Turk J Agric For
32 (2008)


# Determination of the Effects of Hazelnut Husk and Tea Waste Treatments on Urease Enzyme Activity and Its Kinetics in Soil

Rıdvan KIZILKAYA*, İmanverdi EKBERLİ
Ondokuz Mayıs University, Faculty of Agriculture, Department of Soil Science, 55139 Samsun - TURKEY

Received: 05.09.2007

**Abstract:** In this research, the effects of 5% treatment of hazelnut husk (HH) and tea production waste (TEW) to clay loam soil on urease enzyme activity and its kinetics were determined in a 30-day soil incubation experiment. For this purpose, kinetic parameters ($V_{max}$, $K_m$, and $V_{max}/K_m$,) were calculated by determining urease activity in organic wastes treated soils in different substrate concentrations (0%, 1%, 2%, 4%, 6%, 8%, 10%, and 12%), incubation periods (0, 1, 2, 3, 4, 5 and 6 h), and incubation temperatures (0, 10, 20, 30, 40, and 50 °C) at the end of the 30 days of the incubation. The results of the study showed that: a) Treatments of soil with hazelnut husk and tea waste increased urease activity in soil, b) the reaction velocity increased as substrate concentration increased, however this increase continued up to 8% substrate concentration level in control soil and 10% substrate concentration level in organic waste amendment soil, c) While the reaction velocity of control soil became constant at 10% substrate concentration level, it became constant at 12% substrate concentration level in organic waste amendment soil. In both control and soil treated with organic wastes, the highest reaction velocity in substrate concentrations was determined at the incubation temperature of 50 °C. The highest $V_{max}$ in control and soil treated with organic wastes (TEW and HH) was observed at 50 °C. The highest $K_m$ was observed at 40 °C in control and at 50 °C in TEW and HH treatments. The highest $V_{max}/K_m$ was observed at 50 °C in control, at 30 °C in HH treatment, and at 40 °C in TEW treatment soils.

Key Words: Organic waste, hazelnut husk, tea waste, urease, soil, kinetic parameters

## Fındık Zurufu ve Çay Atığı Uygulamalarının Toprakta Üreaz Enzim Aktivitesi ve Kinetiği Üzerine Etkisinin Belirlenmesi

**Özet:** Bu çalışmada, killi tın bünyeli toprağa %5 düzeyinde uygulanan fındık zurufu ve çay atığının toprakta üreaz aktivitesi ve kinetiği üzerine etkisi 30 günlük inkübasyon denemesi ile belirlenmiştir. Bu amaçla 30 günlük inkübasyonun sonunda farklı substrat konsantrasyonları (% 0, % 1, % 2, % 4, % 6, % 8, % 10 ve % 12), inkübasyon periyotları (1, 2, 3, 4, 5 ve 6 saat) ve inkübasyon sıcaklıklarında (0, 10, 20, 30, 40 ve 50 °C) organik atıkların uygulandığı topraklarda belirlenen üreaz aktivitesinden kinetik parametreler ($V_{max}$, $K_m$ and $V_{max}/K_m$,) hesaplanmıştır. Elde edilen sonuçlara göre sırasıyla, a) topraklara fındık zurufu (HH) ve çay atığı (TEW) uygulamasının üreaz aktivitesini artırdığı, b) reaksiyon hızının substrat konsantrasyonunun artışına bağlı olarak arttığı, ancak bu artışın kontrolde % 8 seviyesinde iken organik atık uygulanmasında ise % 10 seviyesine kadar olduğu, c) reaksiyon hızının kontrolde substrat konsantrasyonunun % 10 seviyesinden sonra sabitleşmesine karşın organik atık uygulanmasında ise % 12'den sonra sabitleştiği belirlenmiştir. Hem kontrolde hem de organik atıkların uygulandığı topraklarda en yüksek reaksiyon hızı 50 °C'lik inkübasyon sıcaklığında saptanmıştır. Kontrolde ve organik atık (HH ve TEW) uygulanmış topraklardaki en yüksek $V_{max}$ 50 °C'de belirlenmiştir. Kontrol'de en yüksek $K_m$ 40 °C'de TEW ve HH uygulamalarında ise 50 °C'de saptanmıştır. En yüksek $V_{max}/K_m$ oranları ise kontrolde 50 °C'de, HH uygulamasında 30 °C'de ve TEW uygulamasında ise 40 °C'de belirlenmiştir.

Anahtar Sözcükler: Organik atık, fındık zurufu, çay atığı, üreaz, toprak, kinetik parametreler

## Introduction

Farmers find it easier to regulate precisely the amounts of various nutrients added to the soil by inorganic fertilizers. Their applications are easy, less time-consuming and labor-intensive compared to organic sources. In addition, inorganic fertilizers have an immediate effect on yield output compared to organic sources or fertilizers, which take time to decompose and release nutrients. Therefore, loss of soil organic matter in Anatolian soils, due to intensive agriculture, is responsible

* Correspondence to: ridvank@omu.edu.tr

for a decrease in soil fertility (Kızılkaya, 2005). Declining soil fertility is almost always associated with the decline in organic matter of soil, loss of soil structure, lower water infiltration, soil compaction, increasing erodibility, and leaching leading to a decrease in nutrient holding capacities and a poorer environment for microbial activities (Joergensen and Potthoff, 2005). The most common practice to preserve and/or restore soil fertility is to add organic matter, which, preferentially, should be sufficiently stabilized to produce beneficial effects (Pasquini and Harris, 2005). Organic matter additions will also increase soil biological activity, which will affect the availability of nutrients in the soil. Soil, which has received organic matter, has increased amount of soil microbiological properties, such as enzymes, microbial populations, and their activities (Hu et al. 1999). In general, diversity and activity of soil organisms are influenced by soil organic matter properties. Furthermore, the growth and metabolic activity of soil microorganisms are limited by the availability of nutrients (Marschner et al., 2003).

Several microbiological parameters have been used to define the status and sustainable development of soil productivity in agricultural ecosystems (Visser and Parkinson,1992; Kennedy and Papendick, 1995). There are many methods currently available for studying the microorganisms and their activities at the microhabitat level (Nannipieri et al., 1990). Soil enzymes are potential indicators of soil quality because of their relationship to soil biology, ease of measurement, and rapid response to changes in soil management (Dick and Tabatabai, 1992; Kızılkaya and Bayraklı, 2005). Enzymes catalyze biochemical reactions and are an integral part of nutrient cycling in the soil. Soil enzymes may be of microbial origin (Ladd, 1978) or derived from plants and animals (Bandick and Dick, 1999; Aşkın and Kızılkaya, 2006). Urease is involved in the hydrolysis of urea-type substrates and its origin is basically microbial and its activity is extracellular (Tabatabai, 1994; Dick et al., 1996; Kızılkaya et al., 2004).

Information on the nature of urease activity of a soil and the changes in the urea hydrolysis is beneficial for developing and employing strategies for efficient N management (Ekberli et al., 2006, Kızılkaya et al. 2007). Urease activity in soil is affected by soil physico-chemical properties, agricultural practices, such as organic waste addition, pesticide application, and cultivation. While urease activity as a function of several soil parameters has been studied in detail (McGarity and Myers, 1967; Myers and McGarity, 1968; Gould et al., 1973; Pattnaik, et al., 1999; Kızılkaya and Hepşen, 2004), there has been, to our knowledge, no research on kinetic parameters of urease activity in organic waste added soil. There are many studies on the effects of incubation temperature on the activity of hydrolytic enzymes, although most of these studies have involved finding out the optimum temperature for activity (Kuprevich and Shcherbakova, 1971; Trevors, 1984; Kanazawa and Filip, 1986; Kandeler, 1990; Schinner and von Mersi, 1990; Kramer and Gren, 2000). Furthermore, most published studies on the effect of the incubation time on the activity of soil enzymes refer to hydrolytic enzymes (Beri et al., 1978).

The objective of this study was to assess the effect of hazelnut husk and tea production waste treatment on soil urease enzyme activity, its kinetics in different temperatures, and substrate concentrations in laboratory conditions on studied soil samples.

## Material and Methods

### Soil and organic wastes

Surface soil (0-20 cm) was taken from the experimental station at the Ondokuz Mayıs University Agricultural Faculty campus. The soil used was Vertic Haplustoll and contained 31.2% clay, 36.2% silt, and 32.6% sand. Soil texture was classified as clay loam (CL). The pH was 7.1, the oxidizable organic matter content was 2.26%, and the soil C:N ratio was 16. The study site had been under arable agriculture for 16 years. The site is located in the Black Sea region, northern Turkey (Latitude, 41°21'N; longitude, 36°15'W). The climate is semi humid, ($R_f$ = 47.21) with temperatures ranging from 6.6 °C in February to 23 °C in August. The annual mean temperature is 14.2 °C and annual mean precipitation is 670 mm.

Hazelnut is one of the major agricultural products in Turkey with a yield of 650,000 tons per year; it is especially produced in the Black Sea Region. Hazelnut husk (HH) was collected from hazelnut trees in the eastern Black Sea region, Turkey.

Tea plants are commonly grown in the eastern and middle Black Sea region of Turkey. This organic waste was taken from the industry of tea production in this region. All organic wastes were dried and sieved into less than 0.50 mm. The properties of the organic wastes was expressed on a moist-free basis and analyzed by standard procedures as given in Ryan et al. (2001).

### Experimental procedure

The soil samples were air-dried in a laboratory and sieved through 0-2 mm screens. The samples (750 g air-dried soil) were placed in a 1 L cylindrical plastic container. The organic wastes (HH and TEW) were thoroughly mixed with the soil at a rate equivalent to 5% on an air-dried weight basis. The untreated soil with organic waste served as control. The moisture contents in the soils were adjusted to 60% water holding capacity (WHC) and the containers were incubated at 25 ± 0.5 °C for 30 days in laboratory. The soil moisture was kept at the same level (60% WHC) by adding distilled water at regular intervals throughout the incubation period. At the end of the incubation period, these samples were used to determine urease activity of soils at the moisture condition.

### Urease activity in soil

Urease (EC 3.5.1.5) activity (UA) was measured by the method of Hoffmann and Teicher (1961). To determine urease activity, 0.25 ml toluene, 0.75 ml citrate buffer (pH, 6.7), and 1 ml of urea substrate solution were added to the 1 g soil sample and the samples were incubated. The formation of ammonium was determined spectrophotometrically at 578 nm and results were expressed as µg N $g^{-1}$ dry soil. All determinations of urease activities were performed in triplicate, and all values reported were averages of the 3 determinations expressed on an oven-dried soil basis (105 °C).

### Determination of urease kinetics ($K_m$ and $V_{max}$)

Kinetic parameters were determined using 8 different concentrations of the substrate (0%, 1%, 2%, 4%, 6%, 8%, 10% and 12%, w/v), different incubation times (1, 2, 3, 4, 5, and 6 h) and incubation temperatures (0, 10, 20, 30, 40, and 50 °C). Michaelis-Menten equation (Eq. 1) linearized by Lineweaver-Burk (Eq. 2) was used to determine $V_{max}$, $K_m$, and $V_{max}/K_m$ kinetic parameters (Tabatabai and Bremner, 1971; Tabatabai, 1973; Palmer, 1991; Atkins, 1998).

$$v = V_{max} [S] / ([S] + K_m) \quad (1)$$

where,

v = initial velocity, µg N $g^{-1}$ $min^{-1}$

[S] = Substrate urea concentration, %

$V_{max}$ = Maximum initial velocity, µg N $g^{-1}$ $min^{-1}$

$K_m$ = Michaelis constant, µg N $g^{-1}$

$$[S] / v = [S] / V_{max} + K_m / V_{max} \quad (2)$$

### Statistical analysis

The results from urease assays were examined and kinetic parameters were calculated. Means and standard deviation of triplicates were determined and all the figures presented including standard errors of the data. Analysis of variance (2-way ANOVA) was carried out using the 3 factors arranged in a randomized complete plot design. The means were compared using LSD (Least Significant Difference) test, with a significance level of $P < 0.01$. All statistical calculations were performed using MSTAT and SPSS 11.0.

## Results

It has been determined that tea waste (TEW) used in the experiment as organic waste contains 53.78% organic carbon, 2.46% nitrogen, 0.21% phosphorus, and 4.84% potassium; and hazelnut husk (HH) used in the experiment as organic waste contains 49.50% organic carbon, 0.96% nitrogen, 0.12% phosphorus, and 4.29% potassium.

The effects of adding tea waste and hazelnut husk to the activity of urease in soil at different substrate concentration and incubation periods are illustrated in Figures 1, 2, and 3. It was determined that after the addition of organic wastes (TEW and HH) in soil, increase of urease activity was higher than the control treatment, and urease activity increased in both waste and control treatments depending on substrate concentration increase.

## Discussion

### Urease activity in control and organic waste added soils

It was determined that changes in urease activity occurred by addition of different organic wastes to soil demonstrated significant variations in different temperatures and incubation periods (Figures 1, 2, and 3). It was determined that organic treatment increased urease activity of the soil compared to control. The synthesis of enzymes by microorganisms (Bremner and Mulvaney, 1978; Bandick and Dick, 1999) as a result of

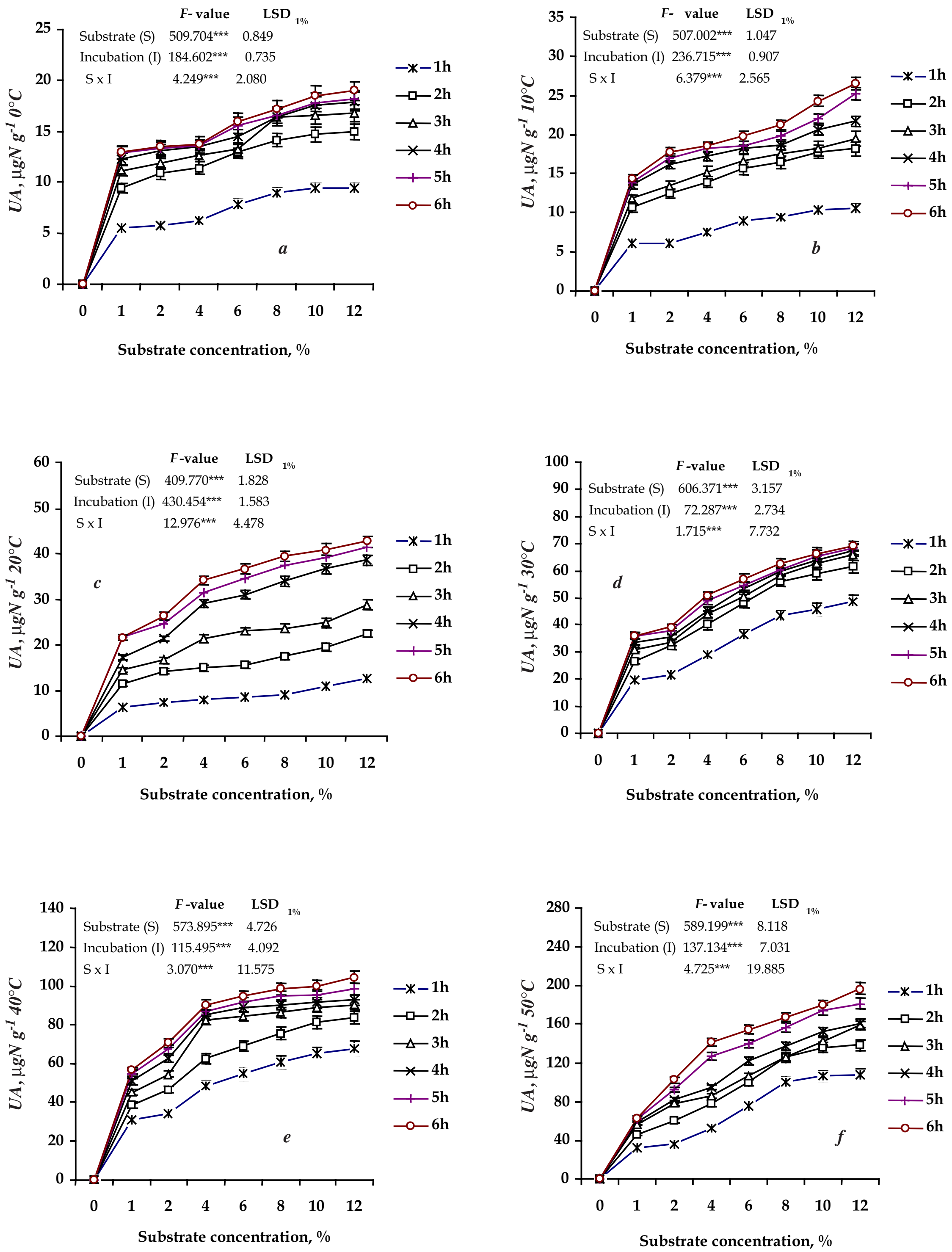


Figure 1. The variance in urease activity (UA) of the control soil determined in different substrate (urea) concentrations and incubation periods (1, 2, 3, 4, 5 and 6 h). 0 °C (b) 10 °C (c) 20 °C (d) 30 °C (e) 40 °C (f) 50 °C.

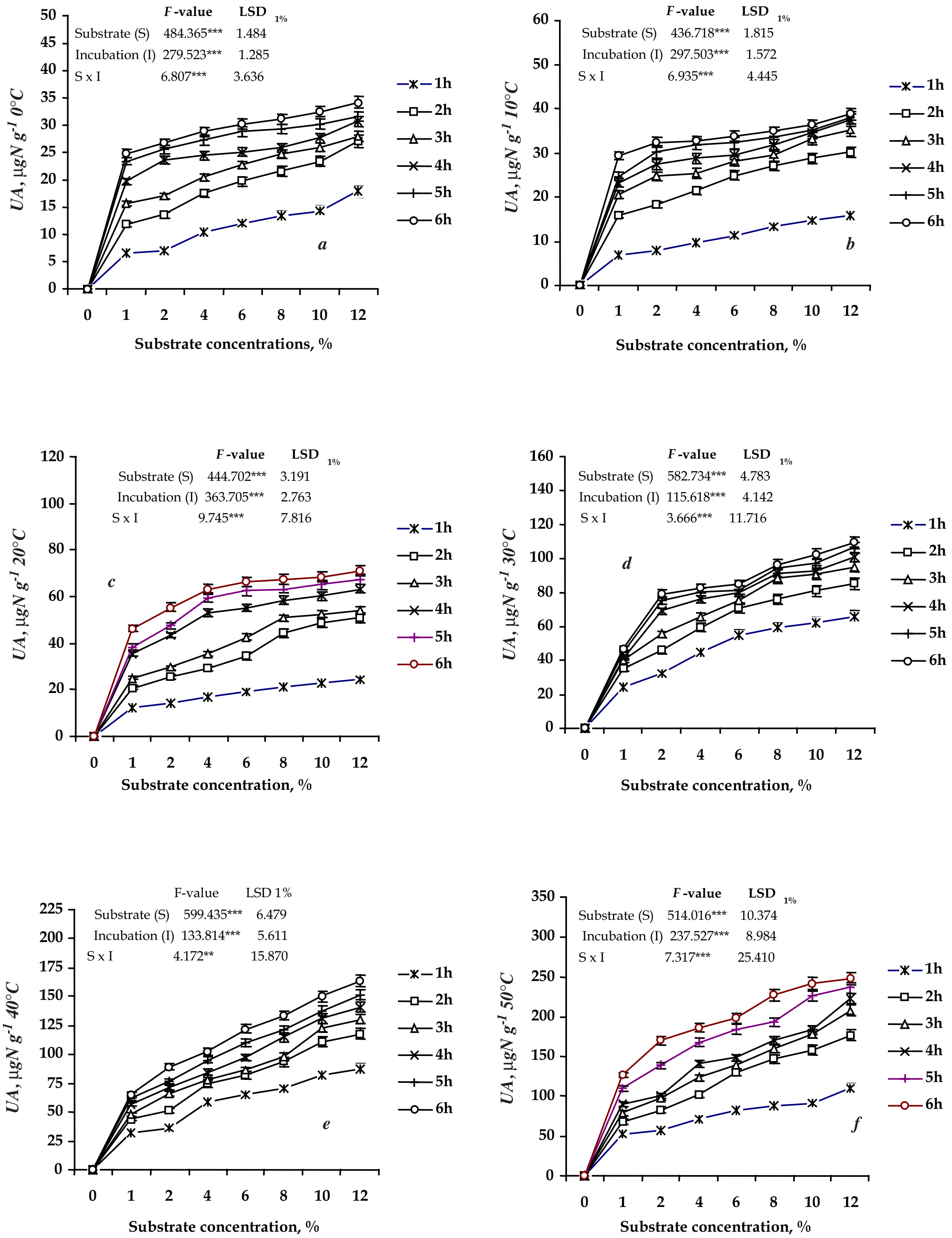


Figure 2. The variance in urease activity (UA) of hazelnut husk added soil determined in different substrate (urea) concentrations and incubation periods (1, 2, 3, 4, 5 and 6 h). (a) 0 °C (b) 10 °C (c) 20 °C (d) 30 °C (e) 40 °C (f) 50 °C.

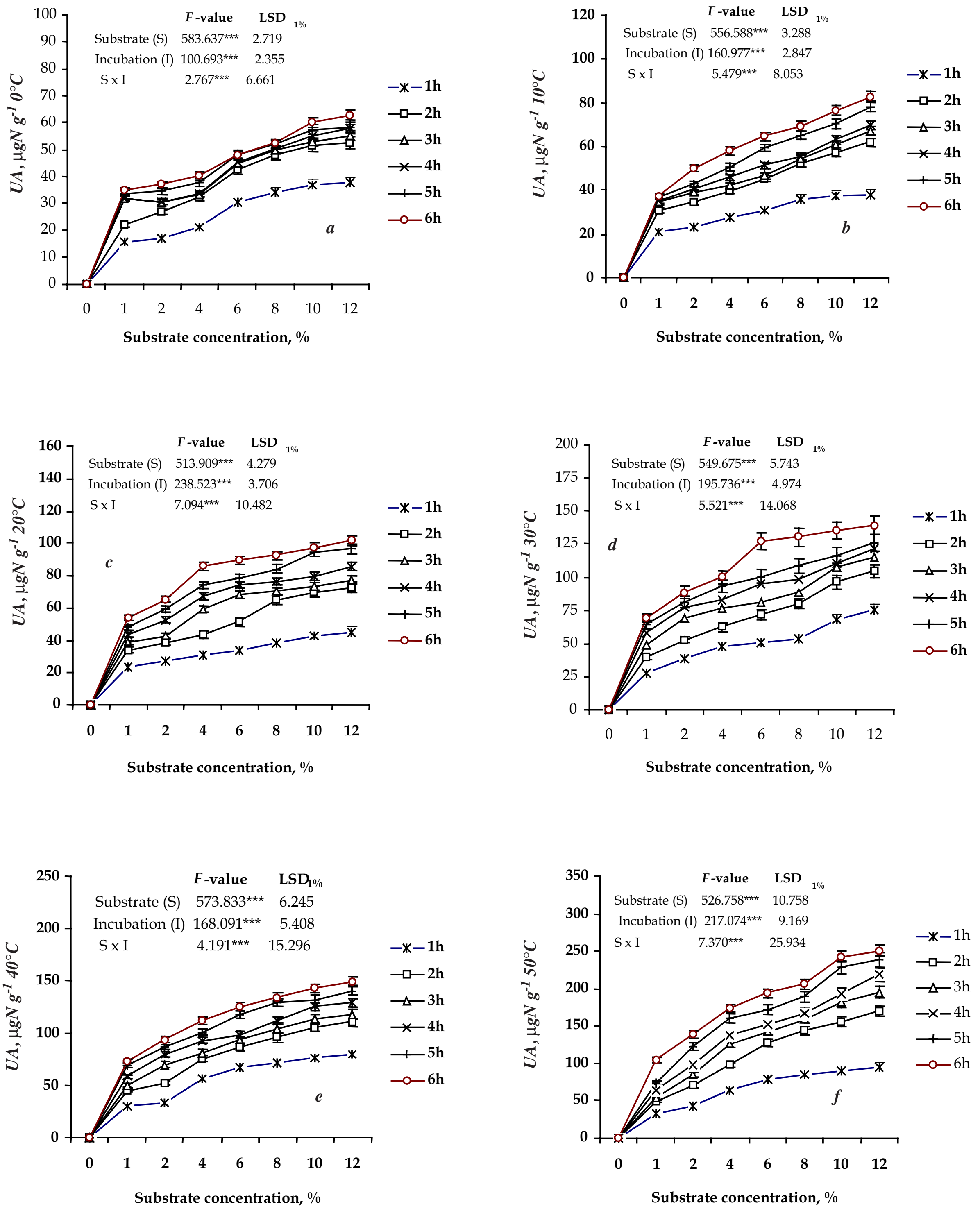


Figure 3. The variance in urease activity (UA) of tea waste added soil determined in different substrate (urea) concentrations and incubation periods (1, 2, 3, 4, 5 and 6 h). (a) 0 °C (b) 10 °C (c) 20 °C (d) 30 °C (e) 40 °C (f) 50 °C.

increasing heterotrophic microbial activity or addition of materials granting substrate characteristics to urease enzyme inside used organic wastes is a cause of increase in urease activity occurring as a result of organic waste treatment of soil (García et. al., 1993). Many other similar studies (Goyal et al., 1999; Hadas et. al., 2004; Kızılkaya and Bayraklı, 2005; Kızılkaya and Hepşen, 2007) have concluded that significant increases in the number of microorganisms in soil, consecutively in enzyme activities, due to microorganisms' activity; occurs as a result of the addition of organic compounds to soil.

It was determined that urease activity increased more with TEW treatment compared to HH, and both of which caused more urease activity compared to control. This is related with the chemical composition of the organic wastes added to the soil. The TEW used in the experiment was not only enriched with nourishment elements, such as organic carbon, N, P, and K, but also its C:N ratio was low. C:N ratio is the most important factor controlling microbiological decomposition of organic wastes in soil (Fog, 1988). If all other environmental and soil factors are appropriate, dispersion of organic compounds with lower C:N ratio occurs faster and they affect biological properties of the soil (Gunapala et al., 1998). Similar studies (Aggarwal et al., 1997; Moreno et al., 1999; Kızılkaya and Bayraklı, 2005) have determined that different organic wastes affect soil biological characteristics such as enzyme activity, number of microorganisms, microbial biomass, and respiration; organic wastes with lower C:N ratio decompose in soils and affect biological properties in a faster manner.

It was determined that urease activity both in control and organic wastes treated soils increase d with increasing substrate concentration. Besides, the occurrence of increase in urease activity was determined to take place in parallel with increase in incubation period and temperature. It was determined that most urease activity in treated soils was taken place at 50 °C incubation temperature during 6 h of incubation period. The enzyme activity increases when substrate is added to soil and it becomes constant when substrate runs out (Palmer, 1991). Therefore, the increase occurred in the urease activity in ascending substrate concentration during the incubation period is an expected result. Additionally, the main cause of the increase in urease activity along with the increase in the temperature is related with the fact that enzyme approaches to the temperature at which it demonstrates maximum activeness. In their study researching kinetics and thermodynamics of clay loam soil's urease enzyme, Ekberli et al. (2006) found similar results.

### Kinetic parameters in control and organic waste added soils

The variance of reaction velocity (v) based on ascending substrate concentration in different incubation temperatures in control soil and soil treated with organic wastes is given in Figure 4. It was determined that relationships between v and substrate concentration [S] were hyperbolic and that reaction velocity increased, as substrate concentration increased along with incubation temperature. In $[S] > 8\%$ level of substrate concentration in the control soil, v started to become constant and it became constant in the 10% level of substrate concentration. Moreover, in applications added with organic wastes, v started to become constant in $[S] > 10\%$ level and becomes constant in 12% level of substrate concentration. The highest velocity was observed in the incubation temperature of 50 °C in all applications including control.

The kinetic parameters of urease activity of soils treated with organic wastes and control soils are given in Table 1, and the Lineweaver-Burk graphic, which is a linearized form of Michaelis-Menten equation, is given in Figure 5. It was determined that $V_{max}$ increased with incubation temperature increase in organic waste treated soils. In all treatments, the highest $V_{max}$ was observed at 50 °C. At the same time, an increase in $K_m$ was observed when the incubation temperature reached at 40 °C. However, $K_m$ started to decrease when the temperature reached at 50 °C. It was also determined that variances in $K_m$ became irregular along with the increase in temperature. In both HH and TEW treated soils, the highest $K_m$ was observed at 50 °C. The lowest $K_m$ in TEW and HH treated soils was observed at 40 °C and 10 °C, consecutively. $V_{max}/K_m$ showed significant variances in different treatments. The highest level of $V_{max}/K_m$ in control and in HH and TEW treatment was observed at 50 °C, 30 °C, and 40 °C, respectively.

The determination of enzyme kinetics ($V_{max}$ and $K_m$) is often undertaken with the purpose of characterizing free enzymes in solution; $V_{max}$ is the maximum rate of activity when all enzymes are substrate-saturated, and $K_m$, also called as Michaelis constant, is equal to the substrate

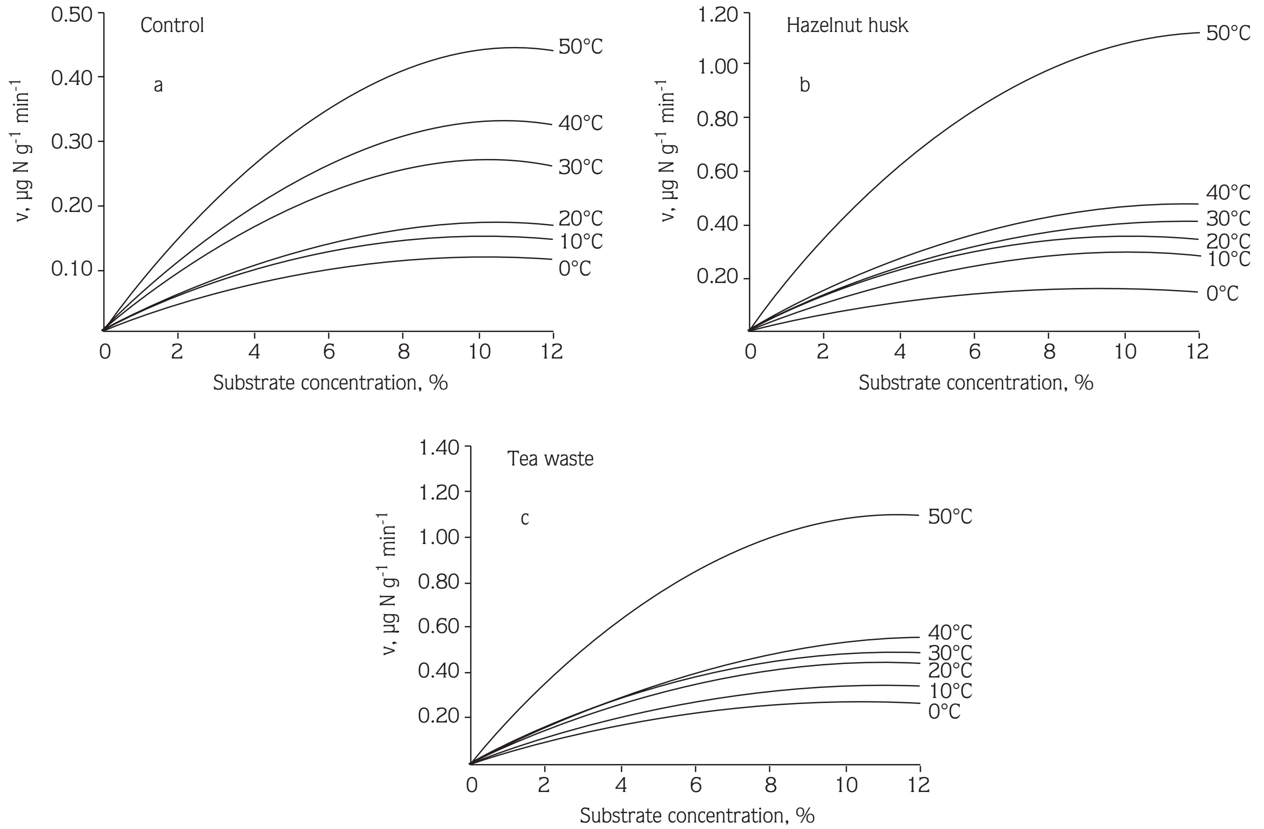


Figure 4. The relationships between substrate concentrations and v in organic waste such as hazelnut husk and tea waste added soil and control (a) control (b) hazelnut husk added soil (c) tea waste added soil.

Table 1. Kinetic parameters of clay loam soil in different incubation temperatures.

| Kinetic parameters | | Temperature, °C | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 °C | 10 °C | 20 °C | 30 °C | 40 °C | 50 °C |
| Control soil | $V_{max}$, µg N $g^{-1}$ $min^{-1}$ | 0.094 | 0.129 | 0.149 | 0.237 | 0.304 | 0.537 |
| | $K_m$, µg N $g^{-1}$ | 0.396 | 0.641 | 0.744 | 0.939 | 1.329 | 1.177 |
| | $V_{max}$ / $K_m$, $min^{-1}$ | 0.236 | 0.201 | 0.200 | 0.253 | 0.229 | 0.456 |
| Hazelnut husk | $V_{max}$, µg N $g^{-1}$ $min^{-1}$ | 0.151 | 0.243 | 0.404 | 0.309 | 0.418 | 1.372 |
| added soil | $K_m$, µg N $g^{-1}$ | 0.779 | 0.633 | 1.989 | 0.659 | 1.123 | 4.325 |
| | $V_{max}$/ $K_m$, $min^{-1}$ | 0.194 | 0.385 | 0.203 | 0.469 | 0.372 | 0.317 |
| Tea waste | $V_{max}$, µg N $g^{-1}$ $min^{-1}$ | 0.276 | 0.314 | 0.414 | 0.459 | 0.450 | 1.406 |
| added soil | $K_m$, µg N $g^{-1}$ | 1.652 | 1.069 | 1.549 | 1.521 | 0.847 | 4.131 |
| | $V_{max}$/ $K_m$, $min^{-1}$ | 0.167 | 0.294 | 0.267 | 0.302 | 0.531 | 0.340 |

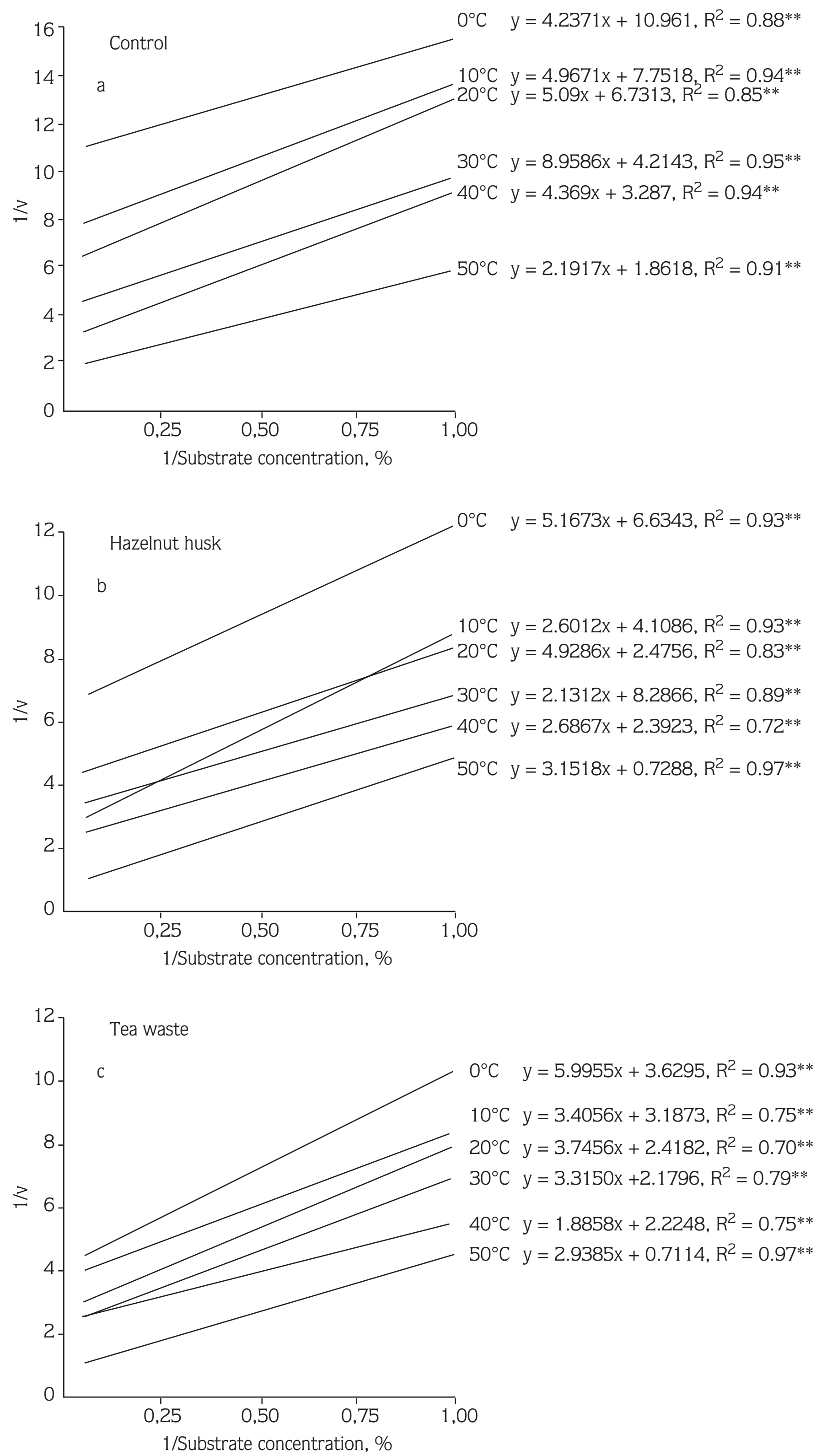


Figure 5. Curves of Lineweaver – Burk equations (a) control (b) hazelnut husk added soil (c) tea waste added soil.

concentration at $V_{max}/2$. $V_{max}$ and $K_m$ are considered to be constant for a specific enzyme under defined experimental conditions (Marx et al., 2005). $V_{max}$ shows dispersion speed of enzyme-substrate complex into enzyme and reaction outputs. The higher or lower value of this indicator implies to speedy or slow enzymatic procedures in soil. $K_m$, which is related with substrate, is a criterion of enzyme amount. $K_m$ represents the endurance of an enzyme-substrate complex. The reverse proportion was found between the value of $K_m$ and enzyme-substrate complex meaning that the endurance of an enzyme-substrate complex is high, when $K_m$ value is low, and vice versa. $V_{max}/K_m$ represents the formation of an enzyme-substrate complex in soil and the comparison of dispersion of this complex in the soil. The higher value of this proportion implies to the fact that dispersion of enzyme-substrate complex occurs faster than its formation (Paulson and Kurtz, 1969; Tabatabai and Bremner, 1971; Tabatabai, 1973; Aliyev et al., 1981; Ekberli and Kızılkaya, 2006; Kızılkaya et al., 2007).

It was determined that organic waste treatments caused significant variances in urease activity and kinetic parameters derived from urease activity. These variances occurred as a result of an increase in $V_{max}$, $K_m$, and $V_{max}/K_m$ due to the organic waste treatments (Table 1). The most important causes of this increase are: i) The occurrence of appropriate environment for enzyme activities due to changes in physico-chemical properties of soil caused by organic waste treatments (Dick and Tabatabai, 1992; Sarkar et al., 2003); ii) The increase in the enzyme activity due to compounds containing N, which is also located in the chemical structure of organic wastes and considered to be the source of substrate for enzymes (García, et al., 1993; Kızılkaya and Bayraklı, 2005); iii) the stimulation of microbial activity in soil by organic wastes including compounds containing N, as well as nourishment substances, such as N, P, and K; and synthesis of enzyme by increasing microbial population (Bremner and Mulvaney, 1978; Bandick and Dick, 1999).

It was determined that there were variances in the effects of HH and TEW treatments on urease enzyme and its kinetics (Table 1). This occurrence is probably related with the differences in the chemical compositions of organic wastes and their decomposition in soil. The decomposition rate of TEW was lower compared to HH because C/N ratio (22:1) of TEW was less than C/N ratio (52:1) of HH. As a result, it was determined that $V_{max}$ level of TEW was higher than $V_{max}$ level of HH. Besides, the highest $V_{max}$ level was observed at 50 °C. The reason for high $V_{max}$ values occurring along with temperature increase in all soil treatments is thought to be related with the activation of enzymes with low bonds or immobilized enzymes by their decomposition into soil solutions as a result of temperature increase and with the probability of the catalyst effect of organic waste treatments on this process.

It was determined that $K_m$ values in HH treatment were lower in 10 and 20 °C and higher in 40 and 50 °C when mixed with TEW (Table 1). The main reason of variance occurred in $K_m$ as a result of treatment of soil with the same level of organic wastes composing of particles with equal magnitudes was for sure related with difference in chemical compositions of organic wastes. This situation implies that HH, which has a greater C/N ratio and lower decomposition rate in soil, can be produced faster because it decomposes quickly due to the loose enzyme-substrate complex formed as a result of the increase in the incubation temperature from 40 °C to 50 °C. The obtained result shows that contribution of more resistible organic materials like HH, compared to TEW, will increase the amount of inorganic-N as a result of urease activity depending on the temperature level increase in soil. In addition, the product with a formation of enzyme-substrate complex originated as a result of HH addition to soil was higher than the one originated as a result of TEW with lower C/N ratio, under the condition of temperature raise up to 50 °C. This condition plays an important role in the treatment of soil with organic wastes and its management. The highest values of $V_{max}/K_m$, which indicate how fast this complex originated as a result of enzyme-substrate complex formation decomposes, were observed at 50 °C in control, at 30 °C in HH treatment, and 40 °C in TEW treatment.

It was determined that the organic waste treatment of soils not only affected urease enzyme activity in soil, but also significantly affected formation, decomposition, and resistance of enzyme-substrate complex. The resistance of enzyme-substrate with no organic waste treatment can last in a temperature up to 40 °C and the optimum temperature level for transformation of this complex to yield is 40 °C. However, the optimum level of temperature for decomposition of enzyme-substrate complex as a result of organic material treatment to soils is lower. This condition implies that climate related factors have to be taken into consideration in agricultural practices, such as organic waste treatment of soil and its management. In addition to all outputs of this research, soil physico–chemical properties demonstrate significant variance along very short distances in field and significant effect of agricultural practices such as organic waste treatment on enzyme activities of soil. The determination of urease activity and its kinetic parameters in an experimental hazelnut husk and tea waste treated soil (clay loam) is not adequate. Also, further studies are needed to determine the effects of other organic wastes and textural class on enzyme activity and their kinetic parameters.

## References


Aggarwal, R.K., P. Kumar and J.F. Power. 1997. Use of crop residue and manure to conserve water and enhance nutrient availability and pearl millet yields in an arid tropical region. Soil Till. Res. 41: 43-51.

Aliyev, S.A., D.A. Gadzhiyev and F.D. Mikaylov. 1981. Kinetic indexes of catalase activity in the main soil groups of Azerbaijan. Soviet Soil Sci. 13: 29-35.

Aşkın, T. and R. Kızılkaya. 2006. Assessing spatial variability of soil enzyme activities in pasture topsoils using geostatistics. Eur. J. Soil Biol. 42: 230-237.

Atkins, P.W., 1998. Physical Chemistry, 6$^{th}$ Edition. Oxford University Press. UK.

Bandick, A.K. and R.P. Dick. 1999. Field management effects on soil enzyme activities. Soil Biol. Biochem. 31: 1471-1479.

Beri, V., K.P. Goswami and S.S. Brar. 1978. Urease activity and its michaelis constant for soil systems. Plant Soil. 49: 105-115.

Bremner, J.M. and R.L. Mulvaney. 1978. Urease activity in soils. In: Soil enzymes (Ed: R.G. Burns). Academic Press- New York, pp. 149-196.

Dick, W.A. and M.A. Tabatabai. 1992. Potential uses of soil enzymes. In: Soil microbial ecology: applications in agricultural and environmental management (Ed: F.B. Meeting). Marcel Dekker-New York, pp. 95-127.

Dick, R.P., D. Breakwill and R. Turco. 1996. Soil enzyme activities and biodiversity measurements as integrating biological indicators. In: Handbook of methods for assessment of soil quality (Eds: J.W. Doran and A.J. Jones). Soil Science Society of America- Madison, pp. 247-272.

Ekberli, İ., and R. Kızılkaya. 2006. Catalase enzyme and its kinetic parameters in earthworm *L.terrestris* casts and surrounding soil. Asian J. Chem. 18: 2321-2328.

Ekberli, İ., R. Kızılkaya and N. Kars. 2006. Urease enzyme and its kinetic and thermodynamic parameters in clay loam soil. Asian J. Chem. 18: 3097-3105.

Fog, K. 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Rev. 63: 433-462.

García, C., T. Hernández, C. Costa, B. Ceccanti, G. Masciandaro and C. Ciardi. 1993. A study of biochemical parameters of composted and fresh municipal wastes. Bioresource Technol. 44: 17-23.

Gould, W. D., F. D. Cook and C. R. Webster. 1973. Factors affecting urea hydrolysis in several Alberta soils. Plant Soil. 38: 393-401.

Goyal, S., K. Chander, M.C. Mundra and K.K. Kapoor. 1999. Influence of inorganic fertilizers and organic amendments on soil organic matter and soil microbial properties under tropical conditions. Biol. Fertil. Soils. 29: 196-200.

Gunapala, N., R.C. Venette, H. Ferris and K.M. Scow. 1998. Effects of soil management history on the rate of organic matter decomposition. Soil Biol. Biochem. 30: 1917-1927.

Hadas, A., L. Kautsky, M. Goek and E.E. Kara, 2004. Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. Soil Biol. Biochem. 36: 255-266.

Hoffmann, G.G. und K. Teicher. 1961. Ein Kolorimetrisches Verfahren zur Bestimmung der Urease Aktivitat in Böden. Z. Pflanzenernähr. Bodenk. 91: 55-63.

Hu, S.J., A.H.C. van Bruggen and N.J. Grünwald. 1999. Dynamics of bacterial populations in relation to carbon availability in a residue-amended soil. Appl. Soil Ecol. 13: 21-30.

Joergensen, R.G. and M. Potthoff. 2005. Microbial reaction in activity, biomass, and community structure after long-term continuous mixing of a grassland soil Soil Biol. Biochem. 37: 1249-1258

Kanazawa, S. and Z. Filip. 1986. Distribution of microorganisms, total biomass, and enzyme activities in different particles of brown soil. Microb. Ecol. 12: 205-215.

Kandeler, E. 1990. Characterization of free and adsorbed phosphatases in soils. Biol. Fertil. Soils. 9: 199-202.

Kennedy, A.C. and R.I. Papendick. 1995. Microbial characteristics of soil quality. J. Soil Water Conserv. 50: 243-248.

Kızılkaya, R., 2005. The role of different organic wastes on zinc bioaccumulation by earthworm *Lumbricus terrestris* L. (Oligochaeta) in successive Zn added soil. Ecol. Eng. 25: 322-331.

Kızılkaya, R. and Ş. Hepşen. 2004. Effect of biosolid amendment on enzyme activities in earthworm (*Lumbricus terrestris*) casts. J. Plant Nutr. Soil Sci. 167: 202-208.

Kızılkaya, R. and Ş. Hepşen. 2007. Microbiological properties in earthworm *Lumbricus terrestris* L. cast and surrounding soil amended with various organic wastes. Commun. Soil Sci. Plant Anal. 38: 2861-2876.

Kızılkaya, R., T. Aşkın, B. Bayraklı and M. Sağlam. 2004. Microbiological characteristics of soils contaminated with heavy metals. Eur. J. Soil Biol. 40: 95-102.

Kızılkaya, R. and B. Bayraklı. 2005. Effects of N-enriched sewage sludge on soil enzyme activities. Appl. Soil Ecol. 30: 192-202.

Kızılkaya, R., İ. Ekberli and N. Kars. 2007. Tütün atığı ve buğday samanı uygulanmış toprakta üreaz aktivitesi ve kinetiği. Ankara Üniversitesi Ziraat Fakültesi Tarım Bilimleri Dergisi. 13: 186-194.

Kuprevich, V.F. and T.A. Shcherbakova. 1971. Comparative enzymatic activity in diverse types of soil. In: Soil Biochemistry (Eds: A.D. McLaren and J. Skujins) Marcel Dekker- New York, pp. 167-201.

Kramer, S. and D.M. Gren. 2000. Acid and alkaline phosphatase dynamics and their relationship to soil microclimate in a semiarid woodland. Soil Biol. Biochem. 32: 179-188.

Ladd, J.N. 1978. Origin and range of enzymes in soil. In: Soil Enzymes (Ed: R.G. Burns) Academic Press- London, pp. 51-96.

Marschner, P., E. Kandeler and B. Marschner. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biol. Biochem. 35: 453-461.

Marx, M.C., E. Kandeler, M. Wood, N. Wermbter and S.C. Jarvis. 2005. Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. Soil Biol. Biochem. 37: 35-48.

McGarity, J.W. and M.C. Myers. 1967. A survey of urease activity in soils of Northern New South Wales. Plant Soil. 27: 217-238.

Moreno, J.L., T. Hernandez and C. Garcia. 1999. Effects of a cadmium-contaminated sewage sludge compost on dynamics of organic matter and microbial activity in an arid soil. Biol. Fertil. Soils. 28: 230-237.

Myers, M.C. and J.W. McGarity. 1968. The urease activity in profiles of five great soil groups from Northern New South Wales. Plant Soil. 28: 25-37.

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

Palmer, T. 1991. Understanding enzymes. Chichester, Ellis Horwood, USA.

Pasquini, M.W. and F. Harris. 2005. Efficient use of resources: urban waste ash and soil fertility on the Jos Plateau, Nigeria. AREA. 37: 17-29

Pattnaik, P., K. Mallick, B. Ramakrishnan, T.K. Adhya and N. Sethunathan. 1999. Urease activity and urea hydrolysis in tropical flooded soil unplanted or planted to rice. J. Sci. Food Agr. 79: 227-231.

Paulson, K.N. and L.T. Kurtz. 1969. Locus of urease activity in soil. Soil Sci. Soc. Amer. Proc. 33: 897-901.

Ryan, J., G. Estefan and A. Rashid. 2001. Soil and plant analysis laboratory manual. International Center for Agricultural Research in the Dry Areas (ICARDA). Syria.

Sarkar, S., S.R. Singh and R.P. Singh. 2003. The effect of organic and inorganic fertilizers on soil physical condition and the productivity of a rice–lentil cropping sequence in India. J. Agr. Sci. 140: 419-425.

Schinner, F. and W. von Mersi. 1990. Xylanase-, CM-Cellulase- and invertase activity in soil, an improved method. Soil Biol. Biochem. 22: 511-515.

Tabatabai, M.A. 1973. Michaelis contants of urease in soil and soil fractions. Soil Sci. Soc. Amer. Proc. 37: 707-710.

Tabatabai, M.A. 1994. Enzymes, In: Methods of soil analysis. microbiological and biochemical properties, Part 2 (Eds: R.W. Weaver, S. Augle, P.J. Bottomly, D. Bezdicek, S. Smith, M.A. Tabatabai and A. Wollum) Soil Science Society of America-Madison, pp. 775-783.

Tabatabai, M.A. and J.M. Bremner. 1971. Michaelis constants of soil enzymes. Soil Biol. Biochem. 3: 317-323.

Trevors, J.T. 1984. Dehydrogenase activity in soil. A comparison between INT and TTC assay. Soil Biol. Biochem. 16: 673-674.

Visser, S. and D. Parkinson. 1992. Soil biological criteria as indicator of sol quality: soil microorganisms. Am. J. Alternative Agr. 7: 33-37.