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Amylases and Phosphatases in Soil

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ABSTRACT
The activities of soil enzymes express the metabolic status of soils at a given time. The present study is conducted to determine the activities of α and β amylases as well of acid and alkaline phosphatases in various soils collected. The enzymes were extracted from soil samples by using various buffers in presence of toluene and the activity was determined by incubating the enzymes with their respective substrates. The released products were estimated by colorimetric and spectrophotometric methods. Different activities were observed in different soils ranging from 1.2 to 1.6×10^{-3}µmoles/min/g soil for α -amylases and 1.8 to 9.6×10^{-3}µmoles/min/g soil for β- amylases. The activities of acid phosphatases were observed ranging from 0.05 to 8.6×10^{-2}µ moles/min/g whereas for alkaline phosphatase 1.3 to $6.8X10^{-2}$µ moles/min/g soil. Effect of temperature on phosphatases was also carried out in different soil samples which were incubated at temperatures ranging from 20 to 70°C for 1hour under same assay conditions. The effect of temperature on both the enzymes was found to be different. The present study provides effective and feasible assay methods for determining amylase and phosphatase activities in various soil samples which in turn are useful to assess soil ecosystem, thereby for better crop management.
Keywords: soil enzymes, amylases, phosphatases, soil fertility

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INTRODUCTION

Soil enzymes play a very important role in organic matter decomposition and nutrient cycling. Soil enzymes are the immediate soil quality indicators compared to other soil quality indicator changes are detectable. Though there are many enzymes present in the soil, those involved in the group of hydrolases and those involved in the degradation of main litter components, which includes enzymes such as glucosidases, phosphatases and urease are well studied as indicators of soil quality [1]. Glucosidases like alpha and beta amylases facilitate the breakdown of organic matter whereas phosphatases are involved in nutrient mineralization [2].Glycosidases are a group of enzymes that catalyze the hydrolysis of glycosides [3]. They are highly diverse enzymes owing to the wide diversity of glycosidic bonds and variations in their substrates [4]. Among the glycosidases, α- and β-glucosidase, as well as α- and β-galactosidase are the main members, widely distributed in the soil [5]. β-glucosidase is, however, the most common, important and widely used soil quality indicator [6]. β-glucosidase acts in the last phase of the cellulose degradation process by hydrolyzing the cellobiose residue [7]. These reactions produce glucose as the final product, an important C energy source for the growth and activity of soil microorganisms [8]. α and β-glucosidase·s importance in their involvement in C cycling has remarkably facilitated its adoption for soil quality testing.

Soil phosphatases catalyze the hydrolysis of esters and anhydrides of phosphoric acid. The main sources of phosphatase enzymes in the soil are of plants and microorganisms. The microbial count, extent of organic materials, mineral and organic fertilizers, tillage and other agricultural practices alters the amount of phosphatases present in the soil [9]. A large amount of soil P is organically bound and since plants make use of only inorganic P, the mineralization of this organic portion can be a vital influence in plant nutrition [10]. Potential activities of individual or groups of enzymes have been correlated with plant growth in different soils or in soils with different crop histories, and are thought to serve as useful indices of soil fertility.

The objective of the present work is to establish an easy method of enzyme assays for both amylases and phosphatases and apply to various soils to measure the quality of soil.

MATERIAL AND METHODS

Sample Collection: -The soil samples used in the present study were collected from different places such as grapes garden, agricultural farm, plant Pot, roof top garden and from laboratory sewage water flooded land and all these areas are located around Bhavan's Vivekananda College, Sainikpuri, Secunderabad. The pH of all these soil samples was determined and samples have been kept in sealed polythene bags and stored at 4^0C until further use.

Assay ofsoil amylase activity: Soil amylase activity was determined on the hydrolysis of starch according to the method by Tu [11] with modifications to suit for our laboratory conditions. In order to determine amylase enzyme activity in soils, 3 ml of toluene was added to 10 gram of soil samples to inhibit activities of microorganisms during assay. The contents in the flasks were mixed thoroughly and after 15 minutes 20 ml of 0.2 M acetate - phosphate buffer ,pH 5.5containing1% starch was added to the soil samples and the flasks were held for 1 hourat 30°C. At the end ofthis period, the soils were centrifuged at 6000 rpm and the filtrate was tested for reducing sugars by dinitro salicylate method as described by Seshidharrao et al [12].

One unit of Amylase activity was defined as the amount of enzyme which liberated 1µmole of maltose per min per gram of soil in this assay system.

$$\text{Amylase Activity } [\mu \text{ moles/min/g}] = \frac{\mu\text{moles of maltose released}}{[\text{Reaction Time in minutes}] \times \text{g of soil}}$$

Assay of soil Phosphatase activity: The activity of acid and alkaline phosphatases was determined with some modifications by method described by Tabatabai *et al* [13]. 2grams of soil samples were taken in a conical flask and 1 ml of toluene was added to the soil samples and mixed thoroughly. To the samples, 4ml of 0.2 M sodium citrate buffer, 4ml of 0.115 p-nitro phenyl phosphatase was added. The soil mixture was incubated for one hour at 37^0C. The reaction was stopped by adding 2 ml of 0.5M calcium chloride and 4ml of 0.5M NaOH. The solution was centrifuged at 5000 rpm for5 minutes and the absorbance of the product p-nitrophenol in the supernatant was measured at 430 nm in UV-Vis spectrophotometer [Elico ltd, India].

$$\text{Enzyme activity}[\mu \text{ moles/min/g}] = \frac{\text{Absorbance [Test – Blank]X reaction volume}}{\text{Time in minutes x18.3 x volume of enzyme}}$$

Effect of temperature on Phosphatases: The assay conditions were maintained as same as earlier described for phosphatase activity except that the temperature was altered ranging from 20^0C to 70^0C for both acid (soil 5)and alkaline phosphatase [soil 4] at the respective pH values of the assay system.

RESULTS AND DISCUSSION:

Soil enzymes act as biological index of soil quality as they catalyse and facilitate decomposition and nutrient cycling. Depth of the soil, type of soil, temperature, moisture, pH, quality and quantity of available substrate, as well as management regimes influence the soil enzyme's activities.

Soil amylases are responsible for the major breakdown of complex polysaccharides like starch to a readily available form of glucose. The results in the present study shows that both α and β amylases are widely present in the soils tested, with a variation in their activities. α- activity of soil from roof top garden (soil 4) has shown highest activity of $1.6x10^{-3}$µ moles/min/gcompared to plant pot soil, (soil 3)which has shown least activity of $1.1x10^{-3}$ µ moles/min/g of soil [Table.1]. The results indicate good C- cycling in roof top soil compared to other soils and in turn suitable for better growth of the plants. The roof top garden is being supplemented with organic manure prepared in the college and the difference in the quality of soil is evident from the presence of good activity of α- amylase. The activity of β-amylase was found maximum in acidic soil (soil 5) indicates that the enzyme's existence is with relation to its optimum pH range [4-6]. The other soils are having varying levels of activity of both α and β amylases (Fig.1).

Many organisms, including soil fungi, release phosphatases into their environment [14]. The phosphatases are introduced into the soil by active excretion, leakage or cell lyses. P deficiency often enhances extracellular phosphatase activity from plant roots, fungi and other microorganisms [15]. Organic phosphorus compounds in soil can constitute 5 – 50 percent of total phosphorus and the assimilation of this phosphorus by plants and microorganisms is preceded by soil enzymes [16].

Phosphatase activity depends on soil pH, seasons, vegetation cover, variation of soil salinity and mycorrhizal association [17].

Results in the present study clearly demonstrate the higher activity of acid phosphatases (8.6 $x10^{-2}$µ moles/min/g) in the acid soils (soil 5, pH 4.0) and less activity of alkaline phosphatise in the same soil(1.3 $x10^{-2}$ µ moles/min/g). On the other hand, the activity of acid phosphatase was found very negligible (0.05 $x10^{-2}$ µ moles/min/g)in the alkaline soil, Soil4. (Table.1)

The highest activity of both acid and alkaline phosphatases in neutral soil (Soil 1) indicates that the phosphatase activities observed in these soils might belong to neutral phosphatases(fig.2). Soil 1 from grapes garden indicates good phosphorus assimilation by plants and microorganisms and P cycle is very active in this soil.

The amylases are more temperature resistant in their activities even after longer incubation times [18]. The reason for this could be high concentration of the non-reducing end of starch would act by protecting the enzyme against thermal denaturation. So the present study is aimed at understanding the thermal stability and temperature optima for the phosphatases and it was found that acid phosphatase has shown maximum activity at 40^0C with a very narrow thermal stability range and alkaline phosphatase has shown optimum temperature at 60^0C and thereby thermal stability up to that point (fig.3).

Table.1. Nature and Activities of soil enzymes- Amylases and Phosphatases

Soil Type	Soil pH	Soil Location	α-amylase activity (µ moles/ min/g soil)	β-amylase activity (µ moles/ min/g soil)	Acid phosphatase activity (µ moles/ min/g soil)	Alkaline phosphatase activity (µmoles/ min/g soil)
1	Neutral[7.0]	Grapes garden	$1.2x10^{-3}$	$3.5x10^{-3}$	$5.9 x10^{-2}$	$6.8 x10^{-2}$
2	Neutral[7.2]	Agriculture land	$1.3x10^{-3}$	$2.7x10^{-3}$	$1.0 x10^{-2}$	$3.4 x10^{-2}$
3	Neutral[7.4]	Plant pot	$1.1x10^{-3}$	$2.6x10^{-3}$	$3.3 x10^{-2}$	$2.9 x10^{-2}$
4	Alkaline[8.0]	Roof top garden	$1.6x10^{-3}$	$1.8x10^{38}$	$0.05 x10^{-2}$	$3.5 x10^{-2}$
5	Acidic[4.0]	Sewage water flooded soil	$1.3x10^{-3}$	$9.6x10^{-3}$	$8.6 x10^{-2}$	$1.3 x10^{-2}$

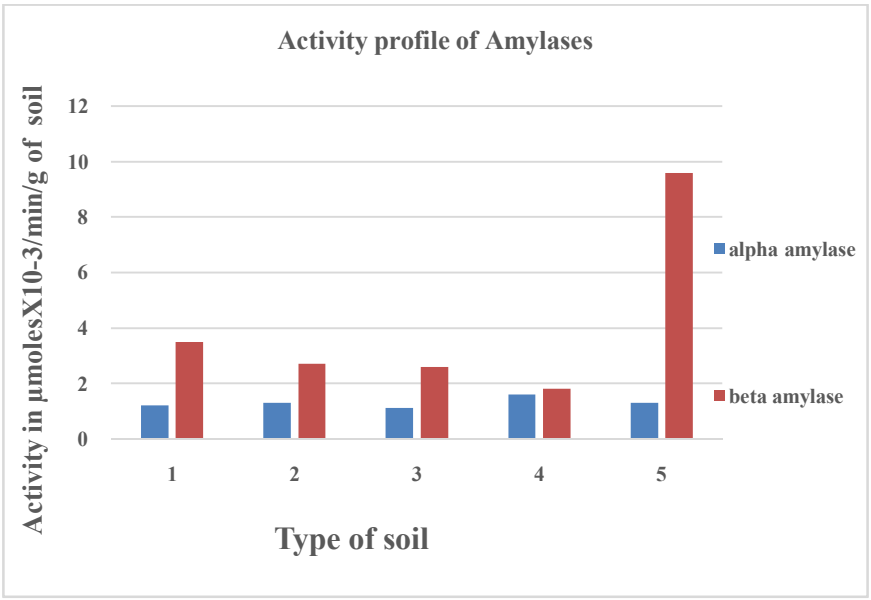

Figure1. Activity profile of Amylases from different soils

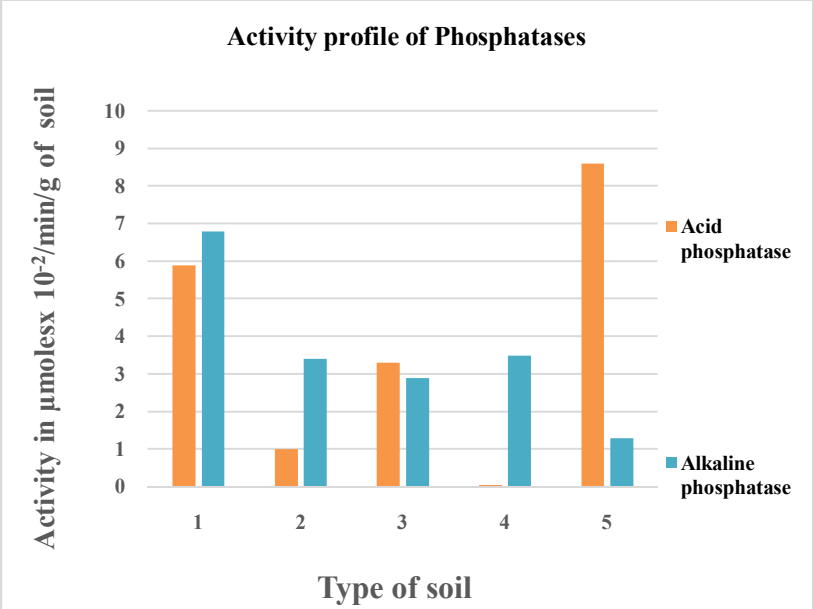

Figure2. Activity profile of Phosphatasees from different soils

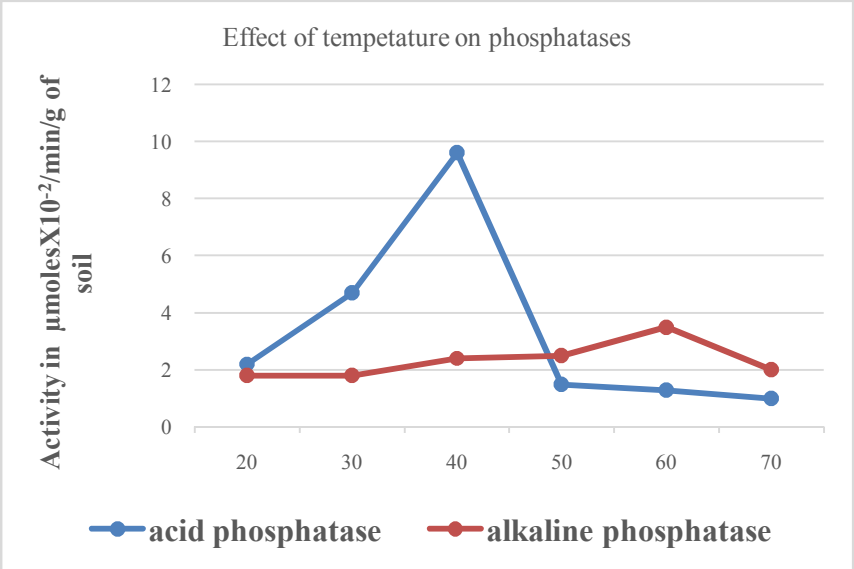

Fig.3: Effect of temperature on acid and alkaline phosphatase activity of soil samples

Though the other soil enzymes like ureases would add up more insight into soil quality, the present study clearly forms a basis for understanding soil quality by assessing activities of both amylases and phosphatases as indicators and this will help in understanding the ecology of soils and further planning for crop management.

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