



## Seasonal variation of acid phosphatase and dehydrogenase activity in natural and artificial habitats of hazel

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### Abstract

The hazels (*Corylus avellana*) are seen in north region of Iran and many stands grow in upper area of northern slop of Alborz Mountains. Soil is an effective living factor in ecosystem balance. There are many biological and biochemical process in soil which rapidly reacts to environmental stresses. Enzymes have essential roles in these processes. The aim of this research was to study the activities of acid phosphatase and dehydrogenase in two natural hazel habitats (Makesh and Fandoghlo) and compare to their activities in an artificial habitat (Alborz). Soil sampling was done in spring and summer. The activity of acid phosphatase and dehydrogenase was evaluated by enzyme- substrate reaction. Enzymes had more activity in summer in all studied habitats. The more activity of acid phosphatase can be related to growth of hazel roots and secretion of enzyme during growth season. Enzymes showed more activity in Fandoghlo habitat in compared to Makesh ones. The amount of nitrogen, phosphorus and organic matter was less in Fandoghlo habitat which causes more activity of microorganisms to supply plants needs and compensation of low nutrients in soil.

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## Introduction

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 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).

Measurements of soil enzyme activity have been used extensively for assessment of different process occurring in nutrient cycles in soils (Tabatabai & Dick, 2002). The activity of more than 100 enzymes has been determined in soil (Tabatabai & 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), 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. It is improved that these activities are important to determine soil quality under different usages, anthropogenic and non- anthropogenic destruction and different types of habitats (Waldrop, 2000; Grierson&Adams, 2000; Sinsabaugh *et al.*, 2002; Caldwell *et al.*, 1999; Ajwa *et al.*, 1999 ).

Phosphatases are key enzymes in phosphorus cycle and their activity is a suitable indicator to determine organic phosphorus mineralization potential and soil biological activity (Tabatabai & Dick 2002). Dehydrogenase are only found in alive microbial cell and used as a microbial activity indicator (Dick,

1994). Dehydrogenase activity is a good indicator to determine microbial metabolism in soil (Tabatabai, 1994).

Several studies have been done about use of soil to evaluate soil biological potential in Iran. Shirnany (2004) reported decreasing of enzyme activity with increasing soil depth in healthy ecosystem of *Ulmus glabra* but the pattern was opposite in unhealthy ecosystem of *Ulmus glabra*. The enzyme activity was studied in touched and untouched habitats of oak in Iran by Matinizadeh *et al.*, 2008. They reported the more enzyme activity in untouched habitats of oak. Moraghebi *et al.*, 2011 showed more acid and alkaline phosphatase activity in summer in compared to spring in hazel habitats. Their results did not show significant difference between natural and artificial habitats of hazel.

Ten years ago hazel seeds was sampled from two natural habitats and sown in Alborz research center known as an artificial habitat. The aim of this study was to measure acid phosphatase and dehydrogenase activity in two natural habitats of hazel and compare to artificial ones.

## 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. 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 (Bremner&Mulvaney, 1982), and the organic-C was resolute by dichromate digestion (Walkley & Black, 1934). Olsen's bicarbonate extractable P ( $\text{PO}_4^{3-}$ ) was also measured (Olsen *et al.*, 1954).

#### *Enzyme activities*

##### *Acid phosphatase activity*

The activity of acid 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  $\rho$ - nitrophenyl phosphate 0.115 MM) and 2.0 ml MA buffer (maleate buffer 0.1 M, pH 6.5). 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  $\text{CaCl}_2$  and 4 ml of 0.5 M NaOH. Concentration of  $\rho$ - nitrophenol (NP) produced in the

assays of phosphatase activities was calculated from a  $\rho$ -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 ( $\mu\text{g}$ )  $\rho$ -nitro phenol per gram (g) soil per hour at 37°C.

#### *Dehydrogenase assay*

By using enzyme- substrate reaction, dehydrogenase changes its buffered substrate triphenyl tetrazolium chloride to triphenyl formazan at 25 °C. This product is extracted by acetone and its absorbance is measured at 546 nm by a spectrophotometer (Ohlinger, 1996b). The enzyme activity expressed as  $\mu\text{g}$  triphenyl formazan  $\text{g}^{-1} \text{h}^{-1}$  at 25 °C.

#### *Statistical analysis*

All data analyzed by T-test.

## **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 32.41 in Makesh, Fandoghlo and Alborz habitats, respectively. The nitrogen percentage was 0.43 and 0.17 and 0.25 in Makesh, Fandoghlo and Alborz habitats, respectively.

#### *Variation of acid phosphatase activity in spring and summer*

The acid phosphatase activity was measures in spring and summer in all studied habitats. It is evident from Figures 1 that acid phosphatase activity changed with sampling time in all studied habitats. Acid phosphatase activity ranged from 112.24 ( $\pm 8.23$ )  $\mu\text{g}$   $\rho$ -nitrophenol  $\text{g}^{-1} \text{h}^{-1}$  in spring to 295.54 ( $\pm 15.64$ )  $\mu\text{g}$   $\rho$ -nitrophenol  $\text{g}^{-1} \text{h}^{-1}$  in summer in Makesh habitat (Figures 1). This enzyme activity varied from 288.53 ( $\pm 18.79$ )  $\mu\text{g}$   $\rho$ -nitrophenol  $\text{g}^{-1} \text{h}^{-1}$  in spring to 678.02 ( $\pm 29.52$ )  $\mu\text{g}$   $\rho$ -nitrophenol  $\text{g}^{-1} \text{h}^{-1}$  in summer in Fandoghlo. Acid phosphatase activity ranged from 268.40 ( $\pm 29.52$ )  $\mu\text{g}$   $\rho$ -nitrophenol  $\text{g}^{-1} \text{h}^{-1}$  in spring to

620.23 ( $\pm 21.73$ )  $\mu\text{g } \rho\text{-nitrophenol g}^{-1} \text{ h}^{-1}$  in summer in Alborz habitat (Figures 1). Acid phosphatase activity was more in summer samples in all studied habitats compared to spring samples. In addition, acid phosphatase activity was more than two times higher in Fandoghlo samples in compared to Makesh ones in

both seasons. The acid phosphatase activity in spring and summer in Makesh habitat was less and showed significant difference ( $p < 1\%$ ) with two other habitats (table 2). There was no significant difference between Fandoghlo and Alborz acid phosphatase activity in spring but difference was significant ( $p < 5\%$ ) in summer samples activity (table 2).

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

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

*Variation of Dehydrogenase activity in spring and summer*

Dehydrogenase activity changed with sampling time in all studied habitats. Its activity ranged from 251.15 ( $\pm 16.28$ )  $\mu\text{g TPF g}^{-1} \text{ h}^{-1} \text{ g}^{-1} \text{ h}^{-1}$  in spring to 351.72 ( $\pm 11.53$ )  $\mu\text{g TPF g}^{-1} \text{ h}^{-1} \text{ g}^{-1} \text{ h}^{-1}$  in summer in Makesh habitat (Figures 2). This enzyme activity varied from 434.32 ( $\pm 32.29$ )  $\mu\text{g TPF g}^{-1} \text{ h}^{-1}$  in spring to 502.36 ( $\pm 21.38$ )  $\mu\text{g TPF g}^{-1} \text{ h}^{-1} \text{ g}^{-1} \text{ h}^{-1}$  in summer in Fandoghlo (Figures 2). Dehydrogenase activity changed ranged from 410.42 ( $\pm 11.18$ )  $\mu\text{g TPF g}^{-1} \text{ h}^{-1} \text{ g}^{-1} \text{ h}^{-1}$  in spring to 520.33 ( $\pm 16.29$ )  $\mu\text{g TPF g}^{-1} \text{ h}^{-1} \text{ g}^{-1} \text{ h}^{-1}$  in summer in Alborz habitat (Figures 2). Results showed that

dehydrogenase activity was more in Fandoghlo in both seasons in compared to Makesh habitat. The dehydrogenase activity in Alborz was very similar to Fandoghlo ones. There was a significant difference ( $p < 5\%$ ) between Makesh and Fandoghlo habitats in dehydrogenase activity (table 3) but among them difference ( $p < 1\%$ ) was significant, too. Dehydrogenase activity was the least in Makesh habitat and had significant difference ( $p < 1\%$ ). There was no significant difference in dehydrogenase activity between Fandoghlo and Alborz habitats in both seasons (table 3).

**Table 2.** statistical comparison of acid phosphatase activity among habitats.

	Fandoghlo- spring	Alborz- spring	Makesh –summer	Fandoghlo- summer	Fandoghlo- Alborz
Makesh –spring	**	**	**	-	-
Fandoghlo- spring	-	ns	-	**	-
Alborz- spring	ns	-	-	-	**
Makesh –summer	-	-	-	**	**
Fandoghlo- summer	**	-	-	-	*

In all studied habitats both enzymes had more activity in summer in compared to spring (figures 1, 2 and Table 2 and 3).

**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 is time consuming and can't be suitable indicators (Dick, 1994; Turco *et al.*, 1994).

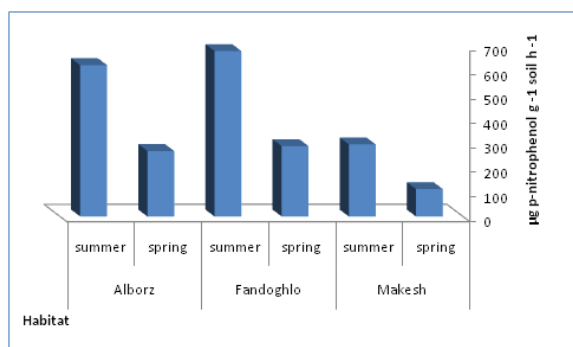
Biochemical and biological properties of soil enzyme

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). 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 & Dick, 2002; Sinsabaugh *et al.*, 2002; Kandeler, 2007).

**Table 3.** Statistical comparison of dehydrogenase activity among habitats.

	Fandoghlo- spring	Alborz- spring	Makesh –summer	Fandoghlo- summer	Alborz- summer
Makesh –spring	**	**	*	-	-
Fandoghlo- spring	-	ns	-	*	-
Alborz- spring	ns	-	-	-	**
Makesh –summer	**	-	-	**	**
Fandoghlo- summer	*	-	-	-	ns



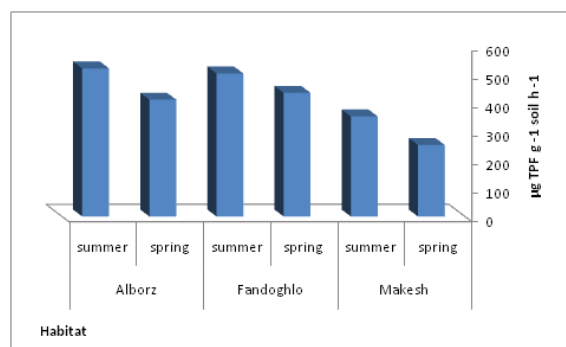
**Fig. 1.** Acid phosphatase activity in Fandoghlo, Makesh and Alborz habitats in spring and summer.

#### *The effect of season on acid phosphatase activity*

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.*, 2002). Acid phosphatase are produced mainly by plants root although microbial community produce but in less extent (Tabatabai, 1994). The acid phosphatase activity in Fandoghlo habitat in both seasons was higher significantly than in Makesh. The more density of plants in Fandoghlo causes better distribution of roots. Although the phosphorus content was less in Fandoghlo but microorganisms have better growth and activity and balanced situation in temperature and humidity resulting in more activity of microorganisms which are source of these enzymes. There is a direct relationship between extracellular

enzyme activity and plant cover as with increasing of plant cover enzyme activity increase (Amiaud & Benizi, 2005; Bastida *et al.*, 2006).

Then the more activity of this enzyme in summer can be related to more secretory activity of root in summer during their growth season that is in agreement with Kaiser & Heinemeyer (1993) finding. In addition enzyme activity was almost same that in conformity with Moraghebi *et al.*, (2010) finding.



**Fig. 2.** Dehydrogenase activity in Fandoghlo, Makesh and Alborz in spring and summer.

#### *The effect of season on dehydrogenase activity*

Dehydrogenase is only produced by alive cells (Dick, 1994) and is a good indicator of microbial metabolism in soil (Tabatabai, 1982).

The enzyme activity in Fandoghlo and Alborz habitat in both seasons was higher than in Makesh. The more density of plants in these habitats causes more balanced situation in temperature and humidity

resulting in more activity of microorganisms which are source of these enzymes. Although organic matter and carbon content in Fandoghlo is less than two other habitats but according to dehydrogenase activity, microorganisms have more activity which has conformity with Kramer & Green, (2000), Sedia & Ehrenfeld, (2006) and Moraghebi *et al.*, (2012) findings.

Summer samples had more activity in all studied habitats that shows suitable moderation in temperature and humidity. These conditions are suitable for microorganisms and increase their activity and consequently soil enzyme activity. Kaiser & Heinemeyer (1993) reported increase activity of soil enzyme activity in the end of summer.

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#### References

**Adams MA.** 1992. Phosphatase activity and phosphorus fractions in Karri (*Eucalyptus diversicolor* F. Muell.) forest soils. *Biology and Fertility of Soils* **14**, 200-204.

**Ajwa HA, Dell CJ, Rice CW.** 1999. Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. *Soil Biology and Biochemistry* **31**, 769-777.

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

**Caldwell BA, Griffiths RP, Sollins P.** 1999. Soil enzyme response to vegetation disturbance in two lowland Costa Rican soils. *Soil Biology and Biochemistry* **31**, 1603-1608.

**Clarholm M.** 1993. Microbial biomass P, labile P and acid phosphatase activity in the humus layer of a spruce forest, after repeated additions of fertilizers. *Biology and Fertility of Soils* **16**, 287-292.

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

**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. *Biological Fertilizers and Soils* **6**, 158-164.

**Gil-Sotres F, Trasar-Cepeda C, Leiros MC, Seoane S.** 2005. Different approaches to evaluate soil quality using biochemical properties. *Soil Biology and Biochemistry* **37**, 877-887.

**Grierson PF, Adams MA.** 2000. Plant species affect acid phosphatase, ergosterol and microbial P in a Jarrah (*Eucalyptus marginata* Donnex Sm.) forest in south-western Australia. *Soil Biology and Biochemistry* **32**, 1817-1827.

**Kandeler E.** 2007. Physiological and biochemical methods for studying soil biota and their function. In: Paul E. A. *Soil Microbiology, Ecology and Biochemistry*. 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. Analysis. application. Marcel Dekker, New York, . 273-340 P.

**Kramer S, Green DM.** 2000. Acid and alkaline phosphatase dynamics and their relationship to soil microclimate in semiarid woodland. *Soil Biology and Biochemistry* **32**, 179-188.

**Matinizadeh M, Korori SAA, Teimouri**

- M, Praznik W.** 2008. Enzyme Activities in Undisturbed and Disturbed Forest Soils Under Oak (*Quercusbrantii* var. *persica*) as Affected by Soil Depth and Seasonal Variation. *Asian Journal of Plant science* **7(4)**, 368-374.
- Moraghebi F.** 2001. Study and introduction of *Corylus maxima* by use of morphological characters and enzymatic. *Pajouhesh and Sazandegi* **52**, 45-51.
- Moraghebi F, Matinizadeh M, Khanjani-Shirazi B.** 2010. Mycorrhizal symbiosis in *Corylus avellana*L.and acid phosphatase activity in Makesh and Fandoghlo habitats. *Plant and Ecosystem* **24**, 13-23.
- Moraghebi F, Matinizadeh M, Khanjani-Shirazi B, Teimouri M, Afdideh F.** 2012. Seasonal variation of urease and alkaline phosphatase activity in natural and artificial habitats of hazel.*Journal of Medicinal Plants Research* **6(14)**, 2714-2720,  
<http://dx.doi.org/10.5897/JMPR10.707>
- Ohlinger R.** 1996. Acid and alkaline phosphomonoesterase activity with the substrate p-nitrophenyl phosphate. In: (Eds) *Methods in soil biology*. Springer-Verlag Berlin. 210-214.
- Olsen SR, Cole LV, Watanabe FS, Deanm 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. *Biology and Fertility of Soils* **43**, 177-189.
- Shirvany A.** 2004. Investigation of healthy and unhealthy *Ulmus glabra* Hudson to find resistant genotypes against in north of Iran. PhD thesis, natural resources faculty, Tehran university, Iran. 550 p.
- 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, 249-266 P.
- Staddon WJ, Duchesne LC, Trevors JT.** 1998.. Acid phosphatase, alkaline phosphatase and arylsulfatase activities in soils from a jack pine (*Pinusbanksiana* Lamb.) ecosystem after clear-cutting, prescribed burning, and scarification. *Biology and Fertility of Soils* **27**, 1-4.
- Tabatabai MA.** 1994. Soil enzymes. In: Page, A. L. Miller, R. H. Keeney, D. R. (Eds.) *Methods of Soil Analysis Part 2, Agronomy 9*, American Society of Agronomy Madison Wis. 903-947 P.
- Tabatabai MA, Dick WA.** 2002. Enzymes in soil. In: Burns, R. G., Dick, W. A. (Eds) *Enzymes in the environment*. Marcel Dekker, New York, 567-596.
- 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 Science Society of America Journal., Special Publication. **35**, 73-90.
- Walkley A, Black IA.** 1934. An examination of degtjareffmethod for determining soil organic matter and a proposed modification of the chromi acid titration method. *Soil Science* **37**, 29-37.
- Waldrop MP, Balser TC, Firestone MK.** 2000. Linking microbial community composition to function in a tropical soil. *Soil Biology and Biochemistry* **32**, 1837-1848.