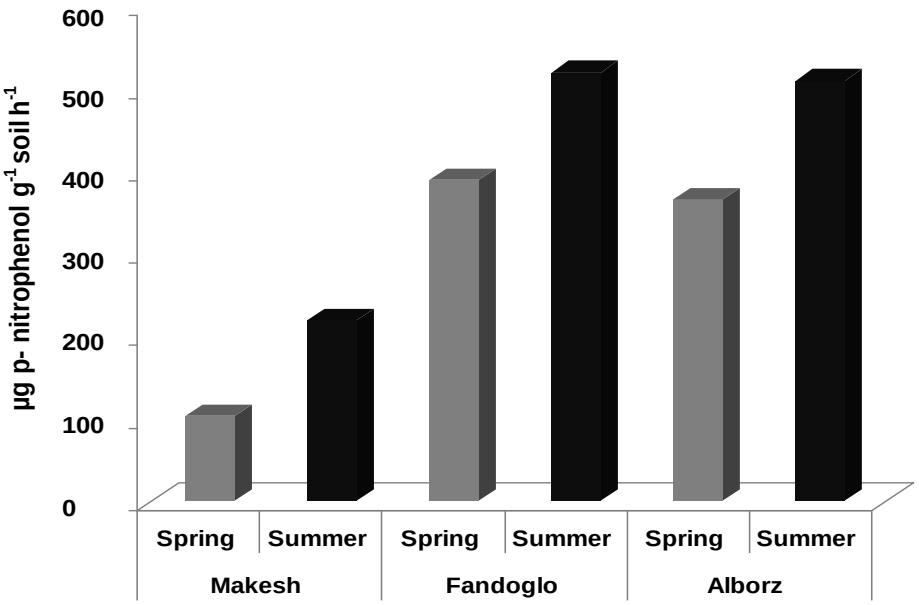

Figure 5. Comparison of alkaline phosphatase activity in three Makesh, Fandoghlo and Alborz habitats in spring and summer.

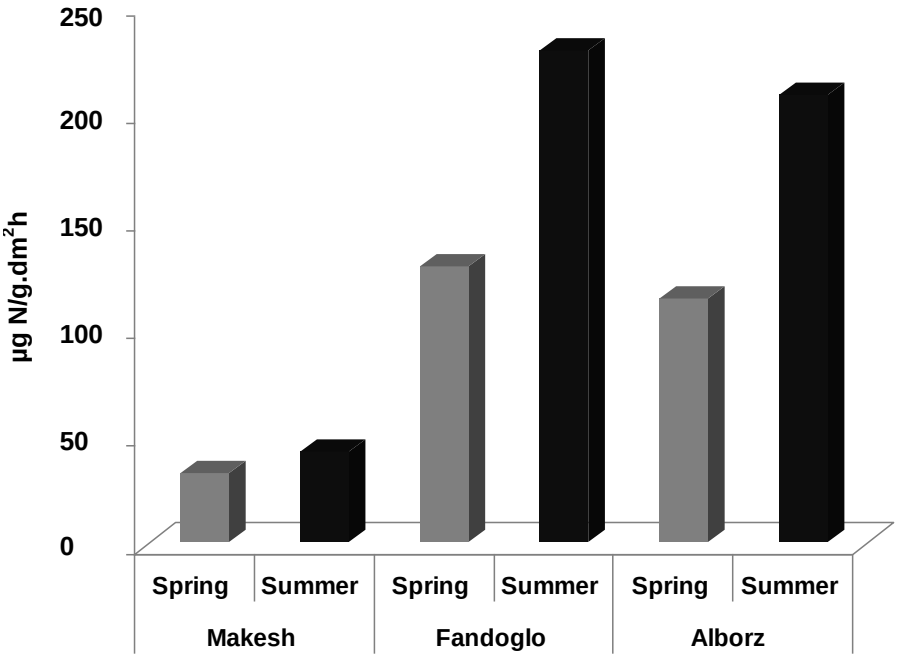

Figure 6. Comparison of Urease activity in three Makesh, Fandoghlo and Alborz habitats in spring and summer.

nitrophenol g^{-1} soil h^{-1} in summer in Alborz habitat (Figures 3 and 4). It is clear that alkaline phosphatase activity was more in summer samples in comparism to spring samples in both natural and artificial habitats.

Variation of soil enzymatic activities based on the sampling habitat

According to Figures 5 and 6, there are differences in

enzyme activity based on sampling habitats. Urease activity was 128.81 (±8.29), 32.05 (±5.28) and 113.82 (±11.18) µg N g^{-1} h^{-2} in Fandoghlo, Makesh and Alborz habitats in spring, respectively. Urease activity increased to 229.04 (±11.38), 41.92 (±6.53) and 208.35 (±16.29) µg N g^{-1} h^{-2} in Fandoghlo, Makesh and Alborz habitats in summer samples, respectively. It is evident that in both season urease activity in Fandoghlo and Alborz habitats is higher than its activity in Makesh habitat. The alkaline phosphatase activity was 389.98 (±23.18), 102.29 (±8.11) and 365.20 (±11.18) µg ρ-nitrophenol g^{-1} soil h^{-1} in Fandoghlo, Makesh and Alborz habitats in spring, respectively (Figures 5 and 6). This enzyme activity changed to 218.76 (±11.52), 520.35 (±21.38) and 510.17 (±16.29) in Fandoghlo, Makesh and Alborz habitats in summer, respectively (Figures 5 and 6). Inconsequently, alkaline phosphatase activity was less in Fandoghlo habitat than its activity in two other habitat.

DISCUSSION

Assessing the long term effects of human activities on forest soil is difficult. Forest soils are complicated as regards the physical, chemical and biological point of view and there are a lot of challenges in determination of soil quality parameters (Staddon et al., 1998). Monitoring of these effects by use of trees growth or soil organic matter are time consuming and can not be suitable indicators (Dick, 1994; Turco et al, 1994).

Biochemical and biological properties of soil enzyme activity, changing in response to environmental stresses, can be used to assess the soil potential and monitor the effects of anthropogenic activities or environmental stresses (Klein et al., 1985; Nannipieri et al., 1990). Soil enzyme activities are sensitive to deterioration effects of human and environment and measurement their activity can be used as a valid tool to assessment of soil metabolically response to management practices, climate changes and environmental stresses and bring valuable information on nutrient cycles (Tabatabai and Dick, 2002; Sinsabaugh et al., 2002; Kandeler, 2007).

The effect of season on a urease and alkaline phosphatase activity in studied habitats

The key to understanding seasonality in enzyme activity may be in the factors that regulate various enzyme systems. Some of soil enzymes are regulated primarily by microclimate and soil chemical factors, whereas other enzymes are more regulated by substrate availability (Sinsabaugh et al., 1992). Soil organic matter increases through the growing season even as soil moisture decreases. This may explain the more enzyme activity in the autumn compared to spring. Source of urease and alkaline phosphatase are soil microorganisms and fauna (Findenegg and Neiemans, 1993; Tarafdar and Chhonkar, 1979) and plants can not produce. Then it can be used as a good tool to measure microbial metabolisms in the soil (Tabatabai, 1982). The enzyme activity in Fandoghlo habitat in both seasons was higher than in Makesh. The more density of plants in Fandoghlo causes more balanced situation in temperature and humidity resulting in more activity of microorganisms which are source of these enzymes. These results had conformity with findings of Kramer and Green (2000) and Sedia and Ehrenfeld (2006).

Relationship between phosphatase and urease with available P and N

In this study, there was a relationship between available phosphorus and nitrogen percentage with alkaline phosphatase and urease activity. Phosphorus content in the Makesh, Fandoghlo and Alborz habitats was 56.8, 23.21 and 32.42, respectively. The nitrogen percentage in the Makesh, Fandoghlo and Alborz habitats was 0/43, 0/17, 0/25 respectively (Table 1). The higher activity of both enzymes in Fandoghlo can be related to higher amount of available phosphorus and nitrogen percentage.

REFERENCES

Bremmer JM, CS Mulvaney (1982). Nitrogen total. In: 'Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties'. (Eds AL Page, RH Mill Keenpp. 595–624. (ASA: Madison).

Dick RP (1994). Soil enzyme activities as indicators of soil quality. In: Doran, J. W., Coleman, D. C., Bezdicek, D. F., Stewart, B. A., (Eds.) Defining Soil Quality for a Sustainable Environment. Soil Sci. Soc. Am. Madison WI, Pp. 108-123.

Dick RP, Rasmussen PE, Herle EA (1988). Influence of long term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. Biol. Fertil. Soils, 6: 158-164.

Dick RP, Sandor JA, Eash NS (1994). Soil enzyme activities after 1500 years of terrace agriculture in the Colca Valley, Peru. Agric. Ecosyst. Environ., 50: 123-131.

Findenegg GR, Neiemans JA (1993). The effect of phytase on the availability of P from myo-inositol hexaphosphate (phytate) for maize roots. *Plant Soil,* 154(2): 189-196.

Gil-Sotres F, Trasar-Cepeda C, Leiros MC, Seoane S (2005). Different approaches to evaluate soil quality using biochemical properties. *Soil Biol. Biochem.,* 37: 877–887.

Kennedy AC, Papendick RI (1995). Microbial characteristics of soil quality. *J. soil water conserve.,* 50: 243–248.

Kandeler E (2007). Physiological and biochemical methods for studying soil biota and their function. In: Paul E.A. *Soil microbial. Ecol. Biochem*. Acad. Press, Oxford, UK. 53-80.

Klein DA, Sorensen DL, Redente EF (1985). Soil enzymes: A predictor of reclamation potential and progress. In: Tate, R.L., Klein, D.A. (Eds.), Soil Reclamation Processes. Microbiol. Anal. Appl. Marcel Dekker, New York, pp. 273–340.

Kramer S, Green DM (2000). Acid and alkaline phosphatase dynamics and their relationship to soil microclimate in semiarid woodland. *Soil Biol. Biochem.,* 32: 179-188.

Nannipieri P, Grego S, Ceccanti B (1990). Ecological significance of the biological activity in soil. Bollag J.M. and Stotzky G. (Eds.) Soil Biochemistry. Vol.6. Marcel Dekker, New York, pp. 293-355.

Ohlinger R (1996). Acid and alkaline phosphomonoesterase activity with the substrate p-nitrophenyl phosphate. In: Schinner F., Kandeler E., Ohlinger R., Margesin R. (Eds) *Methods in soil biology.* Springer-Verlag Berlin. Pp. 210-214.

Olsen SR, Cole LV, Watanabe FS, Dean LA (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circ. United States Dep. Agric. no. 939. Washington DC.

Sedia EG, Ehrenfeld JG (2006). Differential effects of lichens and mosses on soil enzyme activity and litter decomposition. Biol. Fertil. Soils*,* 43: 177–189.

Sinsabaugh RL, Carreiro MM, Alvarez S (2002). Enzyme and microbial dynamics of litter Decomposition. In: Burns R. G., Dick W. A. (Eds) Enzymes in the environment. Marcel Dekker, New York, pp. 249-266.

Staddon WJ, Duchesne LC, Trevors JT (1998). Acid phosphatase, alkaline phosphatase and arylsulfatase activities in soils from a jack pine (*Pinus banksiana* Lamb.) ecosystem after clear-cutting, prescribed burning, and scarification. Biol. Fertil. Soils*,* 27: 1-4.

Tabatabai MA (1982). Soil enzymes. In: Page, A. L. Miller, R. H. Keeney, D. R. (Eds.) *Methods of Soil Analysis Part 2,* Agronomy 9, Am. Soc. Agron. Madison Wis. pp. 903-947.

Tabatabai MA, Dick WA (2002). Enzymes in soil. In: Burns, R. G., Dick, W. A. (Eds) *Enzymes in the environment.* Marcel Dekker, New York, pp. 567–596.

Tarafdar JC, Chhonkar PK (1979). Phosphatase production by microorganisms isolated from diverse types of soils. Zentralbl Bakteriol Naturwiss, 134: 119-24.

Turco RF, Kennedy AC, Jawson MD (1994). Microbial indicators of soil quality. In: Doran JW, Coleman DC, Bezdicek, D. F, Stewart, B. A. (Eds) *Defining soil quality for a sustainable environment.* Soil Sci. Soc. Am., Special Publication no. 35, pp. 73-90.

Visser S, Parkinson D (1992). Soil biological criteria as indicators of soil quality: soil microorganisms. Am. J. Altern. Agric., 7: 33-37.

Walkley A, Black IA (1934). An Examination of Degtjareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromi Acid Titration Method. Soil Sci., 37: 29-37.