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Catalase Enzyme and its Kinetic Parameters in Earthworm *L. terrestris* Casts and Surrounding Soil

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Catalase enzyme and its kinetic parameters (V_{max}, K_m and V_{max}/K_m) were determined in order to assess adding earthworm *L. terrestris* to the soil. As a result, it has been determined that there is a low level of catalase activities, which is an important criterion in evaluation of aerobic micro-organisms, despite the fact that earthworm casts contain higher levels of organic C and nutrients (N, P, K, Ca, Mg, Fe, Cu, Zn and Mn) in comparison with surrounding soil and the control (the soil in which the earthworms are not included). It was also determined that the highest levels of both catalase activities and kinetic parameters occur in surrounding soil.

Key Words: Earthworm, Kinetic parameters, Soil, Catalase.

INTRODUCTION

Earthworms have much importance in soil ecosystem functioning and are ubiquitous soil animals that dominate the invertebrate biomass in agricultural soils[1]. Worms play a major role in altering the development of the soil profile, especially near the soil surface, and contribute significantly to organic matter turnover by ingesting and fragmenting large quantities of litter and incorporating it into the mineral soil and, as a result, fresh casts contain higher nutrient content than non-ingested soil[1–6]. Anecic earthworm species, such as *Lumbricus terrestris* L., are among the most important groups of soil animals involved in fragmenting litter, in incorporating plant residues into the soil and in forming soil aggregates[7, 8]. Earthworms prefer a diet of decomposed and decomposing organic matter, which suggests that micro-organisms are also ingested as food. The invasion by anecic earthworms has been linked to significant changes in organic matter breakdown and nutrient dynamics[9, 10]. These changes can be expected to have significant effects on soil microbial and enzymatic activities[2, 6, 11–15].

Catalase activity in soils is affected by physico-chemical (clay content, soil moisture, soil depth, temperature, organic matter, pH and nutrients) and biological (microbial population and their activity) properties and these properties play a key role among them. As far as physico-chemical soil properties are concerned, numerous studies[16–19] have focussed on the carbon content and its positive impact on catalase activity, relationships between organic matter and pH. Relationships between catalase activity and soil physico-chemical properties (soil depth, soil

organic matter, texture, moisture, etc.) have been described by Formánek and Vranová[17], Arcak *et al.*[20] and Kizilkaya *et al.*[21] On the contrary, not much information on catalase enzyme kinetics in soils and earthworm casts is available.

The objective of the present study is quantifying the catalase activity and its kinetic parameters in soil and earthworm (*Lumbricus terrestris* L.) casts. The standard technique of volumetric analysis has been employed as a measure of catalase activity[22]. We have intended to demonstrate the level of substrate concentration and time to affect catalase activity and its kinetics in earthworm cast and surrounding soil compared the "control" (the soil in which the earthworms are not included). These studies can then help us to interpret related responses of aerobic micro-organisms' activities, both catalase activity and catalase kinetics, in wormcasts and soil.

EXPERIMENTAL

Surface soil (0–20 cm) was taken from the experimental station in the campus of Ondokuz Mayis University Agricultural Faculty. The soil had been developed from basalt and contained 31.2% clay, 36.2% silt and 32.6% sand. Soil texture can accordingly be classified as clay loam (CL). The pH in water was 7.1, the oxidizable organic matter content was 2.26% and the soil C : N ratio was 1 : 6.

The earthworm *Lumbricus terrestris* L. species are considered to be anecic worms. They were collected from the same plot as the soil. Earthworms were washed with distilled water and kept for 2 weeks before starting the experiment in containers with appropriate soil at 20 ± 0.5°C.

Experimental procedure and wormcasts production

The soil samples were air-dried in the laboratory and sieved through 0–2 mm screens. The samples (500 g air-dried soil) were placed in 1 L cylindrical plastic container. Then, three individuals of *L. terrestris* each weighing between 7.0 and 7.5 g were placed in the soil. The soil in which the earthworms were not included was assumed as control soil. The moisture contents in the soils were adjusted to 60% water holding capacity (WHC) and the containers were incubated at 20 ± 0.5°C for 30 d in dark. The soil moisture was kept at the same level (60% WHC) by adding distilled water at regular intervals throughout the incubation period. Five replicates were performed. At the end of the incubation period, samples were collected by hand from earthworm casts deposited on the soil surface and from bulk soil.

Catalase activity in wormcasts and soil

Catalase activity (EC 1.11.1.6) was measured by the method of Beck[22]. 10 mL of phosphate buffer (pH, 7) and 5 mL of a 3% H_2O_2 substrate solution were added to 5 g of the sample. The volume (mL) of O_2 released within 3 min at 20°C was determined. Three replicates of each sample were tested and controls were tested in the same way, but with the addition of 2 mL of 6.5% (w/v) NaN_3. Results were expressed as mL O_2 g^{-1} dry sample.

Kinetic parameters in wormcasts and soil

Kinetic parameters were determined by using ten different concentrations of the substrate, H_2O_2, varying from unsaturated to saturated conditions: 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10% v/v each at different incubation times (0, 0.5, 1, 2, 3, 4 and 5 min). Michaelis-Menten equation (Eqn. 1) linearized by Lineweaver-Burk (Eqn. 2) is used to determine V_{max}, K_m and V_{max}/K_m kinetic parameters.

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

Here, v = initial velocity, mL O_2 g^{-1} min^{-1}; [S] = substrate (H_2O_2) concentration, %; V_{max} = Maximum initial velocity and K_m = Michaelis constant.

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

Organic C and nutrient contents in wormcasts and soil

Organic C (C_{org}) and nutrient contents of soils and wormcasts were determined as oxidizable carbon and organic matter were measured by wet oxidation method with $K_2Cr_2O_7$. Total N was determined by digestion and subsequent measurement was executed by the Kjeldahl method. Available P was extracted from wormcasts and soil samples (0.5 M $NaHCO_3$) by Olsen method. Exchangeable K, Ca and Mg were determined by 1 N NH_4OAc extraction; available micro-nutrients (Fe, Cu, Zn and Mn) were determined by extraction with diethylene triamine pentaacetic acid (DTPA) solution (0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA buffered at pH 7.3) and were analyzed by atomic absorption spectrophotometer[23, 24].

RESULTS AND DISCUSSION

C_{org} and nutrient contents in wormcasts and soil

In the content of C_{org}, macro and micro-nutrient contents were higher in casts of earthworm *L. terrestris* compared to the surrounding soil and the control (Table-1). Earthworm casts, in general, are higher in available nutrients than the surrounding and control, because the organic materials in soil have been partially decomposed during passage through the earthworm gut, converting the organic nutrients to more available forms. Sharpley and Syers[25] and Kizilkaya *et al.*[6] suggested that there was a higher proportion of C_{org} in the casts than in the surrounding soil. On the one hand, the composition of the cast material was a consequence of the effectiveness of the digestion process of the worm. Shaw *et al.*[26] and Daniel *et al.*[13] assumed that there was a higher nutrient concentration in the earthworm casting in an expression of their incomplete resorption. Enrichment of nutrients in earthworm casting is also reported by Scheu[11], Parkin and Berry[27] and Kizilkaya[9]. In this study, the highest level of C_{org}, macro and micro-nutrient contents was observed in wormcasts while it had the lowest level in the control soil. The order of C_{org} and nutrients was as follows: worm casts > surrounding soil > control soil.

TABLE-1
C_{org} AND NUTRIENT CONTENTS IN EARTHWORM CAST AND SOIL

	C_{org} (%)	Macro-nutrients (μg g^{-1})					Micro-nutrients (μg g^{-1})			
		N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
Control soil	1.31	816	7.23	489	608	132	5.85	0.19	0.35	3.81
Surrounding soil	1.32	856	7.52	512	678	165	6.13	0.21	1.21	4.63
Earthworm cast	1.89	1172	16.41	968	1214	257	23.48	0.68	6.40	5.30

Catalase activity in wormcasts and soil

Like C_{org}, macro and micro-nutrient contents, catalase activity showed different values in earthworm casts, surrounding soil and control soil relative to similar substrate concentration and incubation times. The highest level of catalase activity was observed in the surrounding soil while the lowest level was observed in earthworm casts at all substrate concentrations and incubation times. The order of catalase activity was surrounding soil > control > wormcasts. Several researchers suggested that the higher nutrient enrichment of the earthworm casts supported higher extracellular enzymatic activity and microbial population[2, 6, 12–14, 25, 28]. Similarly, various authors[2, 6, 12, 28] also found higher dehydrogenase and extracellular enzyme such as urease, phosphatase and sulphatase activities in nutrient enriched earthworm casts. On the contrary, in this study opposite results were found compared to these studies. It can be explained with origin and sensitivity of enzymatic factors. The CA is sensitive to both natural and O_2 level and shows a quick response to the induced changes. Also it may be affected by cast formation by earthworm in anaerobic conditions. The CA is based on the rates of oxygen release from the added hydrogen peroxide and may be related to the metabolic activity of aerobic organisms[6, 19]. The catalase activity in earthworm casts can be attributed to low levels of catalase activity in the material itself and the inhibition of aerobic microbial activity.

Different substrate concentrations and incubation times significantly affected the levels of catalase activity in the earthworm casts, surrounding soil and the control. Fig. 1 shows that the increasing H_2O_2 concentrations contain higher catalase activity than those of decreasing substrate concentrations at all incubation times in the casts, surrounding soils and the control. In general, substrate concentrations with initial high H_2O_2 concentration had high catalase activity at all samples such as the casts and soils (Fig. 1). Catalase activity showed a similar trend in wormcasts, surrounding soil and the control: an increase in the first 3 min of incubation was followed by a pronounced invariable in catalase activities. The catalase activity is clearly the decomposition of H_2O_2. The rate of catalase activity is influenced by H_2O_2 concentration and incubation times.

Kinetic parameters in wormcasts and soil

Fig. 1 shows the variations in catalase activity of earthworm casts, surrounding soil and control with incubation times at different substrate (H_2O_2) concentrations. Similarly, Fig. 2 shows the relationships, hyperbolic function or relation, between

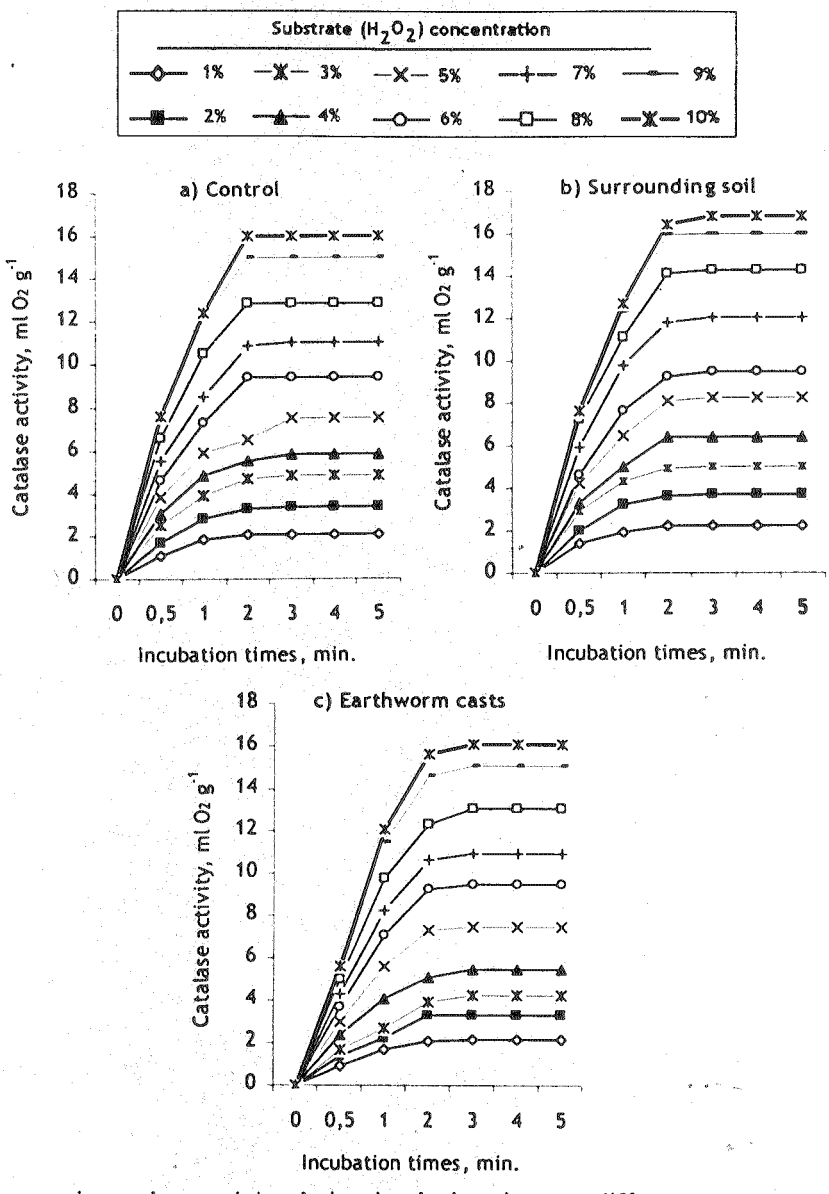

Fig. 1. Changes in catalase activity during incubation times at different substrate (H_2O_2) concentration

velocity and substrate concentration in samples such as wormcasts, surrounding soil and control. Kinetic parameters in earthworm casts, surrounding soil and control are given in Table-2.

TABLE-2
KINETIC PARAMETERS (V_{max}, K_m AND V_{max}/K_m) OF CATALASE ACTIVITY IN WORMCASTS, SURROUNDING SOIL AND CONTROL

	V_{max} (mL O_2 g^{-1} min^{-1})	K_m (mL O_2 g^{-1})	V_{max}/K_m (min^{-1})
Control soil	10.25	14.305	0.72
Surrounding soil	11.68	15.547	0.75
Earthworm cast	8.76	12.033	0.73

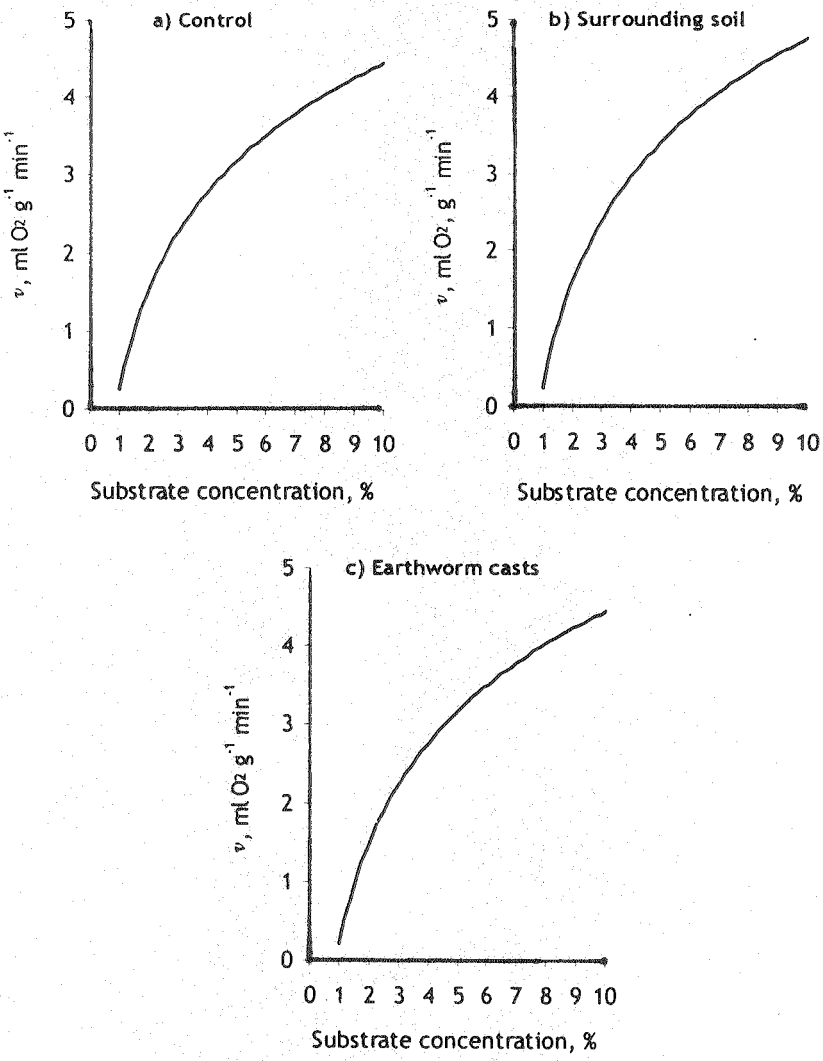

Fig. 2. The relationships between substrate concentrations and v in earthworm casts and soil such as surrounding soil and control

Like catalase activity, the highest V_{max}, K_m and V_{max}/K_m ratios were observed in the surrounding soil. V_{max} and K_m of an enzyme express the quantity of enzyme and substrate affinity, respectively. Enzymes catalyzing the same reactions can have different sources in soil and, thus, different K_m values[29]. In addition, several authors observed that different management systems and agricultural inputs can influence K_m values[15, 30]. In this study, it was determined that earthworm ingesting and living in soils can influence kinetic parameters in soils.

The highest V_{max} value was observed in the surrounding soil compared to the earthworm casts and the control (Table-2). According to the linearized Michaelis-Menten theory by Lineweaver-Burk (Fig. 3), this means a longer vale of catalase activity in soil, which may be the result of higher aerobic micro-organisms. Hence, the addition of earthworm *L. terrestris* in the soil (surrounding soil) may have increased catalase activity and consequently aerobic microbial activity. K_m is independent of enzyme concentration and kinetically reflects the apparent affinity of the enzyme for the substrate: the smaller the K_m value, the greater the affinity. K_m value was lower in earthworm casts than in surrounding soil and

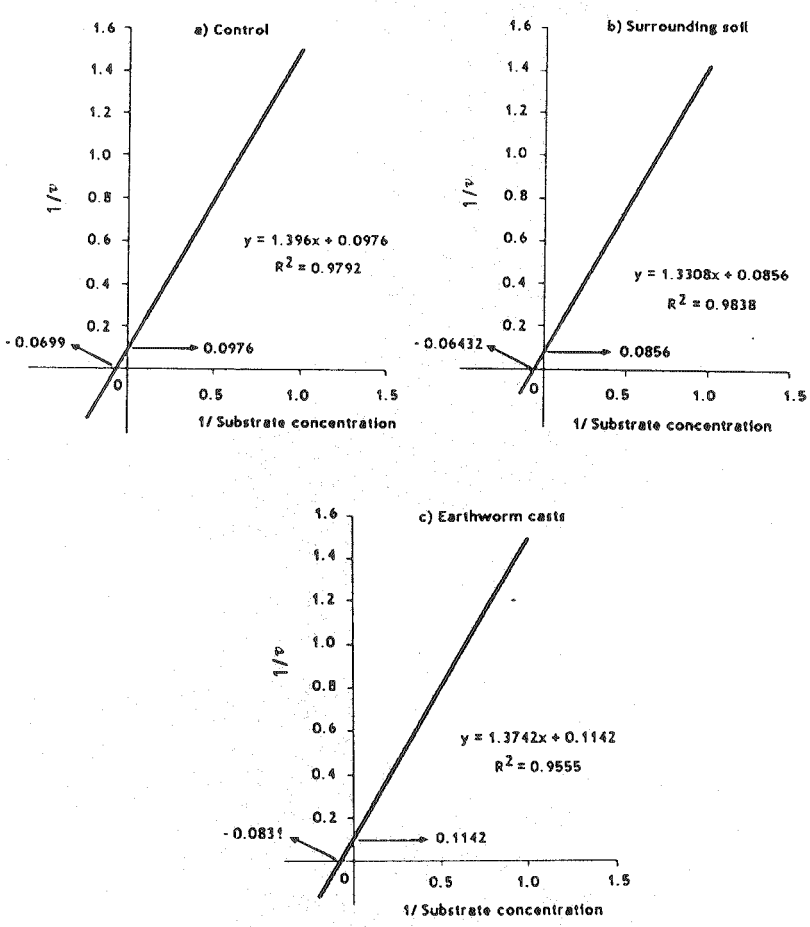

Fig. 3. Curves of Lineweaver–Burk equation

control, suggesting that enzyme affinity for the substrate (H_2O_2) was decreased by earthworm ingesting. Thus, fresh earthworm casts reduced the enzyme-substrate affinity, probably due to a change in the composition of aerobic microflora with change in the community of catalase. V_{max}/K_m shows potential of enzyme process and scattered condition of enzyme-substrate complex compared to the formation of the complex. Based on these results, potential of enzyme process is observed to be higher in surrounding soil than in surrounding soil and control, and enzyme–substrate complex is concluded to be more quickly scattered in surrounding soil compared to the other samples, such as earthworm casts and control, according to this ratio.

In conclusion, the level of earthworm cast has been determined to be lower in comparison with both surrounding soil, where earthworm exists and the control in terms of catalase activity of worm cast, despite the fact that worm cast contains higher level of organic C, macro and micro-nutrient elements in comparison with the surrounding soil, where the worm exists and the control. Most probably, this case has occurred as a result of dominance of anaerobic conditions during worm's cast production. Besides, it was concluded that the highest levels of kinetic parameters, such as V_{max}, K_m and V_{max}/K_m, that are calculated from catalase activities occur in surrounding soils. It was determined that catalase activity, which is an

important criterion in evaluation of aerobic organisms, has been significantly affected by the worm activity and its cast production in the soils.

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