DISTRIBUTION, KINETICS AND SOME INTERACTIONS OF UREASE AND PHOSPHOMONOESTERASES IN SOILS

THESIS SUBMITTED TO THE
ANDHRA PRADESH AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

(Soil Science & Agricultural Chemistry)

By

V. SANKARA RAO M. Sc., (Ag)

DEPARTMENT OF SOIL SCIENCE & AGRICULTURAL CHEMISTRY
COLLEGE OF AGRICULTURE
ANDHRA PRADESH AGRICULTURAL UNIVERSITY
RAJENDRANAGAR, HYDERABAD-500 030
1989

CERTIFICATE

Sri **V. SANKARA RAO** has satisfactorily prosecuted the course of research and that the thesis entitled **"DISTRIBUTION, KINETICS AND SOME INTERACTIONS OF UREASE AND PHOSPHOMONOESTERASES IN SOILS"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

Saroja Raman

Date: 25-3-1989

Dr. (Mrs.) SAROJA RAMAN
MAJOR ADVISOR
ASSOCIATE PROFESSOR/ANALYTICAL CHEMIST
DEPARTMENT OF MICROBIOLOGY AND BIOENERGY
COLLEGE OF AGRICULTURE
RAJENDRANAGAR, HYDERABAD-500 030

CERTIFICATE

This is to certify that thesis entitled **"DISTRIBUTION, KINETICS AND SOME INTERACTIONS OF UREASE AND PHOSPHOMONOESTERASES IN SOILS"** submitted in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY (SOIL SCIENCE & AGRICULTURAL CHEMISTRY)** of the **ANDHRA PRADESH AGRICULTURAL UNIVERSITY**, Hyderabad, is a record of the bonafide research work carried out by Sri **V. SANKARA RAO** under my guidance and supervision. The subject of thesis has been approved by the Students's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. Published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

Saroja Raman

Dr. (Mrs.) SAROJA RAMAN
CHAIRMAN OF THE ADVISORY COMMITTEE

Thesis approved by Student Advisory Committee.

CHAIR PERSON : (Dr.(Mrs.) SAROJA RAMAN) Saroja Raman
ASSOCIATE PROFESSOR/ANALYTICAL CHEMIST,
DEPARTMENT OF MICROBIOLOGY & BIOENERGY
COLLEGE OF AGRICULTURE, RAJENDRANAGAR

MEMBER : (Dr. A. SREENIVASA RAJU)
ASSOCIATE PROFESSOR
DEPARTMENT OF SOIL SCIENCE & AGRL. CHEMISTRY
COLLEGE OF AGRICULTURE, RAJENDRANAGAR

MEMBER : (Dr. M.V. SHANTARAM)
PROFESSOR AND HEAD 27/3/89
DEPARTMENT OF SOIL SCIENCE & AGRL. CHEMISTRY
COLLEGE OF AGRICULTURE, RAJENDRANAGAR

MEMBER : (DR. B. GOPAL SINGH)
ASSOCIATE PROFESSOR
DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE, RAJENDRANAGAR

MEMBER : (Mr. A. SATYANARAYANA)
RESEARCH OFFICER,
COMPUTER CENTRE
COLLEGE OF AGRICULTURE, RAJENDRANAGAR

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Contd.

Contd.

DECLARATION

I, **Mr. V. SANKARA RAO,** hereby declare that the thesis entitled **"DISTRIBUTION, KINETICS AND SOME INTERACTION OF UREASE AND PHOSPHOMONOESTERASES IN SOILS"** submitted to Andhra Pradesh Agricultural Univrsity for the degree of **Doctor of Philosophy** is the result of original research work done by me. I also declare that the material contained in the thesis has not been submitted for a degree of any University.

(V. SANKARA RAO)

ACKNOWLEDGEMENT

It gives me an immense pleasure to express my deep sense of gratitude and indebtedness to **Dr. (Mrs.) Saroja Raman**, Associate Professor/Analytical chemist, Department of Microbiology and Bioenergy, College of Agriculture, Rajendranagar and Chairperson of my advisory committee for her profound interest, inspiring guidance and suggestions with preserving patience in the preparation of manuscript. The help and encouragement received from her is indelible and remains fresh for ever in my memory. I acknowledge all her contributions most gratefully.

I am extreemely thankful to **Dr.A. Srinivasa Raju**, Associate Professor and to **Dr.M.V.Shanta Ram**, Professor and Head, Department of Soil Science and Agricultural Chemistry and members of my advisory committee for their valuable suggestions throughout the period of investigation and in the preparation of manuscript.

My sincere thanks are also due to **Dr.B.Gopal Singh**, Associate Professor, Department of Plant Physiology and to Sri **A.Satyanarayana**, Research Officer, Computer Centre and Members of my Advisory Committee for the help rendered during the period of Investigation.

I am also thankful to All the staff members of the department for their kind cooperation during the period of my study.

The cooperation and uninstincted support received from **Dr.R.N.Pillai,** Professor and Head, Department of Soil Science and Agricultural Chemistry, Agricultural College, Bapatla is gratefully acknowledged.

I am thankful to the authorities of A.P.Agricultural University for deputing me to this course of study.

My warmest thanks are due to my friends **Dr. V.Satyanarayana, Dr. P.C.Rao** and **Dr.D.K.Sarkar** for their suggestions and help offered to me in the preparation of the thesis.

I am thankful to the Staff members of **Prasa Computer Centre** for the pains they have taken in typing and printing the manuscript neatly.

I owe a lot to my wife **Satyavathi** for her understanding and cooperation.

***

(V. SANKARA RAO)

AUTHOR : V.SANKARA RAO

Title of the dissertation : DISTRIBUTION, KINETICS AND SOME INTERACTIONS OF UREASE AND PHOSPHOMONOESTERASES IN SOILS

Degree to which it is submitted : DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty : SOIL SCIENCE & AGRICULTURAL CHEMISTRY

Guide : DR.(Mrs.) SAROJA RAMAN
ASSOCIATE PROFESSOR/
ANALYTICAL CHEMIST
DEPARTMENT OF MICROBIOLOGY
AND BIOENERGY,
COLLEGE OF AGRICULTURE
RAJENDRANAGAR
HYDERABAD - 500 030

University : ANDHRA PRADESH AGRICULTURAL UNIVERSITY

Year of submission : 1989

ABSTRACT

Laboratory, pot and field experiments were conducted to study the distribution, kinetics and some interactions of soil urease, acid and alkaline phosphatase activities. Levels of soil urease and phosphomonoesterases in the surface soils collected from various parts of Andhra Pradesh showed wide variation ranging from 3.8 to 26.5 with an average 10.5 for urease, from 6.4 to 142.7 with an average 34.8 for acid phosphatase and from 10.3 to 155.2 with an average 67.1 for alkaline phosphatase. The enzyme activity correlated significantly with soil organic carbon and clay. The kinetics of enzyme activities were determined at a series of substrate concentration, assuming a pseudo first order kinetics over the initial stages of the reaction. The enzyme activity increased with

substrate concentrations and reached a limiting value (V_{max}) in all the soils. Michaelis Menten equation was applied for enzyme action and V_{max} and the Michaelis constant (K_m) were calculated using the linear transformations of the Michaelis Menten equation. V_{max} and K_m values varied with the type of soil.

Potential urease and phosphatase activities were determined under non rate limiting substrate concentrations from 20-90°C at ten degree intervels. The enzyme activity increased till 60-70°C and sharply decreased there after indicating deactivation beyond 70°C. Energy of activition (Ea) were determined using Arrhenius equation. Urease activity measured in the pH range 3-12 using modified universal buffer (MUB) showed an increase till pH 9.0 followed by a decrease at higher pH. The pH optima was around 8.5 to 9.5.

Urease and phosphomonoesterases were inhibited by Zn (II), Mn (II), Fe (II) and Cu (II). The inhibition due to copper was more compared to other cations. Treatment of soil with commonly used herbicides like Atrazine, Simazine, Methabenzthiazuron and Metoxuron inhibited soil urease activity till 60 days of plant growth with progressive increase in total enzyme activity at each stage. Addition of Jackbean urease over a wide range of concentration to montmorillonite, bentonite, illite and kaolinite resulted in the decrease of enzyme activity. Expanding type of clays inhibit more compared to non expanding type like kaolinite.

Soil urease activity varied with plant cover and decrease in the order: groundnut > sunflower > sorghum > redgram under both sole as well as mixed cropping systems. Fertilization of gingelly with sulphur in the form of elemental sulphur or gypsum did not show any difference in soil urease activity.

***

substrate concentrations and reached a limiting value (V_{max}) in all the soils. Michaelis Menten equation was applied for enzyme action and V_{max} and the Michaelis constant (K_m) were calculated using the linear transformations of the Michaelis Menten equation. V_{max} and K_m values varied with the type of soil.

Potential urease and phosphatase activities were determined under non rate limiting substrate concentrations from 20-90°C at ten degree intervels. The enzyme activity increased till 60-70°C and sharply decreased there after indicating deactivation beyond 70°C. Energy of activition (Ea) were determined using Arrhenius equation. Urease activity measured in the pH range 3-12 using modified universal buffer (MUB) showed an increase till pH 9.0 followed by a decrease at higher pH. The pH optima was around 8.5 to 9.5.

Urease and phosphomonoesterases were inhibited by Zn (II), Mn (II), Fe (II) and Cu (II). The inhibition due to copper was more compared to other cations. Treatment of soil with commonly used herbicides like Atrazine, Simazine, Methabenzthiazuron and Metoxuron inhibited soil urease activity till 60 days of plant growth with progressive increase in total enzyme activity at each stage. Addition of Jackbean urease over a wide range of concentration to montmorillonite, bentonite, illite and kaolinite resulted in the decrease of enzyme activity. Expanding type of clays inhibit more compared to non expanding type like kaolinite.

Soil urease activity varied with plant cover and decrease in the order: groundnut > sunflower > sorghum > redgram under both sole as well as mixed cropping systems. Fertilization of gingelly with sulphur in the form of elemental sulphur or gypsum did not show any difference in soil urease activity.

***

INTRODUCTION

CHAPTER I

INTRODUCTION

Support in favour of the importance of soil biochemical properties in nutrient availability and cycling is on the increase. The views of Quastel (1946) about soil being a biological entity or living tissue aptly describes the role of soil biochemistry. All biochemical reactions are catalysed by enzymes which are proteins with catalytic properties towards specific substrates. Their specificity arises largely from the stereospecific manner in which they combine with the substrate to affect changes in electronic configuration around susceptible bonds.

The bilogical properties of soils are mediated by living soil microflora and fauna, plant roots and soil animals. In addition to these, a number of extracellular enzymes or accumulated enzymes are also present in the soil which account for a number of biochemical transformations. Skujins (1967) coined the term 'Abiontic enzymes' for the accumulated enzymes in soils. These enzymes function extracellularly either free in soil solution or bound to inorganic and organic soil constituents present in particulate cell debris or in dead cells which are viable but non proliferating cells.

The study of soil enzymes is of fairly recent origin and has been progressing fast over the past two decades. The progress has been hampered by slow advance in classical enzymology itself on one hand and on account of many other constraints like the immobilization of the enzymes on soil particles and microbial debris which creates a heterogenous and structurally rigid environment where reactions take place on the solid solution interface. Thus, although several enzymatic studies have been reported, assay procedures for only a few of these enzymes have been standardised and evaluated. Of these the most important are the urease, phosphatase, sulphatase, rhodanese, catalase and cellulase.

Urease is unique among soil enzymes and greatly affects the fate and performance of an important fertilizer like urea; whereas phosphatases deserve special attention because they are involved in the transformation of organic and inorganic phosphorus compounds in soil.

Soil enzymology suffers from lack of well tested and evaluated methodologies. Many of the earlier methodologies have lost relevance due to their failure to seperate microbial enzyme activities from abiontic enzymes. Apart from this, the basic understanding of the manner in which the free enzymes are attached to soil matrix and the manner in which these enzymes act are still not clear. The stabilization of soil enzymes over thousands of years and their survival through most adverse environmental conditions still baffle enzymologists who know enzymes as the most fragile catalysts. Also, how these protein complexes survive the attack of micro organisms proliferating in the soil and that of the proteinaceous enzymes present in the soil itself is amazing.

In addition to these basic questions on soil enzymes, of more relevant and practical importance are the role of these enzymes in mineralisation of nutrients, recycling and humic matter formation. Reports on physico-chemical properties like K_m and V_{max} are limited. In the literature reported too, there have been a number of conceptual fallacies in methodology and interpretations. The effect of temperature on the kinetics of soil enzymes and the consequent determination of energy of activation have found much less attention. The entropy of activation for soil enzymes has not been determined or interpreted.

The subject of enzyme inhibition requires special consideration because soils receive a variety of the manner in which the free enzymes are attached to soil matrix and the manner in which these enzymes act are still not clear. The stabilization of soil enzymes over thousands of years and their survival through most adverse environmental conditions still baffle enzymologists who know enzymes as the most fragile catalysts. Also, how these protein complexes survive the attack of micro organisms proliferating in the soil and that of the proteinaceous enzymes present in the soil itself is amazing.

In addition to these basic questions on soil enzymes, of more relevant and practical importance are the role of these enzymes in mineralisation of nutrients, recycling and humic matter formation. Reports on physico-chemical properties like K_m and V_{max} are limited. In the literature reported too, there have been a number of conceptual fallacies in methodology and interpretations. The effect of temperature on the kinetics of soil enzymes and the consequent determination of energy of activation have found much less attention. The entropy of activation for soil enzymes has not been determined or interpreted.

The subject of enzyme inhibition requires special consideration because soils receive a variety of organic and inorganic chemicals such as fertilizers, herbicides and salts of trace elements as part of crop management and or as industrial pollutants. Clays either inhibit, stimulate or exert no measurable influence on enzyme activity although the inhibitory effects are more prevalently observed. Although initial views regarded clay enzyme interactions as the principal stabilizing mechanism for enzymes in soil, later emphasis centered around the role of soil humic substances as stabilizing agents.

Examination of extracellular enzyme activities in ecosystem has been emphasized in recent years. It has been assumed a *priori* that soil enzymes excreted by plant roots would contribute to the make up of the abiontic soil enzyme system.

Understanding enzyme activity in soils and interpreting its direct contribution to fertility and other applied aspects are difficult even todate. There is a dire need for more advanced methodologies, interpretations and theoritical background to understand fully the total soil enzymatic complex and its role in crop production. The present investigation was carried out with the following objectives:

1. To determine the levels of urease and phosphomonoesterase activities in soils of Andhra

Pradesh, differing widely in physico-chemical properties,

2. To quantify the effect of temperature with a view to determine their Arrhenius activation energy and the thermodynamic activation parameters from Eyring equation,

3. To study the effects of pH and moisture on soil urease activity,

4. To investigate the effect of metallic cations on soil urease and phosphomonoesterases,

5. To study the effect of pure and ion saturated clays on Jackbean urease activity,

6. To study the inhibitory effects of some commonly used herbicides on soil urease activity,

7. To study the effect of gypsum and elemental sulphur levels on soil urease activity,

8. To study the influence of crop cover on urease levels by growing some typical crops of the region.

***

REVIEW OF LITERATURE

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 INTRODUCTION

Although classical enzymology has a long history, the study of soil enzymes may still be considered to be in its infancy. The actual stage for serious work on soil enzymes was set only in 1970's, when a worldwide interest was generated in the subject. Nevertheless, considerable volume of literature has accumulated in a relatively short span of about less than two decades which itself is a testimony to the importance of the subject.

Among the different accumulated soil enzymes, for which Skujins (1967) coined the term "*abiontic enzymes*", soil urease, (urea amydohydrolase) and soil phosphomonoesterases i.e. acid and alkaline phosphatases are the most important in plant nutrition. Urease catalyses the hydrolysis of urea to CO_2 and NH_4^+ a form assimilable by plants and the phosphatases mineralise the organically bound phosphorus to inorganic phosphate. As per the nomenclature of Commission on Enzymes of the International Union of Biochemistry, they have been given the numbers 3.5.1.5 for soil urease and 3.1.3.2 for phosphomonoester hydrolase.

Among the different facets of the subject of soil enzymes, the *in situ* behaviour of these enzymes in the heterogenous environment of the soil system in respect of their thermal sensitivity, pH effects, kinetic parameters, activation and inhibition by different soil amendments, the allelopathic effects of rhizosphere, root exudates and plant residues are of prime importance. The present investigation was specially designed for studying the physico-chemical behaviour of the enzymes, in their soil bound state and their distribution and behaviour in some limited ecosystems with special reference to their kinetics and related activation parametes, effect of different soil conditions like nature of soil components, moisture, temperature, amendments and crop cover which control the efficiency of these enzymes. In addition to this, the effect of different types of clays and the nature of cations in the exchange sites on the activity of these enzymes was also taken up. This review highlights the more recently available literature that is relevant to the work carried out in the present investigation.

2.2 HISTORY

The presence of extracellular enzymes in the soil was first reported by Wood (1899). Most of the early attention was paid towards the enzyme catalase, apparently because of its easy detection and also because of the limited knowledge of other enzymes. The presence of oxidase activity (Peroxidase) in soil was reported by Cameron and Bell (1905) which was later proved to be enzymic in nature by Koning *et al.* (1906 and 1907) using biological inhibitors who also correlated it with microbial activity. Till around 1940, all the reports that appeared on soil enzymic activity were limited to the presence of catalase only. The presence of urease and its enzymatic characteristics in soils were reported in a series of papers by Conrad (1940, 1941, 1942 and 1944) and Conrad and Adams (1940). Rotini (1933) reported the presence of pyrophosphatase in soils. The dephosphorylation of organic phosphorus compounds in soil by extracellular enzymes was examined by Rogers and his co-workers (1941 and 1942).

Since 1950, interest in soil enzyme research started expanding continuously. Imaginative theoretical approaches and methods were introduced and a wealth of information regarding various enzymatic reactions in soil were collected. A number of reviews regarding advancement in soil enzyme research were published notably by Kuprevich (1958) and Hoffmann and Hoffmann *(1966). Several reviews on soil enzymes cover different* aspects of the subject (Burns, 1977; Kiss *et al.*, 1975; Ladd and Jackson, 1982 and Skujins, 1967 and 1976). The book "Soil Enzymes" is the most comprehensive treatise up to-date on the subject (Burns, 1978) and includes chapters on history (Skujins, 1978) origin (Ladd, 1978), urease (Bremner and Mulvaney, 1978) soil phosphatase and sulphatase (Speir and Ross, 1978), interactions between agrochemicals and soil enzymes (Cervelli *et al.*, 1978) and enzyme activity, some theretical and practical considerations (Burns, 1978). Some other reviews include soil enzymes (Skujins, 1978) humus enzymes complexes (Ladd and Butler, 1975) and control of urea transformations in soil (Mulvaney and Bremner, 1981).

To-date about sixty soil enzymes have been detected and assayed which belong largely to the oxido-reductase, hydrolase, transferase and lyases. No investigations on isomerases and ligases have been reported. Of these, the most intensively studied are urease, sulfatase, phosphatases and cellulases from the hydrolase group. Among the transferases, rhodanese was reported by Tabatabai and Singh (1979) and invertase are important. Dehydrogenase is the most important in the oxido reductase group. Thus it is clear that, though the first report on soil enzyme appeared as early as 1899, the development of soil enzymology as a discipline actually gained impetus after 1970, when the Amercian Scientists entered the field. Soil emzymology still desperately needs new methodologies as the methods developed and evaluated have not yet covered many of the important soil enzymes.

2.3 ORIGIN

Though the presence of various enzymes in soils have been reported, the origin of these enzymes in soils is still being debated actively; the main dispute being whether they are of plant origin or are of microbial origin. Most workers subscribe to the view that soil enzymes are largely, if not entirely, of microbial origin (Barthalomew, 1965), but there is no conclusive evidence for this and or for speculations by other workers that they are largely derived from plants (Simonart and Mayaudan, 1961).

As early as in 1899, Wood suggested that soil catalase activity was due to plant exudates. After that, there were many a *priori* assumptions about enzymes being secreted by plant roots which accumulate in the soil. There has been however, some direct and indirect evidence for this view. Vlasyuk *et al.* (1957) showed that activity of enzyme invertase, catalase, phosphatase and protease different in the rhizosphere and nonrhizosphere were also dependant on the nature of crops. The same was also reported for cellulase by Bernhard (1965).

Pancholy and Rice (1973) showed that the activities in soils of some carbohydrases decreased, where as dehydrogenases and urease activities increased during stages of revegetation with prairie grass, and with oak and with oak-pine forest. The type of plant residue rather than the total residue return was considered to be important in determining the activity gradients of the enzymes assayed. Other studies have contrasted the changes in enzyme activities of planted and unplanted soils. Speir *et al.* (1980) showed that the urea hydrolysing activities of moist unplanted soils progressively declined during a five month period, whereas soils planted to ryegrass either increased their activities or exhibited lower rates of decrease. Similar trends were established for soil sulphatases and proteinases. Such experiments implicate plant enzymes as contributors to soil enzyme activities.

Hoffmann (1963) and Hoffmann and Hoffmann (1955) opined that microorganisms were the sole source of abiontic enzymes in soils. Although soil enzyme activities do not correlate with numbers of micro organisms in soils or with soil respiratory activities, the contributions from enzymes of microbial origin are also considered to be of great importance and are reported to be enhanced under conditions favouring microbial growth and turnover. It is also pertinent to add that the earlier evidences of the plant origin of soil enzymes do not eliminate an alternative secondary role of plant residues in promoting conditions in soil favourable for microbial growth and, hence, enzyme synthesis and degredation.

Contributions to soil enzymes by soil fauna like earthworms have also been indicated by Kozlov and Mikhailove (1965). However, the presence of soil enzymes derived directly and specifically from animal source is yet to be demonstrated conclusively.

Soil enzymes or the term "Abiontic enzymes" coined by Skujins (1967) applies only to the accumulated enzymes and excluded enzymes from nonprolific. Early literature on soil enzymes referred to them mostly as extracellular enzymes, thus creating some confusion about their location. Added to this was the uncertainity about the enzymes released by proliferating micro organisms which were not differentiated from the accumulated enzymes due to faulty enzyme assay procedures. Kiss *et al.* (1975) distinguished the activities due to accumulated enzymes from those due to proliferating micro-organisms during assay. Thus abiontic enzymes include which 1. function extracellularly either free in the soil solution or bound to inorganic and organic soil constituents, 2. are present in particulate cell debris and 3. are present in dead cells or in viable but non-proliferating cells. Cells and cell fragments may be animal, plant or microbial in origin.

2.4 PERSISTENCE AND STABILIZATION

There is evidence that although free enzymes *per se* are fragile and easily deactivated and metabolised by proteases, soil enzymes are very persistent and are unaffected by adverse soil environmental conditions like high temperature, low moisture and active microbial attack. Many factors influence the turnover of enzymes in soils. They include (1) direct contribution of plant enzymes to soil (2) growth conditions and the availability of energy sources for *de novo* syntheses by a variety of soil animals and micro organisms, and (3) the availability of substrate for specific induction of enzymes by particular organisms. They also include the properties of the enzymes themselves, whether they are free or complexed and the conditions pertaining to the micro environment in which enzymes are located in soils, thus affording varying measures of protection against denaturation or biological degradation. Undoubtedly the persistence of enzyme activities in soils is partly due to enzymes which turn over relatively rapidly; their renewal being dependent essentially upon a continuing or spasmodic return of energy-rich plant residues to soil. By contrast, some of the enzyme activites of soils are due to stable or stabilized enzymes. For example, urease activity has been detected in stored, geologically preserved soils, carbon dated to be about 9000 years old (Skujins and Mc Laren, 1969).

There are direct and indirect evidences from histochemical and microscopic technique studies that soil enzymes are localized mainly in soil organic and inorganic colloids and dead intact cell and cell debris (Foster and Martin, 1981). It is believed that immobilization of these enzymes on such solid matrices gives rise to their extra ordinary stabilization. Clays and complex organic colloids are known to bind proteins and protect them from easy degradation by micro-organisms or added proteinases (Mayaudon, 1968; Verma *et al.*, 1975). Though enzyme activity is quite often markedly decreased due to adsorption on clays and organic colloids, stability towards denaturating agents and proteinases has been recorded (Ladd and Butler, 1969 and 1975). Thus, till recently the formation of enzyme-clay or enzyme-polymer complexes have been postulated to be the reasons for stability of soil enzymes (Burns, 1977 and Mc Laren, 1963). This is also supported by the fact that very small quantities of pure enzymes are extracted from soil. Of late, however, the role of clay-organic complexes has been shown to be more crucial than the individual component *per se*. Makboul and Ottow (1984) showed that alkaline phosphatase desorbed from clays showed different kinetic properties. Although individually, clays and soil organic concentrates do bind enzymes to various extent and impart stability also, in the soil environment, it is quite reasonable that both of these polymeric entities, which are present intimately associated, should be involved. Review by Burns (1978) emphasised that enzymes are physically and chemically immobilized within the discontinuous organic colloidal material which itself is associated with soil clay particles (the organo mineral complex), an immobilization that occurs during humic matter genesis when exoenzymes and endoenzymes from lysed cells become trapped. Recently Boyd and Mortland (1986) have shown that urease adsorbed on a synthetic montmorillonite -

organic complex, retained its activity and even K_m values.

2.5 ASSAY

The most important beginning in soil enzyme study is the standardisation of assay procedure. Valid measurement of the activities of soil enzymes accumulated at the commencement of assay requires that both enzyme production by growing population of micro organisms and assimilation of reaction products are excluded during the assay period. Further methods adopted to achieve these ends like treating the soil with toluene or sterilization through irradiation should not influence the activities of accumulated enzymes. Toluene is still widely used despite the fact that (1) some fungi and bacteria can utilize toluene as a carbon source (Kaplan and Hartenstein, 1979) (2) toluene does neither sterilize soil nor in some treatments, eliminate completely dehydrogenase or respiratory activities and thus these may remain the potential for energy generation and enzyme syntheses (3) toluene may affect the activities of accumulated enzymes by modes of action which cannot be unequivocally interpreted. For example, the increase in urea hydrolysing activities which some times has been observed following toluene addition to soil may have been due to urease released from plasmolysed microbial cells (Skujins and Mc Laren, 1969). More usually toluene addition has decreased urea hydrolysing activities of soils possibly by destruction of cell bound urease of urea carboxylase or by inhibition of exocellular ureases. Support for this conclusion was provided by Thente (1970) who found that toluene reduced the activity of Jackbean urease.

An alternative treatment to the use of bacteriostatic agent is to irradiate soils at sterilizing or lower dosage levels. Many studies on the effects of high energy radiation on soil urease activity have been reported (Skujins and Mc Laren, 1967; Pettit *et al.*, 1976) but these studies have given contradictory results and are difficult to interpret. To overcome the problem, Kaplan and Hartenstein (1979) suggested neither the use of toluene nor irradiation of the soil and advised to carryout the assay over short periods of time.

2.5.1 Substrate concentration

The choice of substrate is often arbitrary and may involve a compound that does not occur naturally in soil. In enzyme assay, the concentration of substrate in the soil reaction mixture should remain sufficiently high to maintain zero order kinetics (Malcolm, 1983). This may require initial concentration to be as high as 100 times those of the K_m values of the reaction.

2.5.2 Choice of buffer and pH

There has been considerable discussion in the literature concerning the choice of buffers for soil enzyme assays and some have even suggested dispensing with their use alltogether. Tabatabai and Bremner (1972) have debated, at length, the factors influencing their selection of tris-H_2SO_4 (THAM) buffer at pH 9.0 for urease assay of soils with bulk pH values of 6.1 to 7.5. Tabatabai and Singh (1976) when designing an assay for rhodanese, based their final choice on pH activity optima and low extraction of humic matter. Rysavy and Macura (1972) also observed that phosphate buffer solubilized soil organic matter which interfered with subsequent spectrometry. Consequently they used tris-maleate buffer for B-galactosidase determination. Sodium maleate was used as a buffer for 4-nitrophenyl phosphatase determinations because it extracted little or no enzyme activity from the soil (Irving and Cosgrove, 1976). These randomly selected experimental techniques are presented solely to illustrate the plethora of reasons influencing the choice of a buffer in soil enzyme determinations. It is important to emphasise that the choice of method for assay of enzyme activity in soil should depend upon the purpose of assay. For example, the purpose of an investigation is to obtain an index of the ability of the urease in the soil sample under study to hydrolyse urea under natural conditions, non buffer method described is obviously superior to the buffer method. If, on the otherhand the purpose is to detect urease in soils or soil fractions, the buffer method should be preferred because it detects urease activity not detected by the non-buffer method.

2.5.3 Air drying

Most assays of soil enzyme activity have been performed on air dried soils. However, air drying of soils can significantly affect the activities of soil enzymes and the effect depends on the enzyme concerned. For example, Ross (1965) found that air drying of soils led to a reduction in invertase and amylase activities, where as Tabatabai and Bremner (1970) found that it increased arylsulphatase activity. Skujins (1967) reviewed literature pertaining to preservation of soil samples for assay of enzyme activity and concluded that the best method of preservation depends upon the enzyme under study. For comparative studies of enzymes, it is essential to use uniform methods of soil preparation.

2.5.4 Methods of assay

Numerous methods have been used for the assay of urease activity in soils. Most of these methods involve the estimation of NH_4^+ released on the incubation of soils with urea solution (Tabatabai and Bremner, 1972 and Bremner and Mulvany, 1978). Others involve the estimation of urea remaining after hydrolysis using diacetyl monoxide and semicarbazide was given by Douglas and Bremner (1971) and Zantua and Brember (1975).

The methods which have been most thoroughly evaluated are the non-buffer method proposed by Zantua and Bremner (1975) and the buffer method proposed by Tabatabai and Bremner (1972). The non buffer method is essentially a scale down version of the method proposed by Douglas and Bremner (1971), the only significant difference is that toluene is omitted. It involves the determination of the amount of urea hydrolysed on incubation of soil sample with urea at 37°C for 5 hours. Urea hydrolysed being estimated by colourimetric determination of urea in the extract obtained by shaking the incubated soil samples with 2 M KCl containing urease inhibitor and filtering the resulting suspension. The buffer method of Tabatabai and Bremner (1972) involves determination of NH_3 released on incubation of the soil sample with THAM (pH 9.0) urea and toluene at 37^oC for 2 hours, the ammonium released being determined by shaking the incubated soil sample with 2.5 M KCl containing urease inhibitor and steam distilling an aliquot of the resulting soil suspension with MgO. Both methods give precise results but the buffer method gives markedly higher values than the non buffer method and detects urease activity that does not occur when soils are treated with urea in the absence of buffer. The non buffer method provides a very good index of the ability of soils to hydrolyse urea under natural conditions.

2.5.5 Units of enzyme activity

In the soil system, enzyme activity is expressed in units such as micrograms or micromoles of product appeared or substrate disappeared per unit mass of soil. In most of the literature soil enzyme activity has been expressed as u moles or ug of product or substrate g^{-1} soil h^{-1}. The same units have been used in this study. However, Malcolm (1983) suggested enzyme activity should be expressed as u moles product appeared or substrate disappeared g^{-1} soil sec^{-1}. Theoretically measurement of either the amount of product released or the decrease in concentration of substrate is sufficient in enzyme assays, but in practice, measurement of the product released is usually much more precise because the value is based on the difference between zero or close to zero and finite concentration. To measure the decrease in substrate concentration after incubation time, this decrease must be large enough to be determined accurately and at the same time, the difference must not be so large to cause a sigificant change in the concentration of substrate. Assays based on measurement of the decrease in the concentration of the substrate usually are less precise than those based on measurement of product appearance.

2.5.6 Phosphomonoesterases

Assay of phosphomonoesterase activities are based on colourimetric estimation of the 4-nitrophenol released by phosphatase activity when soil is incubated with buffered (pH 6.5 for acid phosphatese actiity and pH 11.0 for alkaline phosphatase activity) sodium 4-nitrophenyl phosphate (Tabatabai and Bremner, 1969). The 4-nitrophenol released is extracted and determined colourimetrically. Other substrates used earlier were phenyl phosphate (Kroll and Krammer, 1955), glycerophosphate (Skujins *et al.*, 1962) and B-naphthyl phenyl phosphate (Ramirez and Mc Laren, 1966). Arylsulphatase was assayed using nitrophenyl sulphate as substrate (Tabatabai and Bremner, 1970) while thiosulphate was used as substrate for the estimation of rhodanese (Tabatabai and Singh, 1976). Rhodanese is assayed using thiosulphate as substrate and SCN^- formed is estimated. The procedure used for the determination of the SCN^- is based on the reaction of SCN^{-1} with Fe^{3+} in acidic medium to form an Fe-SCN coloured complex. The most widely used method for determination of dehydrogenase activity involves the colourimetric determination of 2, 3, 5-triphenyl formazan (TPF) produced by the reduction of 2, 3, 5-triphenyl tetrazolium chloride (TTC) by soil micro organisms.

Soil enzymology still desperately needs new methodologies. Though new methods have been proposed from time to time, they need to be continuously evaluated in view of the increasing awareness of the problems involved in the assays.

2.6 INFLUENCE OF SOIL PROPERTIES ON ENZYME ACTIVITIES

2.6.1 Urease

Studies pertaining to the effect of soil properties on the levels of urease activity have indicated that urease activity tends to increase with organic matter content and that sandy or calcareous soils tend to have a lower activity than heavy textured or non calcareous soils (Skujins and Mc Laren, 1969). Furthermore it has been found that soils under dense vegetation tend to have hihger urease activity and that saline and gleyed soils tend to have low urease activity (Myers and Mc Garity, 1968). Gould *et al.* (1973) and Tabatabai (1977) reported a decrease in urease activity with depth in the soil profiles.

Significant relationship between urease actiity and organic matter content of the soil was found by many workers (Gould *et al.*, 1973 and Tabatabai, 1977). However, Pancholy and Rice (1973) reported that urease activity in nine Oklahoma surface soils was not significantly correlated either with organic carbon or pH and concluded that urease actiity in these soils was determined by the type of vegetation. Dalal (1975) found that urease activity in fifteen Trinidad surface soils was positively correlated with organic carbon and cation exchange capacity. He also found that urease activity was significantly correlated with oxalate extractable (amorphous) iron or aluminium but not with pH and clay. Zantua *et al.* (1977) found that urease activity in twenty one diverse Iowa surface soils was significantly correlated with organic carbon, total nitrogen and CEC. Speir *et al.* (1980) found that urease activity correlated highly signifiantly with organic carbon, total nitrogen, total sulphur and protease activity in nine Newzealand soils while Dash *et al.* (1981) reported a positive correlation between urease activity and total nitrogen, organic carbon, silt + clay and specific conductance and negative correlation with pH and moisture. Sahrawat (1983) found that urease activity in ten Philippine wetland rice soils was highly significantly correlated with total nitrogen and organic carbon but was not significantly correlated with CEC, clay, pH, active iron or active manganese and concluded that organic matter content measured by organic carbon and total nitrogen accounted for most of the variation in urease activity.

2.6.2 Phosphatases

Limited studies of the relationship between phosphatase activity and other soil properties have indicated that phosphatase activity in general increases with increase in organic matter content of the soil. Nannipieri *et al.* (1973) observed a significant positive correlation between phosphatase activity and organic matter content in thirty two Italian soils while

Harrison (1983) reported a significant positive relationship between phosphatase activity and organic carbon content in ten Woodland soils. Speir (1977) showed that phosphatase activity significantly correlated with organic carbon content both within and between sites in a climosequence of soils in Newzealand. However, there have also been reports of non significant correlation (Sanikidze *et al.*, 1973) and even a negative relationship (Kozlov *et al.*, 1973) between phosphatase activity and organic carbon content of the soils.

Soil phosphatase activity has been reported to be significantly correlated to total nitrogen also, presumably because it is closely related to the organic carbon itself (Harrison, 1983).

The relationship of phosphatase activity to soil organic phosphorus content has been the subject of several studies. Khaziev and Burangulova (1965) recorded inverse relationships between organic phosphorus content and soil nuclease, phytase, glyecrophosphatases and phosphatase activities. Mamytov *et al.* (1974) found that in certain Russain cultivated soils, alkaline phosphatase activity was inversely related where as acid and neutral phosphatase activities were directly related to organic phosphorus content.

Soil phosphatase activity has also been shown to vary with soil depth (Harrison, 1983), season (Harrison and Pearce, 1979), and with the type of plants present (Harrison, 1983).

2.7 EFFECT OF pH ON ENZYME ACTIVITIES

A large number of factors control the effect of pH. Changes in H^+ ion concentration influence enzymes, substrate and cofactors by altering their ionisation and solubility. Variations in such properties as ionisation or solubility influence the rate of catalyzed reactions. Enzymes being proteinaceous, exhibit marked changes in ionization from fluctuations in pH. Characteristically each enzyme has a pH value at which the rate is optimal and on either side of this optimum the rate is lower, thus the catalytic action of the enzyme operates in a some what restricted pH range. Enzymes are usually most stable in the vicinity of the optimum pH and they are irreversibly denatured with extremes in acidity or alkalinity.

Studies of the effects of pH on soil enzymes are important because, exposure to extreme pH values may irreversibly inactivate enzymes that play an essential role in nutrient (N, P and S) transformations and humus formation. The hydrogen ion concentration of soils can vary considerably, most soils range between pH 4-8.

Studies involving use of buffers to determine the effect of pH on soil urease activity have been reported by number of investigators. Hoffman and Schmidt (1953) and Pettit *et al.*, (1976) found that the optimum pH for soil urease was 6.5 to 7.0 whereas Tabatabai and Bremner (1972) and May and Douglas (1976) found that it was 8.8 to 9.0. This divergence may be related to differences in the buffers and urea concentrations adopted in these investigations. Though several studies have been reported on the effects of liming and acidifying materials on urease activity in soils, (Zantua and Bremner, 1977), their value with regard to assessment of the effect of pH on soil urease activity is limited.

Soil phosphatase activity, as measured with artificial substrate often appears to have two pH optima, one acid and the other alkaline (Hoffmann and Hoffmann, 1966). Acid and alkaline phosphatases are common in nature and it is not surprising that both should occur in soil. In soils where they are both present (Abramyan and Galstyan, 1975) or where only one or the other is found (Parks, 1974), their pH optima is generally within the range pH 4-6 and 8-10, for acid and alkaline phosphatases, respectively. Frequently, however, neither acid nor alkaline phosphatase is found, but rather a "neutral" phosphatase with an optimum around pH 7.0 (Kishk *et al.*, 1976). The peak may be broad, spanning several pH units and represents a mixture of acid and alkaline phosphatases (Mc Laren, 1963).

The pH optima of soil enzymes differed significantly from those of other sources. For example, optimum pH values for soil urease were significantly higher than corresponding values of jackbean urease (Miller *et al.*, 1968 and Fishbein, 1969).

2.8 ENZYME KINETICS

The two most remarkable properties of enzymes are their specificity and their catalytic efficiency, and it is in these properties that enzymes differ most strikingly from simple catalysts. When it is possible to compare the enzymatic rates wih their non enzymatic counter parts, one finds that enzymes enhance the reaction by several orders of magnitude (Segel, 1975).

Soil enzymes are largely immobilized enzymes in soil colloidal particle and hence are different from homogenous systems. Neverthless with small substrates, the rates of reactions are not expected to be very much reduced as most of the diffusion mobility resides with the substrate.

by forming a complex or compound with the substrate presumably the complex of enzyme and substrate is unstable and proceeds through one or more steps of rearrangement to form the product plus the original enzyme. This theory of enzyme action was proposed by Michaelis and Menten and may be expressed by the following equation:

$$S + E \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \overset{K_3}{\rightarrow} E + P \qquad \ldots\ldots (1)$$

Where S is the substrate, E is the enzyme, ES is the intermediate enzyme substrate complex, P is the product of the reaction and k_1, k_2 and k_3 are the respective reaction velocity constants or rate constants of the three processes.

It can be shown, that with soluble substrate in excess, the rate of reaction i.e. the decrease in concentration of substrate with time or the increase in concentration of the product is given by

$$\frac{-ds}{dt} = \frac{dp}{dt} = k_3 [ES] = \frac{k_3 [E] [S]}{K_m + [S]} = \frac{V_{max} [S]}{K_m + [S]}$$

Where S and ES are the concentration of substrate and enzyme substrate complex, respectively and K_m is Michaelis constant.

$$K_m = \frac{k_2 + k_3}{k_1}$$

$$V_{max} = k_3 E$$

K_m is equal to substrate concentration (expressed in moles per litre) at $V = V_{max}/2$. When $k_2 >> k_3$, K_m may be set equal to the dissociation constant (k_2/k_1) of th enzyme-substrate complex and $1/K_m$ then becomes the "affinity' constant.

Although these equations are basic, it must be kept in mind that pH, Ionic strength, temperature and many other factors influence the values of k_1, k_2 and k_3 (Irving and Cosgrove, 1976).

For the experimental determination of V_{max} and K_m linear form of the Michaelis-Menten equation are generally used. The three linear transformations that commonly used are

1. $$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{[S]}$$ Lineweaver-Burk transformation

2. $$\frac{[S]}{V} = \frac{K_m}{V_{max}} + \frac{1}{V_{max}} \cdot [S]$$ Hanes-Wolf-transformation

3. $$V = V_{max} - K_m \cdot \frac{V}{[S]}$$ Eadie-Hofstee transformation

Plots of the variables of such relationships normally give straight lines. The value of the slope and intercept are commonly used for the determination of the constants from a set of experimental data. Once the K_m and V_{max} are known for a particular enzymatic reaction under a given set of conditions, the reaction velocity, V, can be calculated for any substrate concentration. The Michaelis constant is by far the most fundamental constant in enzyme chemistry. It has the dimensions of concentration (i.e. moles L^{-1}) and it is a constant for the enzyme only under rigidly specified conditions. The K_m value is useful in estimating the substrate concentration necessary to give a maximum velocity.

Although the literature on soil enzymes is on the increase, reports on kinetic constants like Michaelis Constant and V_{max} and their correlation with soil properties are limited. Values for both K_m and

V_{max} vary with the type of soil and also its physical fractions. Than values are also influenced by assay conditions like choice of substrate and buffer, use of shaken or unshaken soil suspensions.

2.8.1 Urease

K_m and V_{max} values for ureases of different particle size fractions of soils differed from each other and from those of unfractioned soils (Tabatabi, 1973). Generally the K_m values of fractions were greater than those of unfractionated soils but no relationship of these values with particle size could be so established. V_{max} values of all fractions were considerably less than those of the unfractionated soils, indicative of urease destruction, perhaps during the sonic vibration treatment used to disperse the soils before isolating the fractions.

K_m values may also fluctuate, depending on whether it is in the free or in an absorbed state (Mc Laren and Packer, 1970). While investigating the enzyme splitting of urea in the presence of bentonite, Durand (1966) obtained higher K_m values for adsorbed than for free enzyme. K_m values also varied with pH of assay, being lowest at the pH optimum. In general K_m for soil enzymes are greater than that for the corresponding pure enzymes. Paulson and Kurtz (1970), indicating a much lower apparent affinity of the adsorbed enzyme for the substrate compared to that of the native enzyme. Shaking of soil suspension during assay decreased K_m values and increased V_{max} values for soil urease (Tabatabai and Bremner, 1971 and Tabatabai, 1973).

Kinetic constants may also differ with origin of the enzyme. Frankenberger and Tabatabai (1982) reported that enzyme urease of plant origin has different kinetic constants and pH_{opt} than that of the native soil enzyme. Also urease of microbial origin may differ in properties from that released by soil microflora.

2.8.2 Phosphomonoesterases

Michaelis - Menten constants of soil acid phosphatase have been reported by several workers (Tabatabai and Bremner, 1971; Cervelli *et al.*, 1973 and Thornton and Mc Laren, 1975). The apparent K_m values of acid phosphatase in soils range from 1.3 to 4.5 mM while the same for alkaline phosphatase range from 0.4 to 4.9 mM. The K_m values obtained for phosphomonoesterases are affected by shaking the soil substrate mixture during incubation, normally the values are more uniform among soils when shaking than when static incubation technique is employed (Eivazi and Tabatabai, 1977). By changing the buffer system and pH used by Tabatabai and Bremner (1969) in assay of phosphatase activity in soils, Cervelli *et al.* (1973) showed that the substrate 4-nitrophenyl phosphate is sorbed by soils in the presence of 0.5 M NaOAc buffer pH 4.7. They used adsorption parameters derived from the Freundlich Isotherm to determine concentration of the free substrate and to calculate the K_m value of acid phosphatase. Irving and Cosgrove (1976) examined the graphical techniques used in calculations of the K_m values of acid phosphatase and concluded that the linear transformation of Eadie-Hofstee is superior. Presumably this graph shows the greatest deviation from the classical Michaelis-Menten equation but recent work shows that the three linear transformations are equally applicable for estimation of the apparent K_m values of enzymes in soils (Dick and Tabatabai, 1978 and Tabatabai and Singh, 1979). Each transformation gives different weightage to errors in the variables (Dowd and Riggs, 1965) and this is reflected in the variation of estimated k_m and V_{max} values derived for any soil enzyme by using different plots.

2.9 EFFECT OF TEMPERATURE ON ENZYME CATALYSED REACTIONS

The activity of any chemical reaction increases with temperature, approximately doubling for every 10°C. The rate of enzyme catalysed reaction increases as the temperature increases until some high temperature is reached above at which the rate begins to decrease because of enzyme inactivation. The same pattern has been observed in soil enzymes by a number of investigators except the fact that the temperature over which the soil enzymes retain their stability is much higher than that for the free enzymes. This is attributed to the stability effect due to the immobilisation of the soil enzymes on soil particulate matter. The effect of temperature was earlier quantified in terms of Q_{10} which is V_2/V_1 for every 10°C interval. Q_{10} is constant till the temperature at which enzyme inactivation sets in.

The more important way of describing the temperature dependence of the rate constant is by the Arrhenius equation

$$K = A \cdot \exp(-E_a/RT)$$

Where, A is the pre exponential factor

E_a is the energy of activation

R is the gas constant and

The Arrhenius equation can be expressed in the logarithmic form as $\log k = \log A - Ea/RT$.

Log A and Ea can be determined from the intercept and slope, respectively of a linear plot of log K vs 1/T at temperature lower than the inactivation stage.

The thermodynamic activation energy parameters i.e. enthalpy and entropy are calculated using the transition state complex theory or theory of absolute reaction rate. According to this, a reaction is represented as follows:

$A + B \rightleftharpoons AB \rightarrow$ products i.e. the reactants A and B form an activated complex which on decomposition gives the products. The rate constant of the reaction is given by

$$k = \frac{k'T}{h} e^{-\Delta H^{\ddagger}/RT} e^{-\Delta S^{\ddagger}/R} \quad \text{- Eyring equation}$$

Where, k = rate constant moles Sec^{-1}

h = Planck's constant = 6.6256×10^{-27} erg Sec^{-1}

k' = Boltzman constant = 1.3805×10^{-16} erg deg^{-1}

ΔH , the entropy of activation is derived from the slope of the plot of ln k/T vs 1/T.

$\Delta S^{\neq}$ is generally calculated by substituting the value of entholpy obtained as above in the Eyring equation. $\Delta^{\neq}H$ and $\Delta^{\neq}S$ are good indicators of the nature of the activated complex. They provide information about the strength of binding of the reactants in the activated complex and also its degree of freedom.

Though the literature on soil enzymes assay is on the increase, the effect of temperature on the kinetics of soil enzymes and the consequent determination of energy of activation has found much less attention. Zantua and Bremner (1977) and Sahrawat (1984) studied the effect of temperature on soil urease activity and found that urease activity increased with increase in temperature from 10-70°C and then decreased drastically with further increase in temperature. Though the inactivation of soil urease has been detected at 65-70°C it is not completely destroyed when heated at 75°C for 24 hours (Zantua and Bremner, 1975) or at 80°C to 90°C for 48 hours (Conrad, 1940). However, soil urease is completely inactivated when soils are heated at 105°C for 24 hours (Zantua and Bremner, 1977). In contrast, Pal and Chhonkar (1979) reported that soil urease activity remained unaffected in a composite sample of cultivated surface soils by temperature treatments from 40-70°C given for 15 mts while exposure to 80^{o}C and above for the same duration completely destroyed the enzyme activity. Soil enzymes were reported by Tarafdar and Chhonkar (1978) to show higher resistence to thermal denaturation in a heterogenous soil system as compared to their behaviour in pure system. The greater thermal stability of urease in soils has been attributed to its adsorption on clay colloids (Pink *et al.*, 1954) or complexing of urease by amorphous organic materials which offers protection against heat denaturation (Ambroz, 1966). O'Toole and Morgan (1984) observed considerable variation between soils in the stabilities of urease enzyme towards thermal inactivation and suggested that these differences were principally due to soil pH status and adsorptive properties of the soils.

The kinetics of urea transformation as a function of temperature was studied by Kumar and Wagnet (1984) who found marginal differences in the values of standard free energy change ($\Delta G^{\ddagger}$), the Arrhenius energy of activation (E_a) and standard of activation entropy ($\Delta S^{\ddagger}$). Similar type of results were reported by Gould *et al.* (1973) while Rachinskiy and Pel'tser (1967) indicated a higher E_a value for soil urease.

For phosphatases, the optimum temperature appears to be around 60-70^{o}C for most soils studied and the temperature optima of these enzymes may be dependent upon incubation time since at higher incubation temperatures, time dependent inactivations could be taking place (Galstyan, 1965). Temperature coefficients calculated for phosphatases depended upon the particular temperature interval and to some extent upon the type of soil (Khaziev, 1975). Engergy of activation varied considerably and the changes in thermodynamic activation parameters were attributed to heterogenity in the composition and state of soil enzymes (Khaziev, 1975; Dick and Tabatabai, 1984).

2.10 EFFECT OF TRACE ELEMENTS ON SOIL UREASE AND PHOSPHATASE ACTIVITIES

Addition of trace elements to soils may affect microbial proliferation and enzymatic activities, possibly leading to a decrase in the rates of biochemical processes in the soil environment. They may also inhibit enzyme reactions by complexing the substrate or by combining with the enzyme substrate complex. The mode of inhibition is dependent on the type of substrate used. The relative effectiveness of trace elements, including heavy metal ions, as inhibitors of enzyme activities, has varied with the soil, the concentration and form of the added element and the enzyme assayed. Bremner and Douglas (1971) found that the amounts of Ag^+, Hg^{2+} and other heavy metal ions needed to effect substantial inhibition of soil urease were much greater than the amounts needed for equivalent inhibition of Jackbean or soybean urease. They also found that the inhibitory effects at 50 ppm (soil basis) of different metallic cations on soil urease activity decreased in the order : $Ag^+ > Hg^{2+} > Au^{3+} > Cu^{2+} > Co^{2+} > Pb^{2+} > 4s^{3+} > Pb^+ > Cr^{3+} > Ni^{2+} >$ others. Similar results were also reported by Tabatabai (1977). They reported that metal ions inhibition was noncompetitive and postulated that the inhibition involved the reaction of metal ions with sulphahydryl groups in the catalytic site of urease Hughes *et al.* (1969) reported that copper ion forming a more stable sulphide is a considerably more powerful inhibitor than zinc ion. Nor (1982) while working with four Malaysian soils, reported that inhibition of urease activity was effectively achieved using Ag^+ while Cu^{2+} was only effective in the other two soils. Badr-El-Din *et al.* (1985) studied the inhibitory effect of metallic cations on urease activity and reported that Cu^{2+}, Fe^{3+}, Al^{3+} and Co^{2+} in their sulphate form reduced the accumulation of NH_4^+-N and gaseous losses by 35-50 per cent.

Studies performed with water soluble metallic salts may not permit one to draw valid conclusions concerning the effects of heavy applications of sewage sludges containing water insoluble forms of heavy metals on the activity of soil urease. Zantua and Bremner (1977) and Frankenberger *et al.* (1983) reported that urease activity in soils was not affected by the addition of large amounts of heavy metals in the form of sewage sludge. In a bid to ascertain the persistence of the inhibitory effects of metal ions on the activity of soil urease, Doelman and Haanstra (1986) found that Cu^{2+}. Ni^{2+} and Zn^{2+} inhibited the enzyme for a period of even more than one and half a year.

Soil phosphatase activity is inhibited by several micronutrient cations. Tylor (1976) found a linear inverse relationship between phosphatase activity and log (Cu+Zn) concentrations. He has also reported that at comparable concentrations, Cu had greater inhibitory effect than Zn. Anderson and Bengtsson (1973) demonstrated that the addition of Cu and Zn cations to soil caused an instantaneous inhibition of phosphatase followed by a slow recovery. Inhibition of phosphatase by Vanadium and Hg^{2+} ions was reported by Tylor (1976) and Kiss *et al.*, (1974), respectively, but at a relatively higher concentration. Juma and Tabatabai (1977) evaluated the effects of 20 trace elements and showed that the relative effectiveness of the trace elements in inhibition of phosphatase activity varies with the soil.

2.11 INTERACTION OF ENZYMES WITH SOIL CONSTITUENTS AND THEIR STABILIZATION

The observation that soil enzymes are extremely long lived, fairly steady in their activity content and stable towards protease and other adverse physical environmental factors shows that these enzymes are some how protected in the soil environments. Elucidation of the mechanism by which these enzymes are protected could contribute significantly to development of immobilized enzymes for commercial use.

Reactions of enzymes with clay or organic polymers usually decrease enzyme activities, but may stabilise the bound enzymes against degradation by proteinases or denaturating agents (Burns, 1978 and Boyd and Mortland, 1980).

Interaction of clay with organic substances including protein was reported by many authors. This has been reviewed extensively by Theng (1979)

Zvyagintsev and Velikanov (1968) reported that adsorption of enzymes by Montmorillonitic minerals was greater than that by kaolinite or soils.

Mohamood and Attiu (1970) concluded that adsorption of amylase by different soils was positively correlated with their clay contents. Ross and Mc Neilly (1972) found that only montmorillonite and allophane could significantly inhibit glucose oxidase activity. The amount of enzyme adsorbed on clay was influenced by the type of clay used and also by the saturating cations (Griffith and Thomas, 1973).

Although initial views regarded clay enzyme interactions as the principal stabilizing mechanism for enzymes in soil, later emphasis centered around the role of soil humic substances as stabilizing agents. A critical review of enzyme humus complex is given by Ladd and Butler, 1975. The forces binding enzymes and soil organic matter have been identified as Ionic, ii-bonding, covalent, hydrophobic and Vander waal's forces. Several references of model, water insoluble organic polymer enzyme derivatives have been given by Ladd and Butler (1975). These are primarily based on adsorption of enzymes on synthetic resins, physical immobilization in gels, covalently cross linked enzyme organic polymers

2.12 EFFECT OF HERBICIDES AND PESTICIDES ON ENZYME ACTIVITIES

Agrochemicals are of critical importance in achieving improvements in agricultural production and the use of agrochemicals in developed countries has undergone tremendous growth in the last few decades. The need for substantial increase in agricultural production is an urgent problem particularly in the less developed areas of the world and further increase in agrochemical use can be foreseen.

Various pesticides and other agricultural chemicals have been studied with a view of ascertaining their effect on soil micro organisms and enzyme activities and discussions concerning the nature and properties of the selected enzyme activities are rarely reported.

The inhibition of soil enzymes by agrochemicals can be of direct or indirect. For example, the inhibition of Jackbean urease by substituted ureas is considered to be due to the complexation of the enzyme with the carboxyl group in the substituted ureas (Cervelli *et al.*, 1975 and 1977). On the other hand any action of the chemicals altering the life functions of soil organisms could indirectly effects soil enzyme activity. Agrochemicals often stimulate or decrease the growth of soil microbial population and thus may alter the enzyme activity. Also these chemicals may exert some physiological effect on living organisms. An agrochemical may also modify the inter relationship between the particular group of organisms and thus influence the amount and type of enzyme produced.

Haunova and Hlebarova (1984) reported that treatment of alluvial meadow soil smolnitza with linuron and metobromuron caused changes in the urease activity of soils. Saksena and Kalyan Singh (1984) studied the effect of agroson GN, aldrin and 2.4-D Na salt on urease activity and reported that all the three herbicides inhibit urease activity among which agroson GN exhibited maximum. Gadkeria (1984) found that when applied at the rate of 50 ppm of soil, Goltia (metamituron) recorded initial increase in urease activity which was later decreased. Liao and Raines (1985) studied the effect of amido derivatives of phosphoric and thiophosphoric acids in four calcareous soils. They observed that the urease activity was inhibited more by di and triamides of phosphoric acid and thiophosphoric acids than by the monoamide. The greatest inhibition was by phenyl phosphoryl triamide and thiophosphoryl triamide.

Contrary to this, no change in urease activity was recorded (Baruah and Misra, 1986) for the herbicides used.

Effects of pesticides on soil phosphatase vary considerably. Some of the pesticides increased activity (Verstraete and Voets, 1974), some had no effect (Calero *et al.*, 1984) while a majority reduced activity (Voets *et al.*, 1974 and Karanth *et al.*, 1975). Voets *et al.* (1974) found the herbicide atrazine significantly reduced phosphatase and other enzyme activities in soil samples eleven months after application but that renewed application caused no further loss. They suggested that the reduction of activity resulted in part atleast from the elimination of vegetative cover and the associated reduction of soil organic matter. Zubets (1973) implicated a similar mechanism to account for the effect of simazine and atrazine. Goskowska and Furezak (1981), reported that long term application of Gestop 50 to soil under maize culture reduced the phosphatase activity whereas application of Aretit in the soil under monoculture of legume and phenoxy acetic herbicides in wheat and barley recorded no change in enzyme activity.

2.13 INFLUENCE OF RHIZOSPHERE ON ENZYME ACTIVITY

The presence of plants and the type of plants grown on a soil will have a marked effect on its enzyme activities. The effect is either direct or indirect. The direct contribution is by way of plant enzymes. In addition to endoenzymes contained in plant residues, extracellular enzymes secreted by living roots may make a significant contribution to the total activity. The indirect effect is caused by many factors, basically, however, changes in enzyme activities are related to changes in soil organic matter content and microbial population brought about by the plants.

Khan (1970) examined enzyme activity in soil as influenced by various crops and fertilizers and noted that invertase, phosphatase, and catalase activities in the surface horizon increased when virgin soils were cultivated. Rhizosphere enzyme activities tend to be higher than activities remote from the plant roots (Kiss *et al.*, 1975). Vlesyuk *et al.* (1959) compared activities in the rhizosphere soil with those in the non-rhizosphere soil and observed a considerable increase in urease activity in the rhizosphere of legume but the differences in urease activity associated with other crops were less pronounced. Similarly they noted that invertase, phosphatase and protease activities were much higher in the rhizosphere of legumes particularly in clover and lupin than in the rhizosphere of oats and wheat.

The stage of plant growth can affect soil enzyme activity; the activity being high when growth is most intensive. Amylase activity in the rhizosphere of finger millet, cultivated on a red loams soil increased with plant age; maximum activity was recorded on 60th day plant growth (Balasubramanyan *et al.*, 1970). The increase was attributed to the growing root system serving as source of amylase. It was also noted that the activities depend on the preceeding crop and also on the nature of present crop. Depending upon the crop, cellulase activity of sod-podzolic soil decreased in the following order maize > winter wheat > potatoes > clover. Similar results were also reported by Dalal (1982), Neal (1982) and Hoult and Mc Garity (1986). Increase in enzyme activity due to tillage was reported by Doran (1980). Enzyme activities are also altered due to addition of residues (Perucci *et al.*, 1984) and with grazing intensity (Dormaar *et al.*, 1984).

***

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The details of the materials used and the methods employed are presented below.

3.1 LABORATORY STUDIES

3.1.1 Soils

Sixty four soil samples widely varying in physico-chemical properties were collected from different parts of Andhra Pradesh. These soils were air dried and passed through 2 mm sieve before use. From these, five soils that have been taxonomically classified i.e. two alfisols from ICRISAT and Agricultural College Farm, Rajendranagar, two mollisols from Tenali and Bapatla of Guntur district and one entisol from Agricultural College Farm, Bapatla were selected for use in the detailed experiments outlined. The characteristics of these five soils are described below.

Soil I: Soil collected from Agricultural College Farm, Rajendranagar, Hyderabad is red sandy loam in texture with good drainage and has been classified as Haplustalf.

Table 1: Physico-chemical properties of the soils

S.No.	Description	depth of sampling	pH	Ec (dSm^{-1})	Organic Carbon (%)	Clay (%)	Silt (%)	Sand (%)
1.	Soil - I	0-15 cm	7.4	0.45	0.41	31	10	59
2.	Soil - II	0.15 cm	7.8	0.55	0.52	55	27	18
3.	Soil - III	0.15 cm	7.2	0.45	0.38	28	12	60
4.	Soil - IV	0.15 cm	7.3	16.65	0.43	52	16	22
5.	Soil - V	0.15 cm	7.0	0.35	0.26	8	13	79

Soil II: Soil collected from Tenali, Guntur district, Andhra Pradesh is a grey brown clayey soil. It occurs on nearly level to gently sloping uplands and poor to imperfect drainage. The soil has been classified as udic pellustert.

Soil III: Soil collected from ICRISAT belongs to Patancheru series, clayey skeletal, mixed isohyperthermic, udic rhodustalf.

Soil IV: Soil collected from Bapatla, Guntur district, Andhra Pradesh comprises dark brown clayey soil with poor drainage. The soil has been classified as ustic pellustert.

Soil V: Soil collected from Agricultural College Farm, Bapatla is sandy soil with perfect drainage and classified as ustic psament.

The physico-chemical properties of these soils are given in Table 1.

3.1.2 Chemicals

Sodium 4-nitrophenyl phosphate: Substrate for the enzyme phosphomonoesterases was obtained from SISCO, Bombay.

Urease was obtained from M/s. Boehringer, Mannhein, West Germany. The specific activity of the enzyme urease was 125 EU 100 mg^{-1}.

Soil II: Soil collected from Tenali, Guntur district, Andhra Pradesh is a grey brown clayey soil. It occurs on nearly level to gently sloping uplands and poor to imperfect drainage. The soil has been classified as udic pellustert.

Soil III: Soil collected from ICRISAT belongs to Patancheru series, clayey skeletal, mixed isohyperthermic, udic rhodustalf.

Soil IV: Soil collected from Bapatla, Guntur district, Andhra Pradesh comprises dark brown clayey soil with poor drainage. The soil has been classified as ustic pellustert.

Soil V: Soil collected from Agricultural College Farm, Bapatla is sandy soil with perfect drainage and classified as ustic psament.

The physico-chemical properties of these soils are given in Table 1.

3.1.2 Chemicals

Sodium 4-nitrophenyl phosphate: Substrate for the enzyme phosphomonoesterases was obtained from SISCO, Bombay.

Urease was obtained from M/s. Boehringer, Mannhein, West Germany. The specific activity of the enzyme urease was 125 EU 100 mg^{-1}.

Table 1: Physico-chemical properties of the soils

S.No.	Description	depth of sampling	pH	Ec (dSm^{-1})	Organic Carbon (%)	Clay (%)	Silt (%)	Sand (%)
1.	Soil - I	0-15 cm	7.4	0.45	0.41	31	10	59
2.	Soil - II	0.15 cm	7.8	0.55	0.52	55	27	18
3.	Soil - III	0.15 cm	7.2	0.45	0.38	28	12	60
4.	Soil - IV	0.15 cm	7.3	16.65	0.43	52	16	22
5.	Soil - V	0.15 cm	7.0	0.35	0.26	8	13	79

All the other chemicals used in the routine analysis were analytically pure reagents.

To study the effect of pH on soil urease activity, universal buffer with a pH range from 3 to 12 was prepared as described by Vogel (1978) by mixing appropriate quantities of citric acid, potassium hydrogen phosphate, boric acid and diethyl barbituric acid.

Distilled water collected using all glass distillation set was used throughout the experiments for the assay of enzyme activities.

3.1.3 Physico-chemical properties of soils

3.1.3.1 Soil pH: The pH of the soil was determined in 1:2.5 ratio of soil to water suspension as described by Jackson (1967) using a glass electrode pH meter (Model LI-120 ELICO).

3.1.3.2 Electrical conductivity: The Ec was measured in 1:2.5 ratio of soil to water extract as detailed by Jackson (1967) using a conductivity bridge (Type CH-84, ELICO).

3.1.3.3 Organic carbon: Organic carbon in the soil was estimated of Walkley and Black (1934) as described by Jackson (1967) and expressed in percentage of organic matter.

3.1.3.4 Cation exchange capacity: The cation exchange capacity of soil samples was determined by the method as described by Richards (1954). A known amount of soil sample was taken in 50 ml centrifuge tube and three washings were given with 1 N sodium acetate (pH 8.2) solution. The excess sodium was removed by washing with alcohal and sodium on the exchange complex was replaced by washings with 1 N ammonium acetate (pH 7.0). The sodium content in the ammonium acetate extract was determined on an ELICO flame photometer and the CEC of soils was expressed in C mole kg^{-1}.

3.1.3.5 Mechanical analysis: Mechanical composition of soils was determined by the international pipette method (Piper, 1950). The relative proportions of sand, silt and clay of soils were determined to describe their textural classes.

3.2 ENZYMES

The experiments outlined below were conducted with urease (Urea amido-hydrolase EC 3.5.1.5) acid phosphatase (Orthophosphoric monoester phosphohydrolase EC 3.1.3.2) and alkaline phosphatase (Orthophosphoric monoester phosphohydrolase EC 3.1.3.1) present in the soils.

3.2.1 Assay of enzymes

3.2.1.1 Urease: Urease activity was assayed by quantifying the rate of release of NH_4^+ from the hydrolysis of urea as described by Tabatabai and Bremner (1972) but with some modifications as outlined below.

Soil sample (5 g) was taken in 50 ml capacity glass tubes to which 9 ml of distilled water was added. The tubes were gently swirled to mix the contents followed by the addition of urea solution (1 ml 0.2 M). The flask was swirled gently again for a few seconds and closed with stoppers. After incubation for a definite time at 37°C in a thermostatic waterbath, the reaction was terminated by adding 15 ml KCl-Ag_2SO_4 solution (100 mg Ag_2SO_4 was dissolved in 700 ml distilled water to which 300 ml water containing 149 g KCl was added). The contents were agitated in a mechanical shaker for one hour to release all the NH_4^+ formed and the suspension was allowed to settle. Control samples were run simultaneously in the same way except adding 1 ml 0.2 M urea solution after termination of reaction.

Tabatabai and Bremner quantified the NH_4^+ by distilling the suspension with MgO for 4 minutes. This method was replaced by the colourimetric method described by Dorich and Nelson (1983).

3.2.1.2 Modified method (Dorich and Nelson, 1983): One millilitre of the supernatant from the soil suspension after incubation with urea and deactivation with Ag_2SO_4 KCl was transferred to a 25 ml volumetric flask and to this was added 1 ml of 6% EDTA, 2 ml phenol-nitroprusside (7 g of phenol and 34 mg of sodium nitroprusside dissolved in distilled water and diluted to 100 ml) and 8 ml buffered hypochlorite reagent (prepared by dissolving 1.48 g NaOH and 4.98 g Na_2HPO_4 in 70 ml distilled water, adding 20 ml of NaOCl and adjusting the pH to 11.4 - 12.0). The volume was made up to the mark, mixed thoroughly by inverting several times, placed in a waterbath maintained at 40°C. 30 minutes was allowed for colour development. The flasks were then removed, brought to room temperature and the absorbance of the coloured complex was recorded at 636 nm by using a spectrophotometr (Varian DMS 90 UV visible spectrophotometer) against a reagent blank solution. The amount of NH_4^+ in the reaction mixture was read from the standard curve.

Urease activity was calculated from the NH_4^+ liberated and expressed as μg of NH_4^+ g^{-1} soil h^{-1}.

3.2.1.3 Standardization and evaluation of the modified indophenol blue method

The modified colourimetric method was evaluated by comparing the urease activity in twenty seven soils of widely different physico-chemical properties with that obtained by the steam distillation method of Tabatabai and Bremner (1972).

Ten grams soil was taken and incubated with 2 ml of 0.2 M urea solution at 37^{o}C. The reaction was terminated after 4 hours of incubation in order to get sufficient quantity of NH_4^+ released to be estimated by distillation method. The contents were agitated in a mechanical shaker for 1 hour and the NH_4^+ liberated was determined separately by distilling 20 ml of the aliquot with MgO and also by indophenol method (Dorich and Nelson, 1983). Urease activity was calculated as the rate of release of μg of NH_4^+ g^{-1} soil h^{-1} obtained from the above estimations.

Urease activity determined by using the above two methods correlated significantly (r = 0.99) and was thereafter used for all the assays.

3.2.1.4 Linear relationship of urease activity with time

To study the linear relationship of soil urease activity with time 5 g soil samples in triplicates were separately incubated with 1 ml of 0.2 M urea and 9 ml of distilled water at 37^{o}C. After 1/2, 1, 2, 3 and 4 hours of time the enzyme activity in the reaction mixture were terminated by adding KCl-Ag_2SO_4 and assayed for urease activity as described in Section 3.2.1.2.

3.2.2 Determination of urease activity at different moisture levels

For studying the effect of moisture on urease activity, 5 g of soils I to V, the moisture of which was already determined were taken in 50 ml capacity glass tubes. To this, 0.5 ml urea (0.2 M) and calculated quantities of distilled water were added separately, so that the moisture levels of the soil were 20, 40, 60, 80 and 100 per cent. The soils were incubated at 37°C for two hours in a thermostatic water bath and assayed for urease activity as described in Section 3.2.1.2.

3.2.3 Effect of pH on soil urease activity

In studying the effect of pH on soil urease activity, modified universal buffer with a pH range from 3-12 was prepared as described in the Section 3.1.2. Buffer solutions (9 ml) of different pH were added to 5g soil in 50 ml capacity glass tubes in quadruplicates, equilibrated for 24 hours to obtain stable equilibrium soil pH conditions which were measured. The soil suspensions were then incubated with 0.2 M urea and the urease activity was assayed as described in Section 3.2.1.2.

3.2.4 Assay of phosphomonoesterases

The procedure of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) were adopted for the assay of the acid and alkaline phosphatase activities, respectively.

Soil samples (1 g) were taken in 50 ml capacity glass tubes to which 4 ml modified universal buffer (pH 6.5 for assay of acid phosphatase and pH 11.0 for assay of alkaline phosphatase) and 1 ml of 4 - nitrophenyl phosphate solution made in the same buffer were added. The glass tubes were swirled for a few seconds to mix the contents, stoppered and incubated for two hours at 37°C in a waterbath. The stoppers were removed and 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added to deactivate enzyme and extract the 4-nitrophenol liberated. The glass tubes were swirled for a few seconds and the soil suspension was filtered through Whatman No.2 filter paper. The absorbance of the yellow colour of 4-nitrophenol liberated due to the hydrolysis of the substrate by the enzymes was measured at 420 nm against a reagent blank. Controls were run simultaneously following the same procedure except adding 1 ml 4-nitro-phenyl phosphate after the addition of 1 ml 0.5 M $CaCl_2$ and 4 ml 0.5 M NaOH. Corrections were made for the control/blank values.

The amount of 4-nitrophenol liberated was computed, with reference to the standard curve of the colour intensity of 4-nitrophenol. The standard curves were run parallel with each set of phosphatase determinations.

The enzyme activities were calculated from the liberated 4-nitrophenol and expressed as μg of 4-nitrophenol released g^{-1} soil h^{-1}.

3.2.5 Relationship between soil urease and phosphomonoestarase activity and soil properties

Sixty four surface (0-15 cm) soil samples from different parts of Andhra Pradesh were collected. Each sample was air dried, crushed to pass through a 2 mm sieve. pH, electrical conductivity, organic carbon, CEC and texture were determined as described in Section 3.1.3. Enzyme activities viz. urease and acid and alkaline phosphatase activities were assayed as described in the Sections 3.2.1.2 and 3.2.4, respectively. Correlation and multiple regression analysis were carriedout on a computer.

3.2.6 Determination of K_m and V_{max} values

The method described by Tabatabai and Bremner (1971) was used for the determination of the K_m and V_{max} values of soil urease and phosphomonoesterases.

For the determination of K_m and V_{max} values, the emzyme activities were determined at a series of substrate concentrations essentially in the same manner as described before. The time of incubation was reduced to 15 minutes so that there is no significant change in the substrate concentration at lower urea concentration. This was necessary to ensure that the enzyme activity was linear with time interval. The experiment is explained in detail below.

3.2.6.1 Urease: Soil samples (5 g) were taken in 50 ml capacity glass tubes to which 9 ml distilled water was added. Substrate i.e. urea solutions (1 ml) of 2, 4, 6, 8, 10, 20, 30, 40, 60, 80 and 100 mM strength were added to different glass tubes so as to obtain 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 and 10.0 mM urea solutions in the incubation mixture. After 15 minutes of incubation at 37°C in a thermostatic waterbath, the reaction was terminated and urease activity (V) was determined by estimating the NH_4^+ liberated as described in Section 3.2.1.2.

3.2.6.2 Acid and alkaline phosphatase: The same procedure as described for soil urease was adopted for the determination of K_m and V_{max} of acid and alkaline phosphatases also.

P = Product

k_1, k_2 and k_3 are velocity constants for the respective steps.

$$V = \frac{k_3 [E] [S]}{K_m + [S]} = \frac{V_{max} [S]}{K_m + [S]} \qquad \ldots (2)$$

Where, V = Velocity of reaction

V_{max} = $k_3 [E]$

K_m = Dissociation constant of the enzyme substrate complex

The three linear transformations of the above equation given below were used to determine V_{max} and K_m.

1. Lineweaver - Burk transformation

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{[S]}$$

V_{max} was calculated from $\frac{1}{\text{Intercept}}$ and

K_m from $\frac{\text{slope}}{\text{Intercept}}$ of the plot of $\frac{1}{V}$ vs $\frac{1}{S}$

2. Hanes - Wolf transformation

$$\frac{[S]}{V} = \frac{K_m}{V_{max}} + \frac{1}{V_{max}} \cdot [S]$$

Soil samples (1 g) were taken in 50 ml capacity glass tubes to which was added 4 ml modified universal buffer (pH 6.5 for assay of acid phosphatase and pH 11.0 for assay of alkaline phosphatase). Different concentrations viz. 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 mM of substrate solution i.e. 4-nitrophenyl phosphate were prepared separately and 1 ml solution was added to each glass tube. The final concentrations of substrate in the incubation mixtues were 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mM. After 15 minutes of incubation in a water bath at 37°C, the glass tubes were removed and 1 ml 0.5 M $CaCl_2$ and 4 ml 0.5 M NaOH were added to each tube. The enzyme activity (V) was determined by estimating the 4-nitrophenol released as described in Section 3.2.4. The experiments were conducted in triplicate.

3.2.6.3 Calculation of K_m and V_{max} values : K_m and V_{max} values were calculated using Michaelis - Menten equation, for enzyme reactions (Eqn.).

$$S + E \underset{k_2}{\overset{k_1}{=}} ES \xrightarrow{k_3} E + P \qquad \ldots (1)$$

Where : S = Substrate

E = Enzyme

ES = Enzyme substrate complex

P = Product

k_1, k_2 and k_3 are velocity constants for the respective steps.

$$V = \frac{k_3 [E][S]}{K_m + [S]} = \frac{V_{max} [S]}{K_m + [S]} \qquad \ldots (2)$$

Where, V = Velocity of reaction

V_{max} = $k_3 [E]$

K_m = Dissociation constant of the enzyme substrate complex

The three linear transformations of the above equation given below were used to determine V_{max} and K_m.

1. Lineweaver - Burk transformation

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{[S]}$$

V_{max} was calculated from $\frac{1}{\text{Intercept}}$ and

K_m from $\frac{\text{slope}}{\text{Intercept}}$ of the plot of $\frac{1}{V}$ vs $\frac{1}{S}$

2. Hanes - Wolf transformation

$$\frac{[S]}{V} = \frac{K_m}{V_{max}} + \frac{1}{V_{max}} \cdot [S]$$

V_{max} was obtained from $\frac{1}{slope}$ and

K_m from $\frac{Intercept}{slope}$ of the plot of $\frac{[S]}{V}$ vs $[S]$

3. Eadie - Hofstee transformation

$$V = V_{max} - K_m \cdot \frac{V}{[S]}$$

Plot of V vs $\frac{V}{[S]}$ gave

V_{max} as the intercept

K_m as the slope

3.2.7 The effect of temperature

3.2.7.1 Urease : For studying the effect of temperature on soil urease activity, 5 g of soil 9 ml distilled water and 1 ml 0.2 M urea were taken in 50 ml capacity glass tubes and incubated in a B.O.D. incubator/constant waterbath at temperatures of 20, 30, 40, 50, 60, 70, 80 and 90°C. After two hours of incubation, the reaction was terminated and urease activity was assayed as described in Section 3.2.1.2. The experiments were conducted in triplicate.

3.2.7.2 Acid and alkaline phosphatase : For studying the effect of temperature on acid and alkaline phosphatase activities 1 g soil with 4 ml modified universal buffer (pH 6.5 for assay of acid and pH 11.0 for assay of alkaline phosphatases), 1 ml 4-nitrophenyl phosphate was taken in 50 ml capacity glass tubes and incubated in a B.O.D. incubator/costant waterbath at temperatures of 20, 30, 40, 50, 60, 70, 80 and 90°C. After two hours of incubation the enzyme activities were determined as described in Section 3.2.4. The experiments were conducted in triplicate.

3.2.7.3 Temperature coefficient (Q_{10}): Temperature coefficient (Q_{10}) values were obtained from the ratio of V_2 and V_1, the velocity of the reactions at ten degree intervals.

3.2.7.4 Arrhenius energy of activation (Ea): Energy of activation (Ea) was calculated using the Arrhenius equation

$$K = A.\ \exp.\ (-Ea/RT) \qquad \ldots.\ (1)$$

Where A = Pre exponential factor
Ea = Energy of activation
R = gas constant
T = Temperature in degrees Kelvin

The logarithmic transformation of equation (1) gives

$$\log k = (-Ea/2.303\ RT) + \log A$$

log A and Ea were determined by the least square analysis of log k vs 1/T.

3.2.7.5 Thermodynamic activation parameters (enthalpy $\Delta^{\neq}H$ and entropy ΔS) : The enthalpy and entropy of activation were determined using the Eyring equation (Eqn 2).

$$k = \frac{k'T}{h} e^{\Delta^{\neq}G/RT} = \frac{k'T}{h} e^{\Delta^{\neq}H/RT} e^{\Delta^{\neq}S/R} \quad \ldots(2)$$

Where,

G = Free energy of activation

H = Enthalpy of energy of activation

S = Entropy of energy of activation

k = the specific reaction rate for the reaction

k' = Boltzman constant, 1.3805×10^{-16} erg degree^{-1}

h = Planck's constant, 6.6256×10^{-27} erg sec^{-1}

The logarithmic transformation of eqn 2 gives

$$\ln \frac{k}{T} = \frac{\log k'T}{h} - \frac{\Delta^{\neq}H}{RT} + \frac{\Delta^{\neq}S}{R}$$

Plot of $\frac{\log k}{T}$ vs $\frac{1}{T}$ gives

$$\text{Slope} = \frac{\Delta^{\neq}H}{R}$$

ΔS was obtained by substituting ΔH in the Eyring equation.

3.2.8 Effect of micronutrient cations on soil urease and phosphomonoesterases

3.2.8.1 Urease: Micronutrient solutions containing i.e. 0, 10, 20, 30, 40 and 50 μ M ml^{-1} of zinc, copper, manganese and iron were prepared from A.R. grade $ZnSo_4$ $7H_2O$, $CuSO_4$ $5H_2O$, $MnCl_2$ and $FeSO_4$, respectively. One milli litre of each of these solutions was added to 5 g soil dropwise so as to moisten the whole soil, followed by 8 ml distilled water and 1 ml substrate (0.2 M urea). After two hours of incubation at 37°C, the reaction was terminated and the urease activity was determined as described in Section 3.2.1.2.

3.2.8.2 Acid and alkaline phosphatases: one ml of solutions each containing 0, 2, 4, 6, 8 and 10 μ moles of zinc, manganese, iron and copper were added to 1 g soil followed by 3 ml modified universal buffer (pH 6.5 for assay of acid and pH 11.0 for assay of alkaline phosphatase) and 1 ml substrate (4-nitrophenyl phosphate). After two hours of incubation at 37°C the enzyme activities were determined as described in Section 3.2.4.

Percentage inhibition of enzyme activity by micronutrient cations was calculated from $(1 - \frac{B}{A})100$, where A is the enzyme activity of the untreated soil and B is the enzyme activity of the respective micronutrient treated soil.

3.2.9 Effect of clay on urease activity

3.2.9.1 Clay sample:

The clay sample predominantly montmorillonite was obtained from Sri Rangapur clay deposit in West Godavari district of Andhra Pradesh. Kaolinite and bentonite were supplied by M/s. Strake and Co., New Delhi and illite from the Wards Museum, New York. The clays were ground to a fine powder using procelain pestle and mortar. A sample was then transferred to a sedimentation cylinder after thorough dispersion in 0.05 N Na_2CO_3 (pH 9.5). The cylinder was filled with Na_2CO_3 solution and was shaken end over end for a minute and set aside after noting down the time. The 2 µ fraction was collected with an upward bent iphon at 30 cm depth. The cylinder was refilled with a_2CO_3 (pH 9.5) and the process of settling for edetermined time and decanting at 30 cm depth were eated till the yields became very small. The sample collected in a large container and sodium chloride added at the rate of 20 g per one litre volume. r flocculation of the clay, the supernatant was easily siphoned off. The clay was collected and stored as suspension after removing the excess salts with distilled water (Jackson, 1956).

3.2.9.2 Preparation of homoionic montmorillonites: Suitable amounts of the suspension of the 2 µ fraction were taken separately in 500 ml conical flasks. To these 1 M solutons of LiCl , CsCl , $CoCl_2$, $CuSO_4$, $ZnCl_2$, $CaCl_2$, $MgCl_2$, $BaCl_2$, KCl and NaCl were added. These were shaken thoroughly and set aside for 24 hours. The ion saturated clays were washed free of excess ions with distilled water and were redispersed in distilled water and used in adsorption studies.

3.2.9.3 Preparation of enzyme: The stock solution of urease was prepared by dissolving 1 tablet of urease (120 mg) in 100 ml of distilled water. Linear relationship between the amount of enzyme protein taken and its activity was asertained by determining the activity in a series of aliquots ranging from 1 to 8 ml of the stock solution.

3.2.9.4 Effect of pure clays on urease activity: The effect of clays on enzymatic activity was studied by equilibriating aproximately 40 mg of each of the clays with urease in glass tubes such that the final volume was 10 ml. The initial enzyme activity added ranged from 1 to 8 mg of NH_4+ released h-1. These were incubated at $30^{o}C$ for 24 hours with intermittant shaking and analysed as follows.

1. The clay-enzyme equilibriated solutions in the glass tubes were centrifuged at 12,000 rpm for 15 minutes. The whole supernatant was transferred carefully to which 1 ml of 0.2 M urea was added. After incubating for half an hour the reacton was terminated by making final volume to 25 ml with $KCl-Ag_2SO_4$. Before estimation of the liberated NH_4^+ the above solution was diluted to 5 times.

2. To the clay settled at the bottom of the glass tube by above operation, 10 ml distilled water and 1 ml of 0.2 M urea solution were added mixed gently and assayed for enzyme activity as described above.

3. In the other case, to the whole clay-enzyme suspension (equilibriated solution), 1 ml of 0.2 M urea was added mixed thoroughly and assayed for enzyme activity as in the case of supernatant.

The total amount of enzyme activity adsorbed was determined by substracting the amount of enzyme activity left from the amount of the activity added initially.

3.2.9.5 Effect of ion saturated clays: The ions used to saturate the clays comprised alkaline earth ions (Ca^{2+}, Mg^{2+}) divalent transition group ions (Cu^{++}, Co^{++}, Li^{++}, Cs^{++} and Zn^{++}) and the monovalent ions (Na^{+}, and K^{+}).

The experiment was carried out by equilibrating 2 ml of the suspension containing 40 mg of ion saturated clay with urease (initial activity is 5 mg of NH_4^+ released h^{-1}) and incubated at 30°C for 24 hours. The enzyme left in the clay suspension was assayed as described above and the quantity of enzyme adsorbed was calculated.

3.4 POT EXPERIMENTS

3.4.1 Effect of herbicides on urease activity

A pot experiment was conducted in Agricultural College farm, Bapatla during *kharif*, 1986 to study the effect of four different herbicides on soil urease activity with maize as test crop. The crop was grown in a black clay loam soil, neutral in reaction and low in organic matter content. For this, 10 kg air dried soil samples passed through 2 mm sieve were filled in glazed pots. A basal dose of 60, 40 and 40 kg ha-1 N, P and K, respectively were given in the form of urea, single superphosphate and muriate of potash. Five levels viz. 0, 1, 2, 3 and 4 mg Kg^{-1} of the active ingradients of four herbicides viz. Atrazine, Simazine, Methabenzthiazuron and Metoxuron were tried. Calculated quantities of these fertilizers and herbicides were applied in the form of solutions to each pot and mixed thoroughly with the soil so as to achieve an uniform distribution of the amendments. The pots were irrigated and left for two days for equilibriation. Surface soil in each pot was then pulverized with a plastic spatula. Six to seven maize seeds (Var. Ganga safed) were sown in each pot. The number of seedlings were later thinned to three per pot. Each concentration of a herbicide was replicated thrice.

The moisture status was maintained by irrigating the pots whenever necessary. Soil samples were collected after 20, 40, 60, 80 and 100 days of sowing, dried under shade, ground, passed through a 2 mm sieve and assayed for urease activity as described in Section 3.2.1.2.

Percentage inhibition of enzyme activity by each herbicide was calculated from $(1 - \frac{B}{A})100$, where A is the urease activity of the untreated soil and B is the urease activity of herbicide treated soil.

3.5 FIELD EXPERIMENTS

3.5.1 Influence of crop cover on soil urease activity

The rhizosphere of different crops has been reported to affect the activity of soil enzymes in different ways. A study was accordingly undertaken in All India Co-ordinated Sorghum Improvement Project farm, Rajendranagar, Hyderabad during *kharif*, 1986 and 1987 to study the influence of rhizosphere of four principal crops of the area viz. Groundnut (*Arachis hypogaea* var. PGN_2) Sorghum (*Sorghum bicolor* var. CSH_6 and CSH_9), Sunflower (*Helianthus annuus* var. EC 68414) and Redgram (*Cajanus cajan* var. C_{11}). The study was carried out in two different experiments outlined separately by growing these crops as sole crops in one experiment and as mixed crops in different combinations with sorghum as base crop in another experiment. The details of the treatments are given below.

Experiment I (Sole crop)

Treatments:

T_1 Sorghum var. CSH_6
T_2 Sorghum var. CSH_9
T_3 Groundnut var. PGN_2
T_4 Sunflower var. Ec 68414
T_5 Redgram var. C_{11}

Experiment II (Mixed crop)

Treatments :

T_1	Sorghum Var.CSH_6	+	Redgram	(1:1 row)
T_2	Sorghum var.CSH_9	+	Redgram	(1:1 row)
T_3	Sorghum CSH_9	+	Redgram	(3:3 rows)
T_4	Sorghum CSH_9	+	Groundnut	(3:3 rows)
T_5	Sorghum CSH_9	+	Sunflower	(3:3 rows)
T_6	Sorghum CSH_9	+	Redgram	(4:2 rows)
T_7	Sorghum CSH_9	+	Groundnut	(4:2 rows)
T_8	Sorghum CSH_9	+	Sunflower	(4:2 rows)

The spacing between two adjacent rows were maintained at 30 cms. The crops received normal agricultural practices recommended for them during crop growth. The soil samples were collected as described in Section 3.5.2 after 20, 40, 60, 80 and 100 days of sowing and assayed for urease activity as described in Section 3.2.1.2.

3.5.2 **Effect of sulphur on urease activity**

A field experiment was conducted to study the effect of different forms of sulphur on urease activity with gingelly as the test crop grown under rainfed conditions during *kharif* 1986 and 1987. The experiment was laid out on a sandy loam soil at the Directorate of Oil Seeds Research, Hyderabad with varying levels of sulphur viz. 10, 20, 30, 40 and 50 kg S ha^{-1} in two different forms, namely, elemental sulphur and gypsum. Each treatment was replicated three times. The plot size was 2.8 m x 1.5 m. A basal dose of 30, 30 and 20 kg ha^{-1} of N, P and K, respectively, were given in the form of urea, single superphosphate and muriate of potash. The crop was sown in rows and thinning was done after 10 days adopting a spacing of 15 cm x 15 cm. Soil samples were collected at 20, 40, 60, 80 and 100 days after sowing and assayed for urease activity as described in Section 3.2.1.2.

For collection of soil samples, wooden pegs were fixed at four different points in every plot and soils at a depth of 10 cm were collected within 30 cm radius at each time of sampling. The soils so collected were ground, mixed thoroughly and passed through a 2 mm sieve before taking up the assay of urease activity. Moisture content of the soil sample was determined and urease activity was assayed as described in Section 3.1.2.2.

RESULTS

CHAPTER IV

RESULTS

4.1 ASSAY OF SOIL UREASE

A variety of methods have been used for the assay of urease activity in soils among which the method proposed by Tabatabai and Bremner (1972) has been widely reported. The method designed for assay of urease activity under optimum conditions involves the determination of NH_4^+ released by the treatment of the incubated soil samples with 2 *M* KCl containing Ag_2SO_4 (to stop urease activity) followed by the steam distillation of an aliquot of the resulting soil suspension with MgO for 4 minutes, absorbing the NH_3 liberated into boric acid and titrating with a standard acid.

The above method was modified in this study and instead of distillation with MgO, the NH_4^+ liberated by the hydrolysis of urea by urease is directly determined using the modified indophenol blue method as described by Dorich and Nelson (1983) because this method is more sensitive and requires shorter incubation time. The adaptability of the method for the estimation of NH_4^+ recovery from soils was evaluated by adding different quantities of NH_4^+ to soils and determining the NH_4^+ in the KCl extract using both the methods. The results presented in Table 2 indicate the validity of the use of indophenol blue method in place of distillation with MgO for the estimation of NH_4^+ in the KCl extract of the soils.

4.1.1 Evaluation of the indophenol blue method for assay of soil urease

The new assay method for soil urease activity was evaluated thoroughly in a number of soils by comparing the results obtained by this method with that of Tabatabai and Bremner (1972) method and the results are presented in Table 3 and are depicted graphically in Fig. 1. Linear regression analysis was carried out for the activity obtained by the indophenol blue method against the distillation method. The following linear equation with regression coefficient r = 0.99 was obtained

In view of the excellent concordance between two methods and the distinct advantages of the indophenol blue method over others, all subsequent assays were carried out by this method only.

4.2 ASSAY OF PHOSPHOMONOESTERASES

Several methods have been proposed for the estimation of phosphomonoesterase activities of soils.

Table 2 : Comparison of NH_4 determination in the 2 M KCl extract

NH added to soil (µg 5 g^{-1} soil)	Distillation with MgO (µg 5 g^{-1} soil)					Indophenol blue method (µg 5 g^{-1} soil)				
	Soil I	Soil II	Soil III	Soil IV	Soil V	Soil I	Soil II	Soil III	Soil IV	Soil V
400	390	320	380	340	386	380	308	374	352	392
800	760	720	745	732	784	740	710	728	740	778
1200	1134	1080	1122	1094	1145	1102	1068	1120	1104	1164
1600	1530	1472	1508	1486	1528	1508	1460	1486	1502	1536

Table 3 : Assay of urease activity by indophenol and distillation method

	Location	Soil pH	Organic carbon (%)	Urease activity (μg of NH_4 released g^{-1} soil h^{-1})	
				Indophenol blue method	Distillation method
1.	Moncompu I	2.90	7.40	1.00	1.05
2.	Moncompu II	4.50	2.34	6.13	6.94
3.	Phoolbagh	7.00	1.17	20.53	19.15
4.	Beni	7.20	1.46	28.93	25.67
5.	Haldi	6.50	1.41	8.78	8.86
6.	Kumarganj	10.10	0.20	4.05	3.58
7.	Faizabad	7.30	0.50	6.03	7.37
8.	Patna	7.40	1.25	26.93	25.46
9.	Kapurthala	8.90	0.44	10.05	9.52
10.	Raipur	6.70	0.82	34.78	32.25
11.	Chiplima	6.40	0.74	26.55	22.93
12.	Chinsurah	7.40	0.78	3.21	2.95
13.	Pusa	8.60	0.34	7.63	7.58
14.	Ghagraghar I	7.50	0.86	1.68	1.47
15.	Ghagraphar II	7.70	0.52	2.44	1.84
16.	Ghagraghar III	7.30	0.98	3.19	4.42
17.	D R R	7.70	0.98	3.50	2.89
18.	R'Puram	8.90	0.55	3.76	4.00
19.	Malam	5.40	1.08	6.20	6.60
20.	ARI Farm	8.40	1.02	5.77	5.55
21.	CF, Bapatla	7.40	0.81	6.05	6.55
22.	Machavaram	8.20	0.62	3.65	3.10
23.	R'Nagar	6.80	0.64	4.10	3.44
24.	Karlapalem	7.40	0.82	3.70	2.66
25.	D O R	7.20	0.46	7.55	7.33
26.	ICRISAT	6.50	0.72	6.20	6.60
27.	Kathevaram	7.10	1.10	7.60	7.77

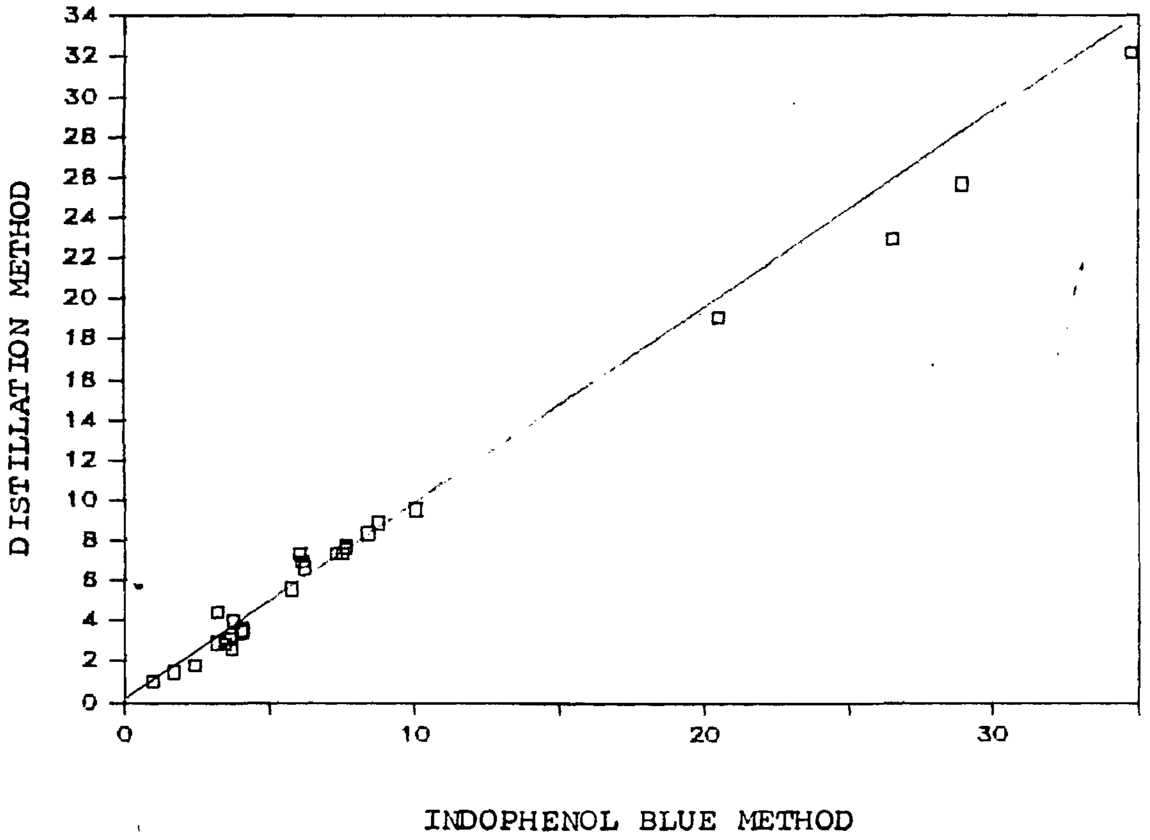


FIG.1 : COMPARISON OF NH_4 DETERMINATION IN THE 2 M KCl EXTRACT

The basic difference is in the substrate used and consequently in the technique employed in measuring the product of hydrolysis of the substrate by the phosphatase enzymes.

Of the various methods available for the assay of acid and alkaline phosphatases, the methods developed by Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) are the most rapid and precise and hence, were used by us. They involve the colourimetric estimation of the 4-nitrophenol released when soil is incubated with disodium 4-nitrophenyl phosphate buffered at pH 6.5 for assay of acid phosphatase activity and pH 11.0 for alkaline phosphatase activities. The $CaCl_2$ - NaOH treatment extracts the 4-nitrophenol released by phosphatase activity.

4.2.1 Levels of urease and phosphomonoesterases in the soils

The basic difference is in the substrate used and consequently in the technique employed in measuring the product of hydrolysis of the substrate by the phosphatase enzymes.

Of the various methods available for the assay of acid and alkaline phosphatases, the methods developed by Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) are the most rapid and precise and hence, were used by us. They involve the colourimetric estimation of the 4-nitrophenol released when soil is incubated with disodium 4-nitrophenyl phosphate buffered at pH 6.5 for assay of acid phosphatase activity and pH 11.0 for alkaline phosphatase activities. The $CaCl_2$ - NaOH treatment extracts the 4-nitrophenol released by phosphatase activity.

4.2.1 Levels of urease and phosphomonoesterases in the soils

Sixty four soil samples, widely varying in physico-chemical properties collected from various parts of Andhra Pradesh, were analysed for urease, acid and alkaline phosphatase activities as discussed in detailed under section 3.2.1.2 and 3.2.4. Physico-chemical properties of soils alongwith enzyme activities are presented in Table 4.

Table 4: Levels of urease and phosphomonoesterase activities in the soils.

S.No.	pH	Ec dSm^{-1}	Organic Carbon %	Clay %	Silt %	Sand %	Available nitrogen kg ha^{-1}	Available P$_2$O$_5$ kg ha^{-1}	Available K$_2$O kg ha^{-1}	Urease*	Acid phosphatase**	Alkaline phosphatase**
1.	7.40	0.45	0.41	31.00	10.00	59.00	120.00	46.00	162.00	10.45	38.00	66.00
2.	7.80	0.55	0.52	55.00	27.00	18.00	86.00	54.00	178.00	8.84	30.60	73.20
3.	7.20	0.45	0.38	28.00	12.00	60.00	92.00	38.00	154.00	7.50	31.00	64.40
4.	7.30	16.65	0.43	52.00	26.00	22.00	94.00	42.00	132.00	1.35	21.10	18.50
5.	7.00	0.35	0.36	8.00	13.00	79.00	86.00	52.00	104.00	2.32	43.30	12.50
6.	8.30	0.26	0.63	30.00	18.00	52.00	139.00	49.00	141.00	9.40	10.40	44.30
7.	8.40	0.22	0.72	26.00	26.00	48.00	75.00	49.00	218.00	10.20	15.10	75.40
8.	7.20	0.26	1.35	28.00	32.00	40.00	144.00	64.00	403.00	16.80	94.60	18.40
9.	8.30	0.22	0.06	40.00	20.00	40.00	95.00	38.00	157.00	9.20	33.80	102.90
10.	7.30	0.42	0.81	36.00	17.00	47.00	208.00	44.00	306.00	10.60	62.10	66.60
11.	7.20	0.42	0.81	42.00	18.00	40.00	144.00	49.00	339.00	11.00	62.60	62.00
12.	8.00	0.20	0.03	47.00	18.00	35.00	154.00	82.00	107.00	7.60	27.20	10.70
13.	7.00	0.16	0.36	47.00	21.00	32.00	141.00	77.00	215.00	7.20	53.50	26.50
14.	7.10	0.13	0.09	20.00	18.00	62.00	144.00	74.00	140.00	6.00	31.40	21.20
15.	7.30	0.12	0.62	27.00	15.00	58.00	136.00	89.00	124.00	8.60	56.20	125.00
16.	9.90	3.04	0.16	19.00	16.00	65.00	159.00	67.00	100.00	4.80	27.40	116.60
17.	9.90	1.63	0.67	21.00	21.00	58.00	146.00	59.00	87.00	8.60	11.20	60.10
18.	9.30	0.40	0.47	49.00	20.00	31.00	108.00	67.00	178.00	7.80	8.50	155.20
19.	7.20	2.48	0.37	50.00	10.00	40.00	126.00	74.00	73.00	6.60	26.60	18.10
20.	7.30	0.14	0.36	50.00	21.00	29.00	113.00	82.00	53.00	7.00	20.10	18.60
21.	7.10	0.09	0.21	36.00	17.00	47.00	62.00	76.00	57.00	6.40	18.40	117.00
22.	6.70	0.46	0.16	42.00	15.00	43.00	67.00	84.00	60.00	4.60	23.60	49.80
23.	6.90	0.12	0.16	41.00	30.00	29.00	54.00	51.00	34.00	3.80	22.50	112.20
24.	8.00	0.22	0.31	51.00	25.00	24.00	39.00	46.00	205.00	6.80	21.50	57.70
25.	8.40	0.14	0.83	41.00	29.00	30.00	129.00	56.00	305.00	9.40	6.40	34.10
26.	8.80	0.32	0.67	35.00	12.00	53.00	172.00	59.00	332.00	8.60	30.60	37.60
27.	8.30	0.19	0.47	21.00	19.00	60.00	139.00	97.00	238.00	6.20	24.50	74.40
28.	7.90	0.08	0.16	21.00	9.00	70.00	100.00	74.00	235.00	5.60	16.40	79.40
29.	7.90	0.10	0.31	35.00	11.00	54.00	105.00	72.00	252.00	6.40	35.60	87.60
30.	8.90	0.18	0.52	50.00	22.00	28.00	124.00	38.00	265.00	7.80	36.70	15.50
31.	8.50	1.12	0.41	29.00	19.00	52.00	77.00	76.00	403.00	6.20	15.10	16.00
32.	7.80	0.11	0.52	20.00	13.00	67.00	157.00	58.00	151.00	7.40	63.80	10.30
33.	7.50	0.11	0.26	20.00	19.00	61.00	149.00	64.00	67.00	6.10	39.90	12.10
34.	6.10	0.26	5.54	33.00	26.00	41.00	115.00	36.00	403.00	21.80	142.70	93.50
35.	7.40	0.10	0.47	30.00	23.00	47.00	159.00	56.00	138.00	6.80	14.30	66.10
36.	8.00	0.08	0.47	37.00	23.00	40.00	103.00	34.00	97.00	7.20	58.90	50.50
37.	8.00	0.22	0.78	26.00	24.00	50.00	157.00	49.00	255.00	8.90	30.10	63.90
38.	7.80	0.14	0.21	38.00	27.00	35.00	165.00	54.00	154.00	5.60	44.30	60.20
39.	6.30	0.10	0.26	43.00	18.00	39.00	134.00	42.00	117.00	4.90	21.60	84.60
40.	7.70	0.08	0.36	40.00	24.00	36.00	128.00	46.00	77.00	6.20	23.50	51.00
41.	8.10	0.11	0.26	30.00	28.00	42.00	149.00	28.00	155.00	5.80	21.90	81.00
42.	8.10	0.13	0.26	44.00	18.00	38.00	54.00	28.00	161.00	4.80	41.00	69.40
43.	7.30	0.10	0.26	36.00	34.00	30.00	124.00	34.00	285.00	6.40	25.70	110.50
44.	7.00	0.09	0.47	43.00	30.00	27.00	116.00	42.00	114.00	8.20	27.40	86.40
45.	7.00	0.18	0.72	40.00	22.00	38.00	95.00	61.00	122.00	10.40	16.70	116.80

Contd..

ontd..

.No.	pH	Ec $dS m^{-1}$	Organic Carbon %	Clay %	Silt %	Sand %	Available nitrogen kg ha^{-1}	Available P_2O_5 kg ha^{-1}	Available K_2O kg ha^{-1}	Urease*	Acid phosphatase**	Alkaline phosphatase**
46.	8.00	15.04	0.87	12.00	31.00	57.00	128.00	48.00	152.00	10.40	44.20	68.00
47.	8.50	7.62	0.68	25.00	7.00	68.00	110.00	60.00	136.00	16.30	56.20	112.00
48.	8.60	3.81	0.34	17.00	4.00	79.00	99.00	56.00	208.00	12.30	18.40	48.00
49.	8.60	7.62	0.24	29.00	3.00	68.00	132.00	32.00	172.00	15.60	66.60	114.60
50.	8.50	6.86	0.22	25.00	1.00	74.00	128.00	52.00	234.00	21.40	26.50	79.40
51.	8.00	22.86	0.91	25.00	31.00	44.00	142.00	42.00	155.00	26.50	21.20	84.60
52.	8.40	2.67	0.50	55.00	14.00	31.00	102.00	34.00	98.00	11.70	10.70	67.40
53.	8.20	9.40	0.80	17.00	8.00	75.00	148.00	67.00	104.00	15.70	34.00	72.00
54.	8.30	7.62	0.77	22.00	7.00	71.00	97.00	64.00	122.00	22.90	55.60	116.00
55.	8.30	3.81	0.57	31.00	4.00	65.00	98.00	54.00	154.00	16.90	37.60	54.00
56.	8.20	2.29	0.62	25.00	5.00	70.00	104.00	48.00	97.00	12.50	79.40	69.00
57.	8.30	14.08	1.09	30.00	11.00	59.00	140.00	52.00	132.00	18.60	87.60	72.00
58.	8.40	7.62	0.90	26.00	1.00	73.00	130.00	44.00	182.00	21.50	15.50	55.60
59.	7.40	7.62	0.99	26.00	4.00	70.00	145.00	56.00	164.00	16.30	34.10	60.10
60.	8.70	8.38	0.48	48.00	10.00	42.00	89.00	32.00	88.00	11.20	79.40	122.00
61.	8.70	7.62	0.45	34.00	21.00	45.00	92.00	28.00	104.00	8.50	18.40	13.40
62.	9.70	2.29	0.56	15.00	47.00	38.00	96.00	24.00	122.00	14.90	49.80	114.00
63.	9.10	3.81	0.25	14.00	3.00	83.00	108.00	32.00	180.00	21.70	18.10	72.60
64.	8.20	2.29	1.05	43.00	2.00	45.00	132.00	22.00	164.00	16.50	24.50	65.40

* μg of NH_4 released g^{-1} soil h^{-1} ** μg of 4-nitrophenol released g^{-1} soil h^{-1}

Urease activity of the surface soils expressed as μg of NH_4^+ released g^{-1} soil h^{-1} ranged from 3.8 to 26.5 with an average value of 10.5. Acid phosphatase activity of the soils expressed as μg of 4-nitrophenol released g^{-1} soil h^{-1} ranged from 6.4 to 142.7 with an average of 34.8 while the activity of alkaline phosphatase ranged from 10.3 to 155.2 with the average of 67.1.

4.2.2 Correlation coefficients between soil properties and soil urease, acid phosphatase and alkaline phosphatase

Correlation coefficients (r) between soil properties and soil urease, acid phosphatase and alkaline phosphatase activities calculated for all the soils showing a wide range in pH, texture and organic carbon are presented in Table 5.

Simple correlation analysis showed that urease activity correlated highly significantly with organic carbon ($r=0.78^{**}$) and clay content ($r=0.62^{**}$). Urease also correlated weakly with silt ($r=0.32^{*}$) and pH ($r=0.27^{*}$). Phosphomonoesterases correlated significantly with organic carbon and clay content and showed no significant correlation with pH and silt. The correlation coefficients obtained with organic carbon

Table 5 : Correlation coefficients between urease, phosphomonoesterases and other soil properties

Soil properties	Correlation coefficient (r)		
	Soil urease	Soil acid phosphatase	Soil alkaline phosphatase
Organic carbon	0.78**	0.62**	0.58**
pH	0.27*	0.12	0.19
Clay	0.62**	0.54**	0.48**
Silt	0.32*	0.21	0.17
Sand	-0.14	-0.11	-0.16
Available nitrogen	0.18	0.13	0.24
Available Phosphorus	0.19	0.29*	0.16
Available potassium	0.13	0.12	0.18

*, ** Significant at 5 per cnent and 1 per cent level, respectively.

($r=0.58^{**}$) and clay ($r=0.54^{**}$) for acid phosphatase and organic carbon ($r=0.62^{**}$) and clay ($r=0.48^{**}$) for alkaline phosphatase are highly significant. However, these enzymes studied, except acid phosphatase with available phosphorus ($r=0.29^{*}$) showed no significant correlation with any of the available major nutrients.

4.2.3 Determination of kinetic parameters of the enzymes

The two fundemental kinetic parameters of an enzyme catalysed reaction are V_{max}, which corresponds to the maximum potential of the enzyme present in the soil and is reached when all the active sites of the enzyme are fully saturated with the substrate and the Michaelis - Menten constant K_m, which is an index of the apparent affinity between the enzyme and its substrate. K_m is a measure of the dissociation constant of the enzyme-substrate complex. V_{max} and K_m were obtained by determining the enzyme activity for a series of substrate concentrations ranging from 0.2 mM to 10.0 mM.

4.2.3.1 V_{max} and K_m for soil urease : Soil urease activity increased with an increase in substrate concentration in the begining and almost reached a plateau at a substrate concentration of > 4 mM for soils I, II and III. For soils IV and V, the limiting value was reached at a higher concentration of urea i.e., 6 and 8 mM, respectively (Table 6 and Fig. 2). With further increase in substrate concentration, minimal change in enzyme activity was observed.

The V_{max} and K_m values were determined for urease using the three linear transformations of the Michaelis - Menten equation as described in section 3.2.4.3. Line weaver - Burk transformation plot of I/V against $1/[S]$, Hanes - Wolf transformation plot of $[S]/V$ against $[S]$ and Eadie - Hofstee transformation plot of V against $V/[S]$ for the five different soils studied are shown in Fig. 3. From the graph it is observed that with all the soils, reasonably linear plots were obtained in all the cases.

The values of V_{max} and K_m obtained from the least square analysis of these plots are presented in Table 7.

Maximum enzyme reaction velocity (V_{max}) of the soil urease was markedly different for different soils. When calculated as μ M of urea g^{-1} soil h^{-1} it varied from 0.044 mM to 0.432 mM and followed the sequence soil I > soil II > soil III > soil V > soil IV.

Table 6: Effect of substrate concentration on soil urease activity

Substrate Concentration (mM)	Urease activity (μg of NH_4 released g^{-1} soil h^{-1})				
	Soil I	Soil II	Soil III	Soil IV	Soil V
0.2	2.20	2.94	2.45	0.08	0.19
0.4	3.70	4.38	3.55	0.10	0.34
0.6	5.10	5.6	4.60	0.22	0.50
0.8	5.70	6.27	5.60	0.29	0.63
1.0	6.60	6.77	6.28	0.41	0.81
2.0	7.90	7.85	6.92	0.71	1.12
4.0	10.45	8.80	7.50	0.96	1.63
6.0	10.45	8.84	7.50	1.34	1.95
8.0	10.45	8.84	7.50	1.35	2.32
10.0	10.45	8.84	7.50	1.35	2.32

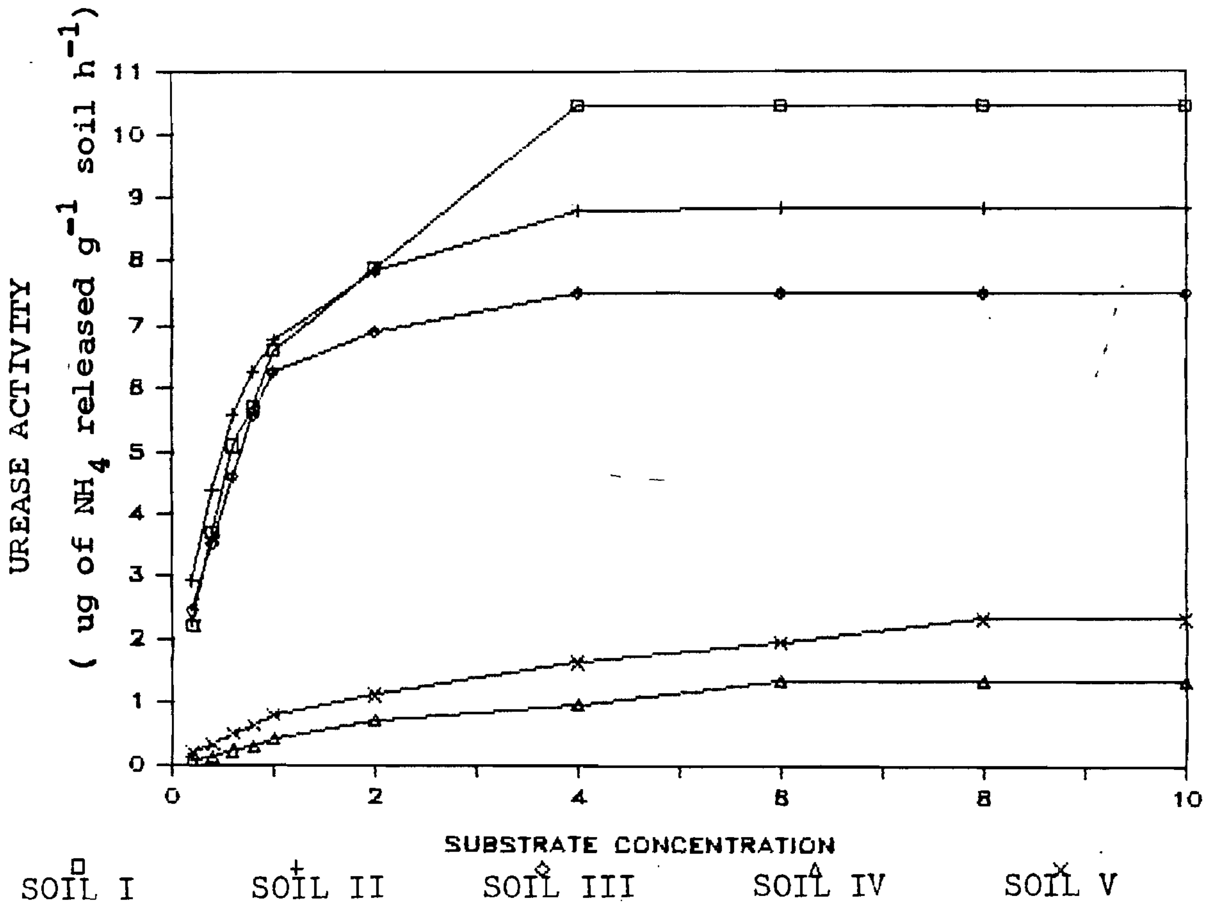
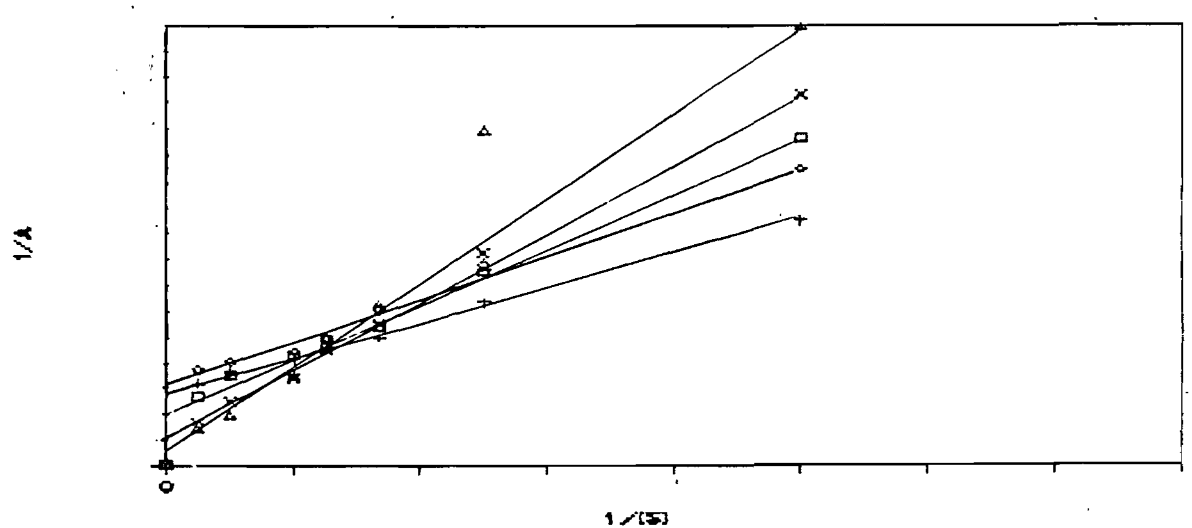


FIG.2 : EFFECT OF SUBSTRATE CONCENTRATION ON SOIL UREASE ACTIVITY

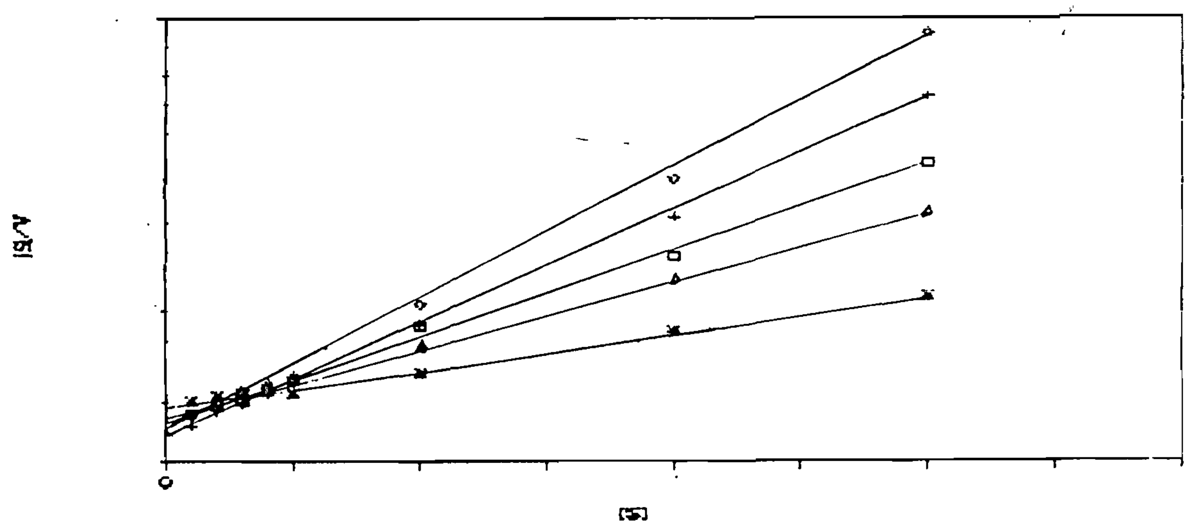
Table 7 : Maximum enzyme reaction velocity (VMax) and Michaelis constant (km) values of soil urease activity

Soils	Maximum enzyme reaction velocity (VMax) (mM of urea hydrolysed g^{-1} soil h^{-1})			Michaelis constant (km) (mM)		
	Lineweaver-Burk transformation	Hanes-Wolf transformation	Eadie-Hofstee transformation	Lineweaver-Burk transformation	Hanes-Wolf transformation	Eadie-Hofstee transformation
Soil I	0.432	0.341	0.368	0.876	0.907	0.898
Soil II	0.343	0.264	0.302	0.452	0.420	0.459
Soil III	0.310	0.225	0.352	0.518	0.412	0.063
Soil IV	0.044	0.049	0.070	3.001	3.292	3.493
Soil V	0.087	0.079	0.078	2.347	2.910	2.768

LINEWEAVER- BURK PLOT OF SOIL UREASE ACTIVITY



HANES - WOLF PLOT OF SOIL UREASE ACTIVITY



EADIE - HOFSTEE PLOT OF SOIL UREASE ACTIVITY

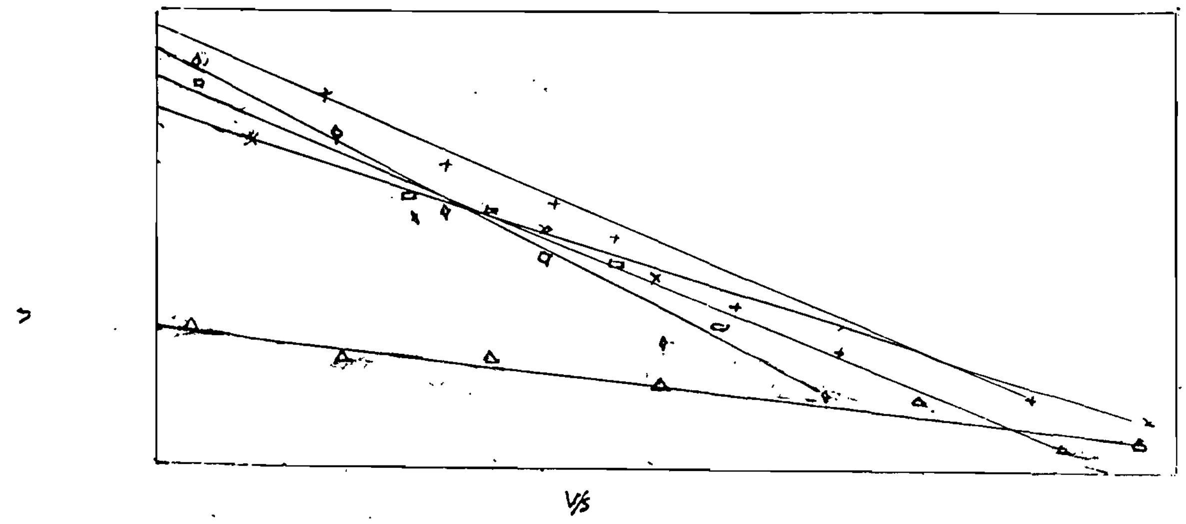


FIG.3 : LINEAR TRANSFORMATIONS OF SOIL UREASE ACTIVITY

Michaelis constant (K_m) of the soil urease calculated using Line-Weavor burk plot varied from 0.452 x 10^{-3} to 3.000 x 10^{-3} M and followed the sequence soil IV > soil V > soil > soil III > soil II. The values compared well with those obtained from Hanes-Wolf and Eadie-Hofstee plots

4.2.3.2 V_{max} and K_m for soil acid phosphatase: The effect of substrate concentration on acid phosphatase activity is presented in Table 8 and is depicted in Fig. 4. Increase in substrate concentration increased the soil acid phosphatase activity and almost reached a plateau at a substrate concentration of > 4 mM in all the soils except soil II where levelling off was observed at a substrate concentration of 3 mM. At higher concentration, the enzyme activity remained almost constant.

Plots of the three linear transformations of Michaelis - Menten equation drawn for acid phosphatase in five different soils are shown in Fig. 5. The linearity of these plots indicated that the values obtained for acid phosphatase activity fitted the three linear transformations of the Michaelis - Menten equation.

Table 8: Effect of substrate concentration on soil acid phosphatase activity

Substrate Concentration (mM)	Acid phosphatase activity (µg of 4-nitrophenol released g^{-1} soil h^{-1}				
	Soil I	Soil II	Soil III	Soil IV	Soil V
0.2	9.3	5.3	7.1	4.5	9.8
0.4	14.6	10.0	11.7	7.1	15.8
0.6	19.0	14.0	15.3	8.8	20.3
0.8	21.8	16.7	17.3	10.4	24.1
1.0	24.9	19.5	20.2	11.4	28.1
2.0	29.0	24.8	23.9	13.7	32.2
3.0	31.6	30.4	27.9	16.1	38.2
4.0	34.8	30.5	30.8	21.1	43.3
5.0	38.2	30.7	30.9	21.0	43.6
6.0	38.0	30.6	30.9	21.0	43.5

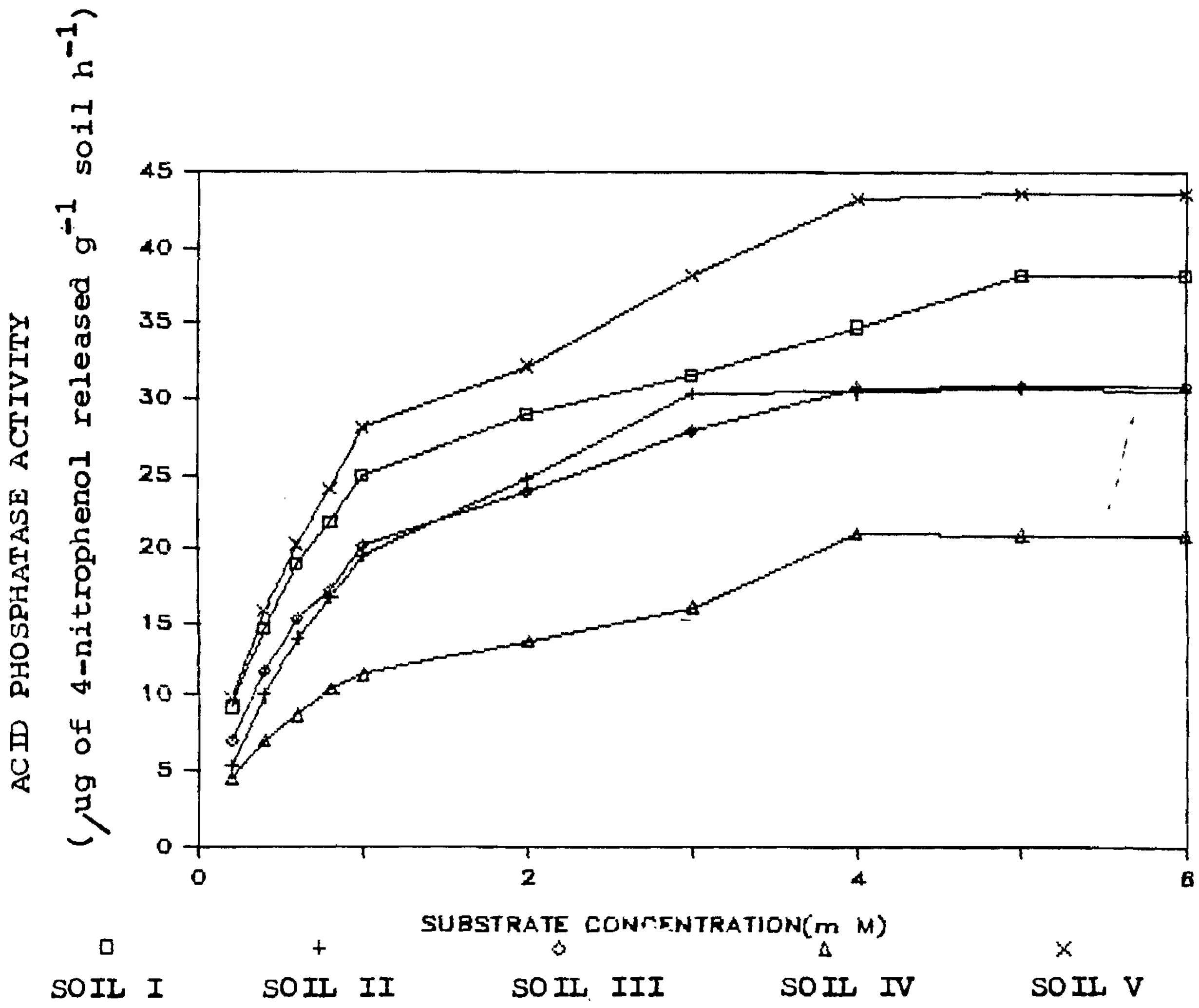
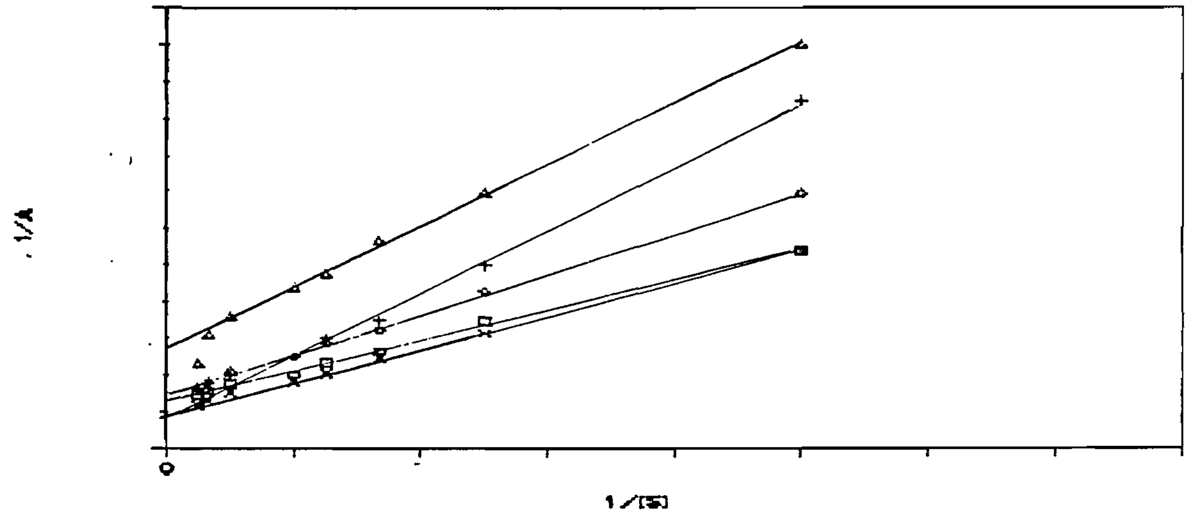
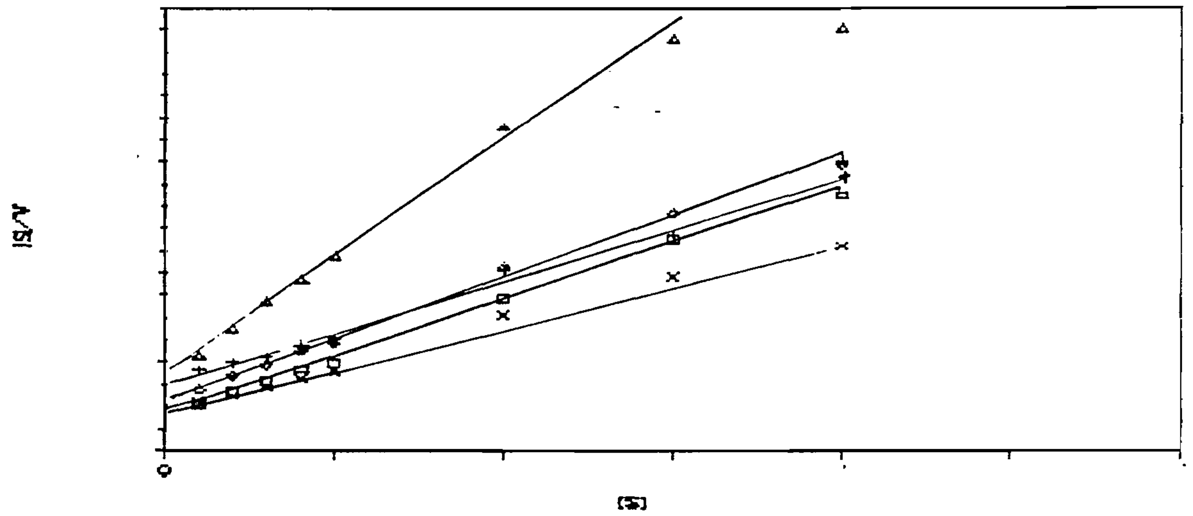


FIG.4 : EFFECT OF SUBSTRATE CONCENTRATION ON SOIL ACID PHOSPHATASE ACTIVITY

LINEWEAVER - BURK PLOT OF SOIL ACID PHOSPHATASE



HANES- WOLF PLOT OF SOIL ACID PHOSPHATASE



EADIE- HOFSTEE PLOT OF SOIL ACID PHOSPHATASE

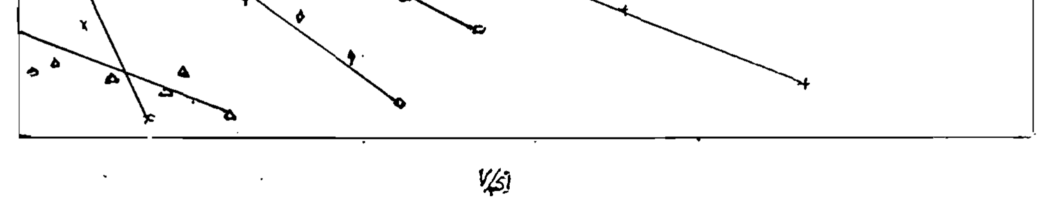


FIG.5 : LINEAR TRANSFORMATIONS OF SOIL ACID PHOSPHATASE

The values of V_{max} and K_m obtained by the least square analysis of these plots are presented in Table 9.

Maximum enzyme reaction velocity (V_{max}) for soil acid phosphatase activity was markedly different for different soils. The V_{max} value for acid phosphatase activity in the five soils studied ranged from 21.1 to 51.1 µg of 4-nitrophenol g^{-1} soil h^{-1} following the sequence of soil V > soil II > soil I > soil III > soil IV.

The Michaelis - Menten constant (K_m) value calculated using Line-Weaver burk for the five soils ranged from 0.68×10^{-3} M to 1.42×10^{-3} M and followed the sequence soil II > soil V > soil III > soil IV > soil I indicating the apparent affinity of the enzyme to soil I and II were maximum and minimum, respectively. The values compared well with those obtained from Hanes-Wolf and Eadie-Hofstee plots.

4.2.3.3 V_{max} and K_m for soil alkaline phosphatase: The results on effect of substrate concentration on alkaline phosphatase activity are presented in Table 10 and are depicted in Fig. 6.

Soil alkaline phosphatase activity increased *with increase in substrate concentration and leveled off*

Table 9: Maximum enzyme reaction velocity (VMax) and Michaelis constant (km) values of soil acid phosphatase activity

Soils	Maximum enzyme reaction velocity (VMax) (μg of 4-nitrophenol released g^{-1} soil h^{-1})			Michaelis constant (km) (mM)		
	Lineweaver-Burk transformation	Hanes-Wolf transformation	Eadie-Hofstee transformation	Lineweaver-Burk transformation	Hanes-Wolf transformation	Eadie-Hofstee transformation
Soil I	40.62	40.00	39.40	0.681	0.658	0.651
Soil II	44.04	40.80	32.60	1.422	1.184	1.204
Soil III	35.19	37.00	32.80	0.793	0.867	0.701
Soil IV	21.08	24.30	19.90	0.759	0.801	0.730
Soil V	51.08	52.60	50.20	0.906	0.947	0.881

Table 10: Effect of substrate concentration on soil alkaline phosphatase activity

Substrate Concentration (mM)	Alkaline phosphatase activity (µg of 4-nitrophenol released g^{1}soil h^{-1})				
	Soil I	Soil II	Soil III	Soil IV	Soil V
0.2	16.3	29.6	12.6	7.0	1.0
0.4	21.2	39.8	24.3	9.8	2.0
0.6	35.6	50.9	31.5	11.8	2.9
0.8	38.4	59.4	37.3	12.8	3.7
1.0	44.7	64.5	42.6	13.8	4.4
2.0	51.7	69.8	54.2	15.8	7.2
3.0	59.9	72.7	58.5	16.6	9.5
4.0	65.5	73.3	64.5	18.6	12.4
5.0	66.1	73.2	64.3	18.5	12.7
6.0	66.0	73.1	64.4	18.5	12.5

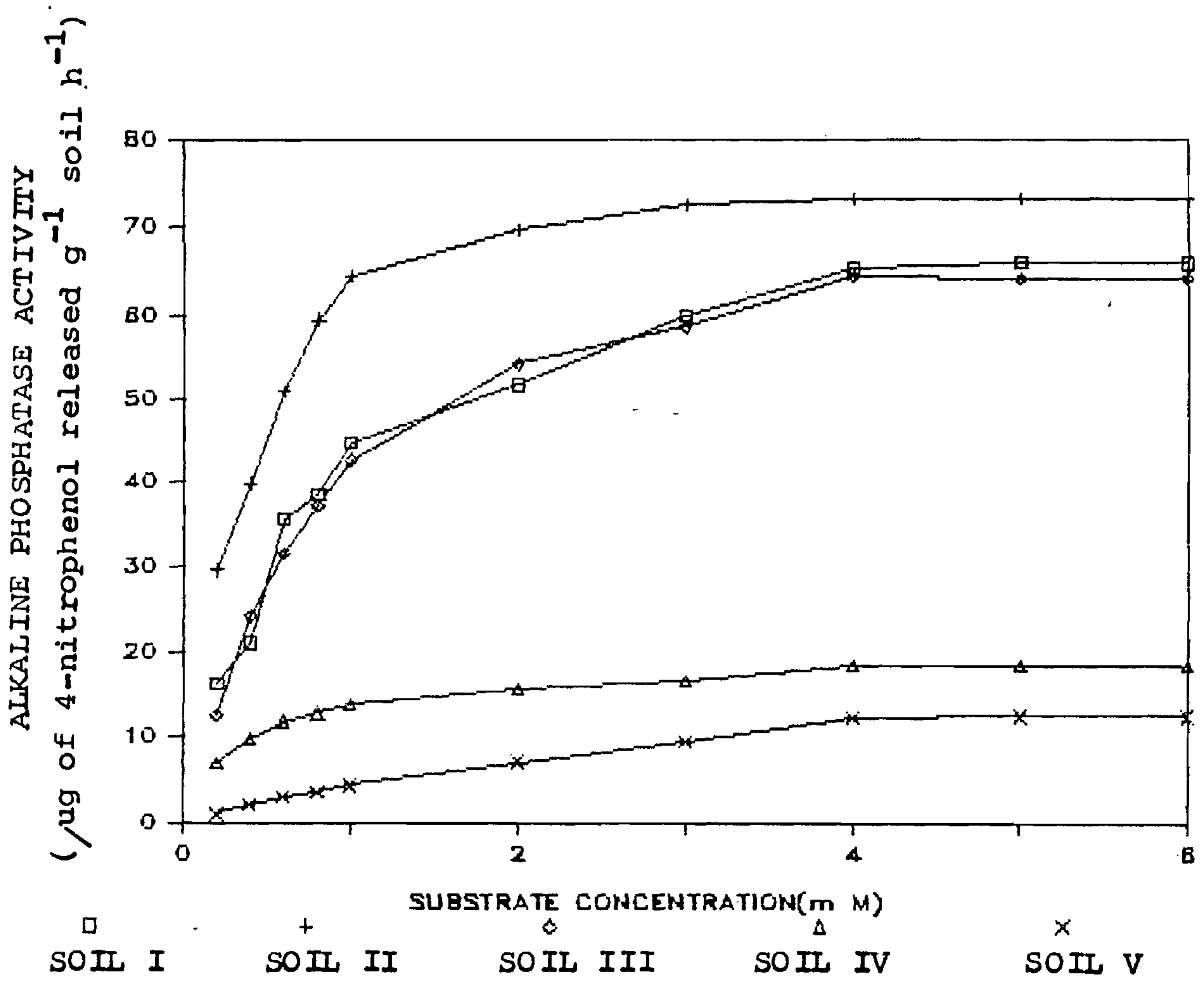


FIG.6 : EFFECT OF SUBSTRATE CONCENTRATION ON SOIL ALKALINE PHOSPHATASE ACTIVITY

at a substrate concentration > 5 mM in soil I and at > 4 mM for the rest of the soils.

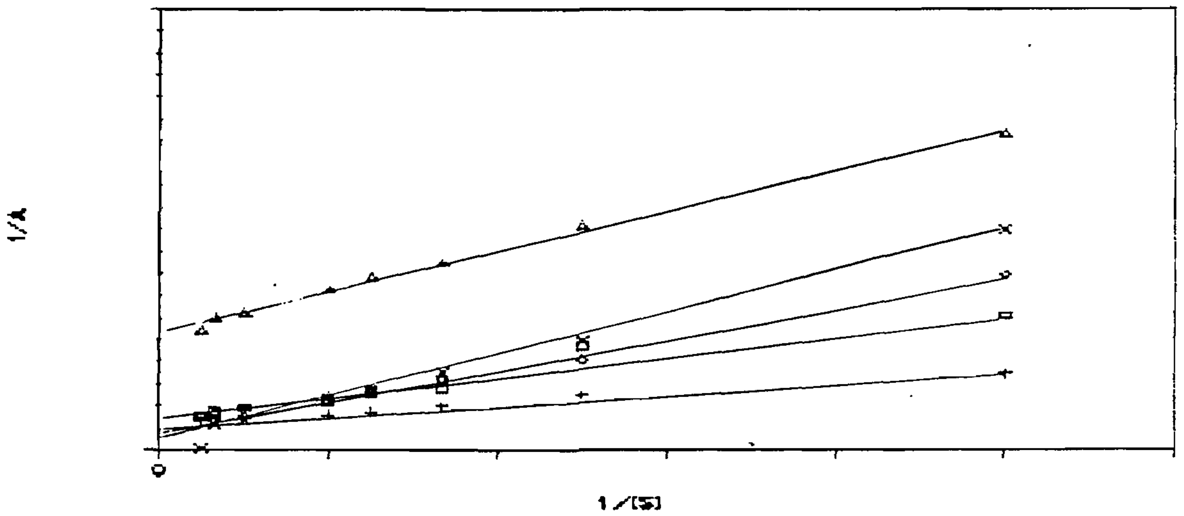
Plots of the three linear transformations of the Michaelis - Menten equation for alkaline phosphatase activity are shown in Fig. 7. The linearity of these plots confirmed that the Michaelis - Menten equation was obeyed.

The values of V_{max} and Km obtained from the least square analysis of these plots are presented in Table 11.

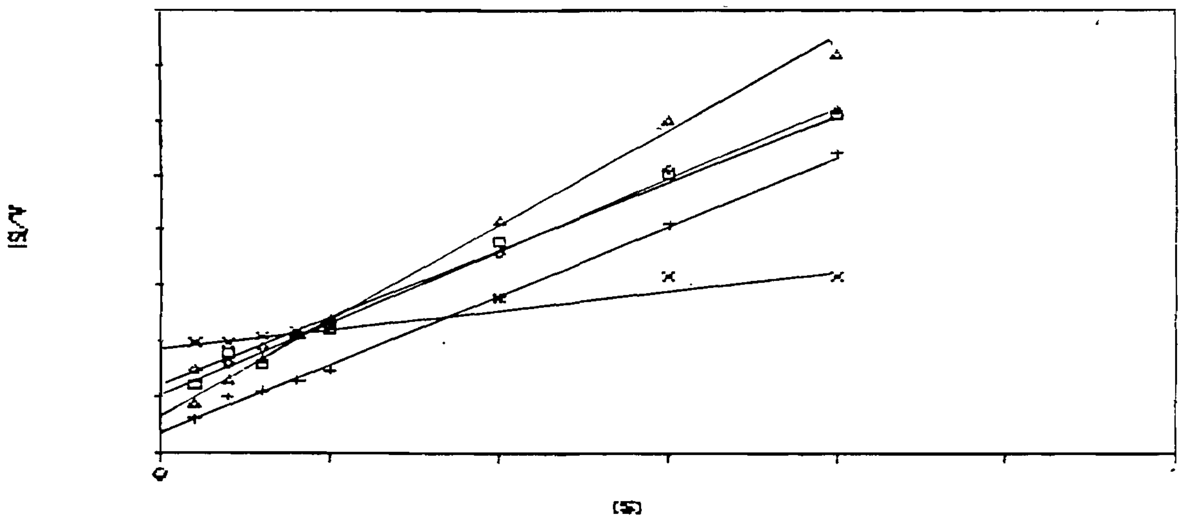
The maximum enzyme reaction velocity (V_{max}) values for soil alkaline phosphatase activity were found to range from 19.0 to 85.67 μg of 4-nitrophenol released g^{-1} soil h^{-1} and followed the order soil III > soil II > soil I > soil V > soil IV. V_{max} values of alkaline phosphatase activity were slightly higher when compared with acid phosphatase activity.

The calculated Michaelis - Menten constant (Km) of soil alkaline phosphatase activity calculated using Line-Weaver burk plot varied from 0.352×10^{-3} M to 4.823×10^{-3} M and followed the order soil V > soil III > soil I > soil II > soil IV. The values compared well with those obtained from Hanes-Wolf and Eadie-Hofstee plots.

LINEWEAVER - BURK PLOT OF SOIL ALKALINE PHOSPHATASE



HANES - WOLF PLOT OF SOIL ALKALINE PHOSPHATASE



EADIE-HOFSTEE PLOT OF SOIL ALKALINE PHOSPHATASE

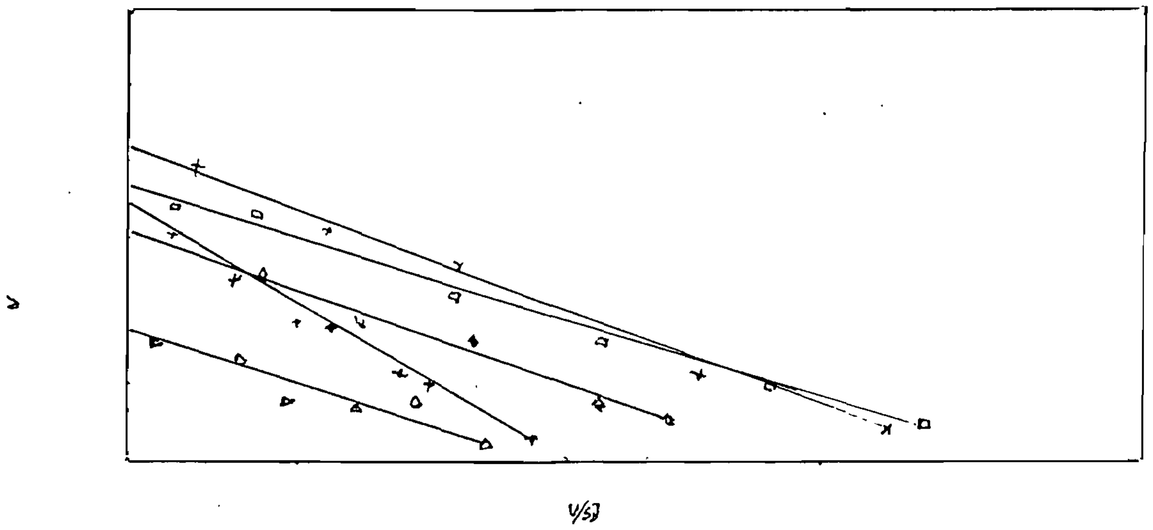


FIG.7 : LINEAR TRANSFORMATIONS OF SOIL ALKALINE PHOSPHATASE

Table 11: Maximum enzyme reaction velocity (V_{Max}) and Michaelis constant (km) values of soil alkaline phosphatase activity

Soils	Maximum enzyme reaction velocity (VMax) (µg of 4-nitrophenol released g^{-1}soil h^{-1})			Michaelis constant (km) (mM)		
	Lineweaver-Burk transformation	Hanes-Wolf transformation	Eadie-Hofstee transformation	Lineweaver-Burk transformation	Hanes-Wolf transformation	Eadie-Hofstee transformation
Soil I	83.033	77.5	83.0	0.708	0.798	0.708
Soil II	81.320	80.0	82.0	0.359	0.278	0.361
Soil III	85.670	85.0	82.8	1.118	1.282	1.080
Soil IV	19.000	20.0	18.9	0.352	0.400	0.358
Soil V	25.370	27.8	25.8	4.823	5.163	4.844

4.3 EFFECT OF MOISTURE ON SOIL UREASE ACTIVITY

The results on effect of moisture on soil urease activity are presented in Table 12. As seen from the results, there were very little differences in the soil urease activities due to different soil moisture levels ranging from 20 to 100 per cent (Fig. 8).

4.4 EFFECT OF pH ON SOIL UREASE ACTIVITY

The results pertaining to the effect of pH, in the range 3 - 12 maintained with modified universal buffer, on the activity of soil urease are presented in Table 13 and are depicted in Fig. 9.

Urease activity in all the soils increased till about 7.0 pH, reached a plateau due to further to about 2 to 3 units of pH and decreased there after. The soils exhibited slightly different pH ranges over which the maximum of urease activity was observed.

4.5 EFFECT OF TEMPERATURE

Temperature is an important factor affecting enzyme - catalyzed reactions. The rate of enzyme catalysed reaction increased with an increase in temperature until at some temperature, the rate begins to decrease because of inactivation of the enzyme. Measurement of the rate of an enzyme - catalyzed

Table 12: Effect of moisture on soil urease activity

Moisture level (%)	Soil Urease activity (µg of NH_4 released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
20	10.12	8.68	7.24	1.30	2.24	5.92
40	10.40	8.74	7.36	1.30	2.30	6.02
60	10.34	8.72	7.40	1.35	2.32	6.03
80	10.42	8.76	7.40	1.34	2.28	6.04
100	10.45	8.80	7.45	1.35	2.34	6.08
Mean	10.35	8.74	7.30	1.33	2.29	

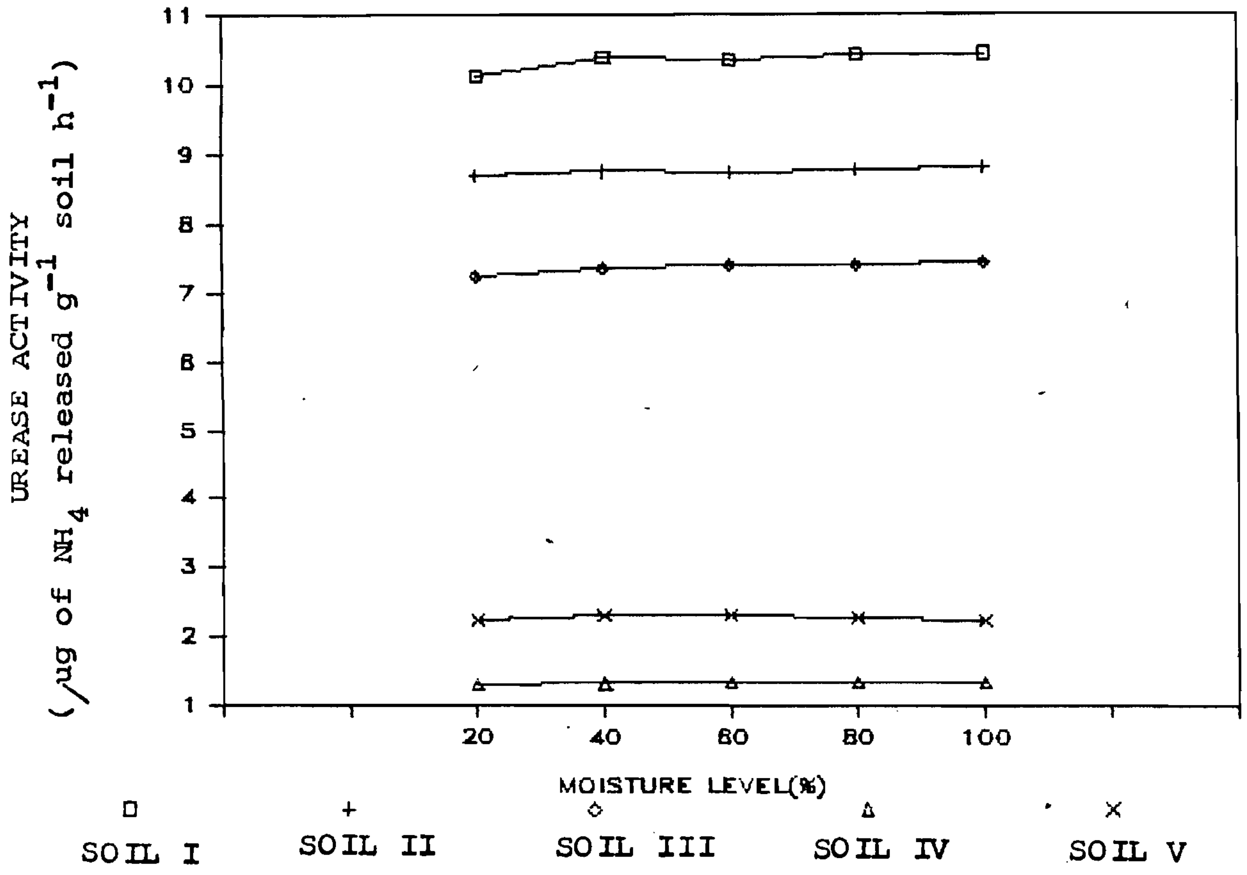


FIG. 8 : EFFECT OF MOISTURE ON SOIL UREASE ACTIVITY

Table 13: Effect of pH on soil urease activity

Buffer added pH	Urease activity (μg of NH$_4$ released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
3.0	2.2	2.1	1.5	0.8	1.2	1.56
4.0	4.7	3.9	3.1	1.0	1.3	2.80
5.0	6.8	5.8	5.7	1.2	1.5	4.20
6.0	8.3	7.3	7.5	1.3	1.7	5.22
7.0	9.6	11.2	8.4	1.4	4.2	6.96
8.0	12.5	11.6	10.5	2.4	4.0	8.20
9.0	12.8	11.8	10.7	2.8	4.2	8.46
10.0	12.4	11.6	10.4	2.9	3.1	8.30
11.0	10.5	10.2	8.2	1.8	2.7	6.68
Mean	8.86	8.34	7.33	1.73	2.26	

C.D. [illegible] 5 per cent

Buffer pH : 1.44
Soils : 1.32
Buffer pH x soils : 1.98

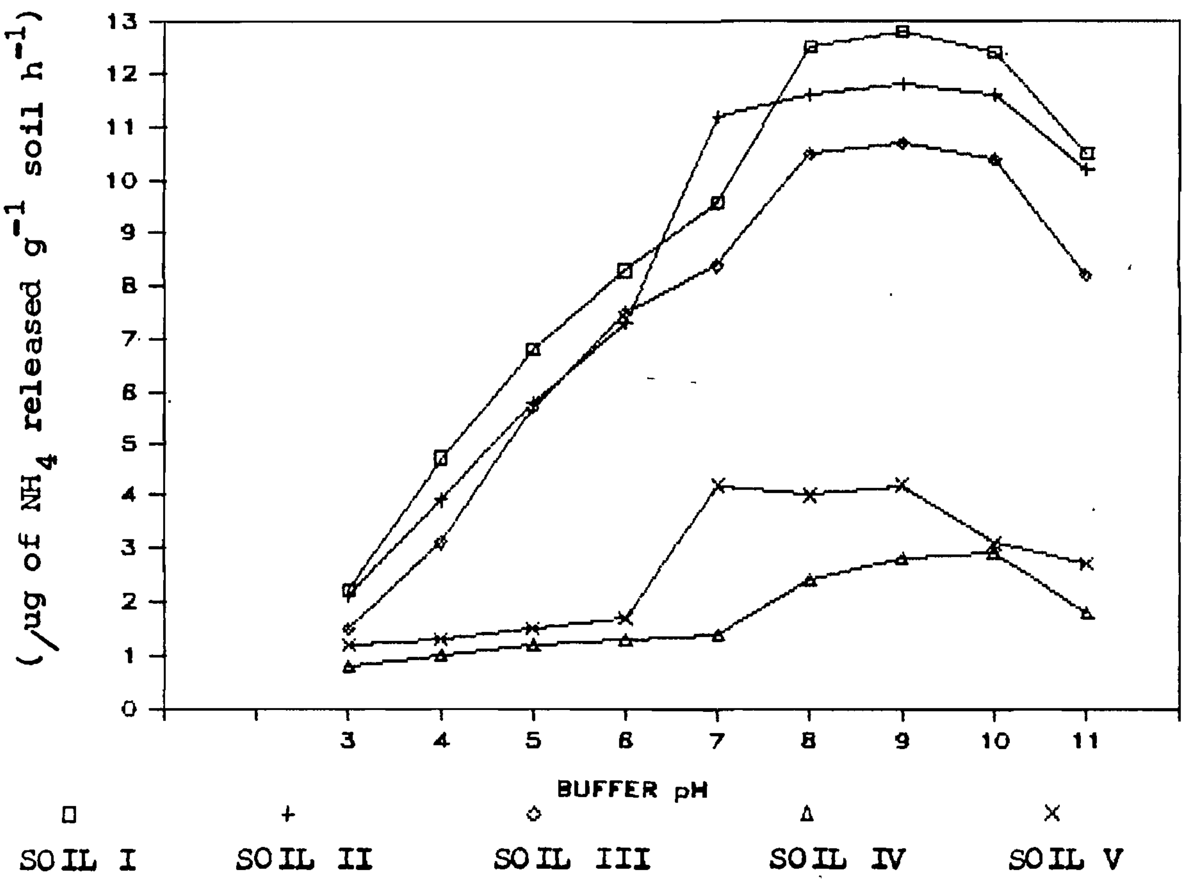


FIG. 9 : EFFECT OF pH ON SOIL UREASE ACTIVITY

reaction upto the temperature inactivation allows calculation of the energy of activation (Ea) of an enzyme. The Ea indicates the energy barrier that must be overcome for the reaction to proceed. A catalyst, such as enzyme, greatly reduces this energy hump and allows the reaction to proceed at a reasonably faster rate at ambient temperature.

4.5.1 Soil urease:

The results pertaining to the effect of temperature on soil urease activities are presented in Table 14 and also are depicted graphically in Fig. 10.

Urease activity of all the soils except soil II increased with increase in temperature from 20 - 70°C and then decreased rapidly with further increase in temperature to 90°C. Urease activity in soil IV increased constantly till 90°C.

4.5.2 Soil acid phosphatase activity:

The data on effect of temperature on soil acid phosphatase activity are shown in Table 15 and are depicted graphically in Fig. 11.

Acid phosphatase activity increased gradually with increase in temperature from 20 - 70° C and then decreased with further increase in temperature to 90°C

Table 14: Effect of temperature on soil urease activity

Temperature ^{o}C	Urease activity (μg of NH_4 released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
20	3.97	3.05	2.38	1.38	1.36	2.43
30	10.19	7.61	7.44	1.51	2.36	5.82
40	19.36	12.53	13.88	2.11	2.96	10.17
50	34.33	26.81	28.89	3.36	4.69	19.62
60	110.08	45.25	79.08	3.94	6.79	41.03
70	145.11	82.17	166.88	8.24	8.70	82.22
80	68.49	35.78	57.83	20.52	5.55	37.64
90	48.00	27.05	47.41	22.25	3.65	29.67
Mean	49.94	30.03	50.46	7.91	4.51	

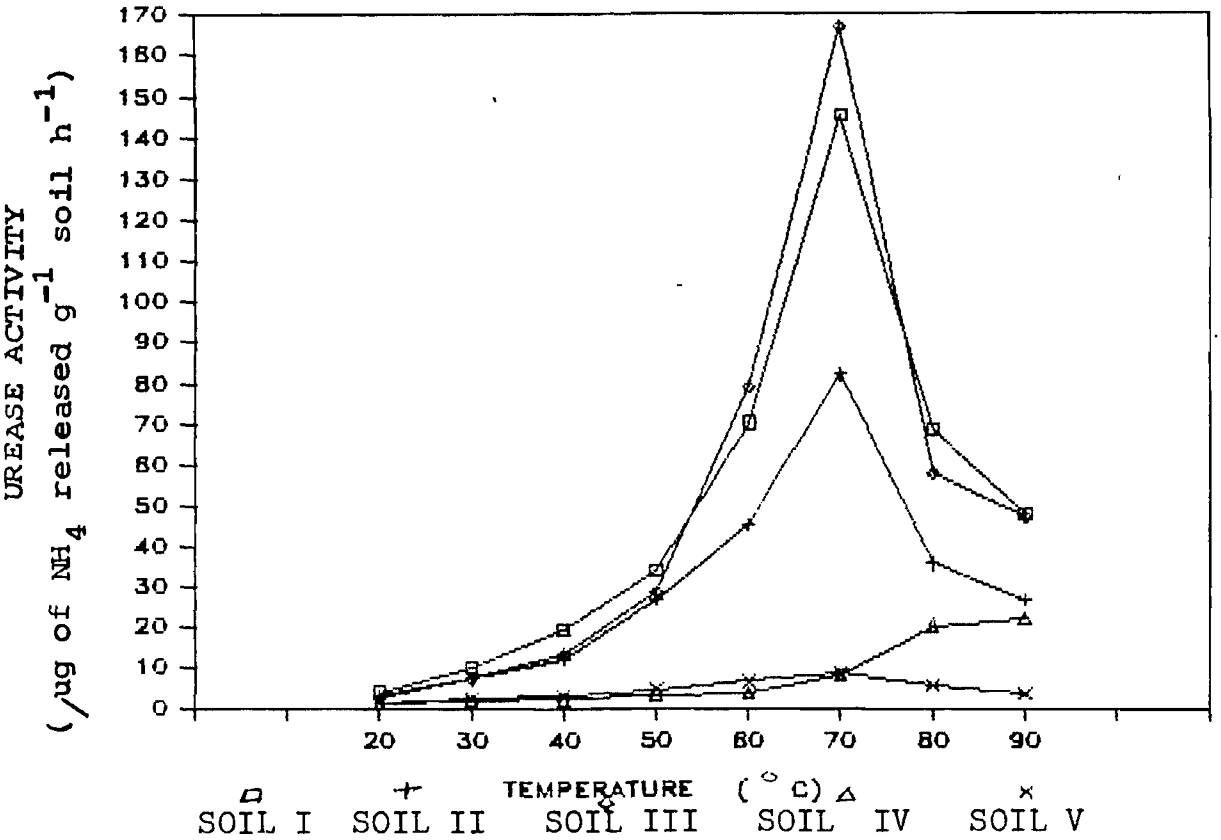


FIG.10 : EFFECT OF TEMPERATURE ON SOIL UREASE ACTIVITY

Table 15: Effect of temperature on soil acid phosphatase activity

Temperature °C	Acid phosphatase activity (μg of 4-nitrophenol released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
20	10.0	8.1	7.2	4.8	8.5	7.72
30	22.0	16.0	15.0	11.1	20.7	16.96
40	42.5	34.2	33.3	24.8	45.0	35.96
50	75.9	60.0	54.2	47.3	79.7	63.42
60	164.3	128.6	116.3	92.7	182.7	136.92
70	282.7	248.1	221.7	177.5	164.5	218.90
80	142.6	112.2	104.4	79.8	124.6	112.72
90	86.0	68.1	65.2	37.2	55.7	62.44
Mean	103.05	84.41	77.16	59.4	85.17	

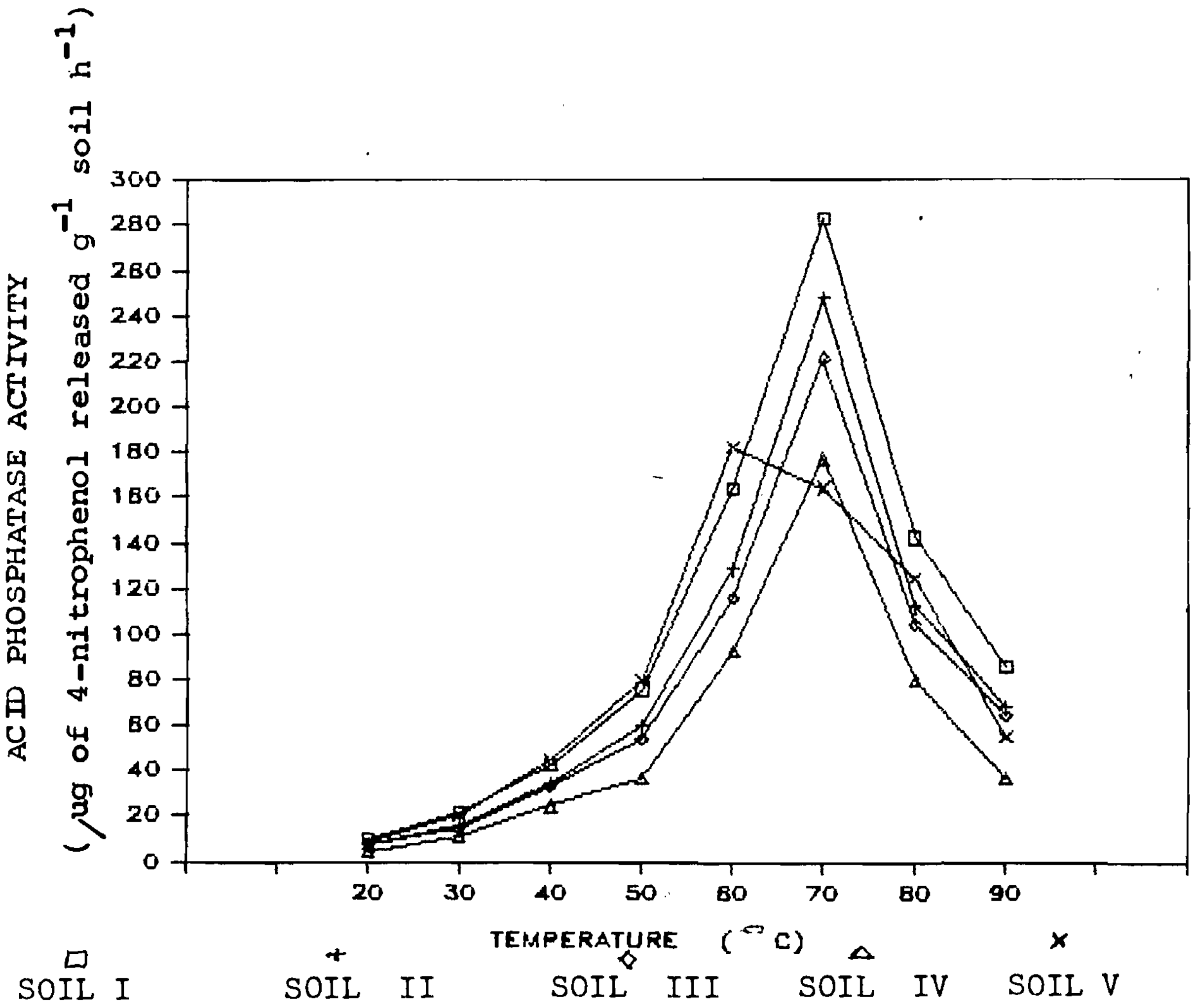


FIG.11 : EFFECT OF TEMPERATURE ON SOIL ACID PHOSPHATASE ACTIVITY

for all except soil V for which maximum activity was recorded at 60°C.

4.5.3 Soil alkaline phosphatase activity

The results on effect of temperature on soil alkaline phosphatase activity are given in Table 16 and are depicted graphically in Fig. 12.

Alkaline phosphatase activity of the soils except soil I and soil III increased with increase in temperature from 20-70°C and then decreased rapidly with further increase in temperature to 90°C. Soil I and III reached maximum activities at 60°C.

4.5.4 Temperature coefficient (Q_{10}) values

It is an empherical practice to estimate Q_{10} values for enzyme reactions over short temperature intervals of 10°C. This is to ascertain the temperature at which enzymes denaturation sets in. At and beyond that temperature, drastic changes would be seen in Q_{10}. The temperature coefficient values (Table 17) varied with enzyme and ranged from 1.46 to 2.38 for soil urease, 1.92 to 2.08 for soil acid phosphatase and 1.77 to 2.09 for alkaline phosphatase. At temperature above 70°C, except in soil IV for urease, these values for the enzymes were less than one.

Table 16: Effect of temperature on soil alkaline phosphatase activity

Temperature °C	Alkaline phosphatase activity (µg of 4-nitrophenol released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
20	14.9	23.1	14.1	8.0	5.1	13.04
30	32.0	43.3	28.9	12.9	9.0	25.22
40	71.1	80.2	69.0	21.1	17.0	51.68
50	135.8	154.9	130.4	45.2	32.2	99.70
60	263.0	292.7	256.5	84.0	70.2	193.28
70	190.3	570.6	174.3	156.8	134.5	245.30
80	120.6	246.0	86.1	66.1	48.2	113.40
90	84.6	118.6	64.0	34.9	18.2	64.06
Mean	114.03	160.4	102.9	53.62	41.8	

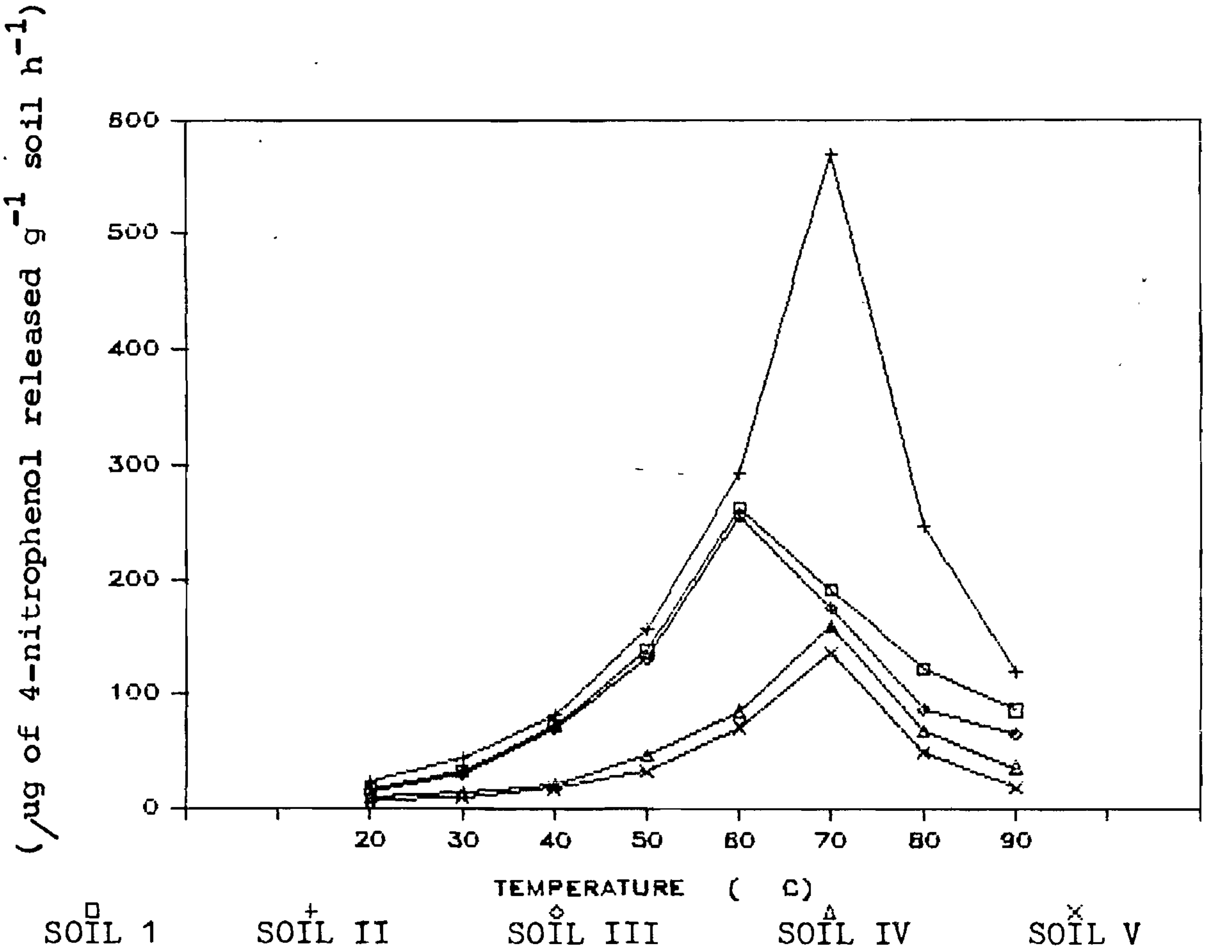


FIG.12 : EFFECT OF TEMPERATURE ON SOIL ALKALINE PHOSPHATASE ACTIVITY

Table 17: Temperature cofficient values (Q_{10}) of soil urease and phosphomonoesterases

Temperature (°C)	Temperature coefficient values (Q_{10})														
	Urease					Acid phosphatase					Alkaline phosphatase				
	Soils					Soils					Soils				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
20-30	2.56	2.49	3.12	1.09	1.73	2.20	2.00	2.08	2.29	2.43	2.13	1.86	2.00	1.61	1.80
30-40	1.89	1.64	1.86	1.52	1.25	1.93	2.13	2.20	2.25	2.25	2.22	1.86	2.30	1.61	1.89
40-50	1.77	2.13	2.07	1.59	1.58	1.78	1.75	1.64	1.96	1.77	1.90	1.92	1.88	2.14	1.88
50-60	2.04	1.68	2.73	1.17	1.45	2.16	2.13	2.15	1.96	2.27	1.95	1.89	1.97	1.86	2.18
60-70	2.07	1.81	2.11	2.09	1.28	1.72	1.94	1.90	1.93	0.90	0.72	2.92	0.68	1.85	1.91
70-80	0.47	0.43	0.35	2.49	0.64	0.50	0.45	0.47	0.45	0.76	0.63	0.43	0.49	0.42	0.36
80-90	0.70	0.75	0.82	1.08	0.66	0.60	0.60	0.62	0.47	0.44	0.70	0.48	0.74	0.53	0.37
Mean Q_{10} (20-70°C)	2.06	1.95	2.38	1.49	1.46	1.96	1.99	1.99	2.08	1.92	1.78	2.09	1.77	1.81	1.93

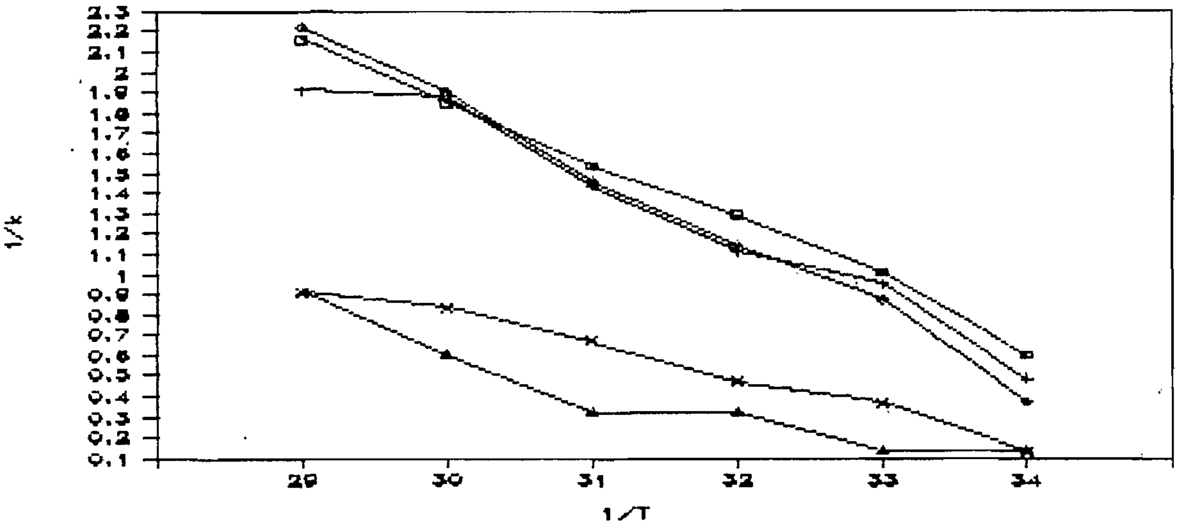
4.5.5 Energy of activation

A better and more rigorous method to express the quantitative effect of temperature is the calculation of the energy of activation from Arrhanius equation as explained in section 3.2.7.4. The plot of log k vs 1/T is given in Fig. 13. Excellent linearity was observed in the temperature range of 20-70°C with $r^2>0.95$ in all the five soils. The values of Ea obtained by least square analysis are given in Table 18. The Ea varied from 16.40 to 6.76 for soil urease, 14.00 to 13.00 for soil acid phosphatase and 14.00 to 9.75 for alkaline phosphatase.

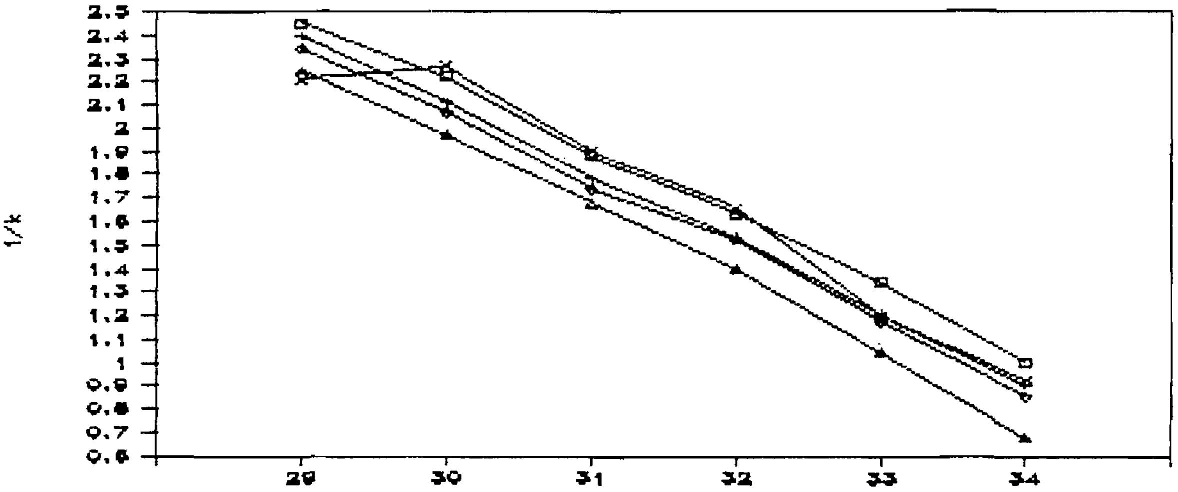
4.5.6 Thermodynamic activation parameters of soil urease, acid phosphatase and alkaline phosphatase

The actual thermodyanamic parameters (enthalpy $\Delta^{\neq}H$ and entropy $\Delta^{\neq}S$) were calculated using Eyring equation as explained in section 3.2.7.5 Enthalpy ($\Delta^{\neq}H$) of activation was obtained by the least square analysis of ln k/T vs 1/T and entropy at each temperature was calculated by substituting the value of $\Delta^{\neq}H$ in the equation. The enthalpy of activation for urease and phosphomonoestarase in different soils are presented in

U R E A S E



ACID PHOSPHATASE



ALKALINE PHOSPHATASE

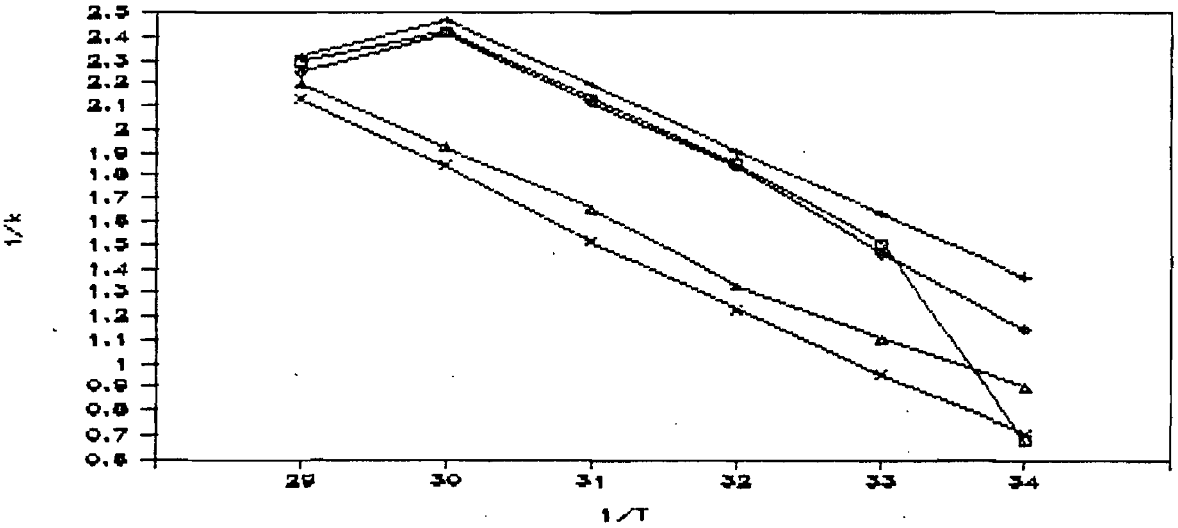


FIG.13 : ENERGY OF ACTIVATION (E_a) OF UREASE AND PHOSPHOMONOESTERASES IN DIFFERENT SOILS

Table 18 : Energy of activation (E_a) of urease and phosphomonoesterases in different soils

Soils	(E_a K cal mole^{-1})		
	Urease	Acid phosphatase	Alkaline phosphatase
Soil I	10.68	10.09	14.00
Soil II	13.18	13.00	9.75
Soil III	16.40	13.34	11.15
Soil IV	6.76	14.00	11.95
Soil V	7.05	10.21	10.00

Table 19. High values of $r^2 > 0.95$ and low standard deviation confirmed that enzyme deactivation did not set in till 70°C.

Enthalpy of activation ($\overset{\neq}{\Delta}H$) in soil urease varied widely from 6.3 ± 1.1 to 13.86 ± 2.44 k cal mole^{-1} and followed the order soil IV > soil V > soil II > soil I > soil III. $\overset{\neq}{\Delta}H$ of acid phosphatases were slightly higher than alkaline phosphatase and varied from 12.07 ± 1.67 to 13.94 ± 0.59 k cal mole^{-1} and followed the order soil V > soil I > soil II and III > soil IV. $\overset{\neq}{\Delta}H$ of alkaline phosphatases ranged from 10.92 ± 1.16 to 12.5 ± 2.04 k cal mole^{-1} and the order was soil III > soil I > soil IV > soil II > soil V.

The entropy of activation ($\overset{\neq}{\Delta}S$) at an interval of 10°C in the temperature range from 20-70°C was calculated for the three enzyme catalysed reactions.

$\overset{\neq}{\Delta}S$ values of urease catalysed reaction system (Table 20) of any given soil did not show much variation at the temperature intervals. However, these values vary widely with the type of soil and followed the order: soil IV > soil V > soil II > soil I > soil III. $\overset{\neq}{\Delta}S$ values of acid phosphatase (Table 21) of any given soil showed either negligible or no differences at the temperature intervals. A slight variation however,

Table 19 : Enthalpy of activation ($\Delta^{\neq}H$) of urease and phosphomonoesterases in different soils

Soils	$\Delta^{\neq}H$ (K cal mole^{-1})		
	Urease	Acid phosphatase	Alkaline phosphatase
Soil I	13.19 ± 1.68	12.85 ± 1.15	10.94 ± 4.62
Soil II	11.15 ± 1.37	10.21 ± 1.37	12.32 ± 1.43
Soil III	13.86 ± 2.44	13.21 ± 1.31	10.92 ± 1.06
Soil IV	6.30 ± 1.10	13.94 ± 0.59	11.39 ± 2.30
Soil V	6.40 ± 1.02	12.07 ± 1.67	12.57 ± 2.04

Table 20 Entropy of activation ($\Delta^{\neq}S$) of urease in different soils

Temperature C	$\Delta^{\neq}S$ cal $mole^{-1}$ deg^{-1}				
	Soil I	Soil II	Soil III	Soil IV	Soil V
20	52.54	55.86	44.08	77.92	76.64
30	52.12	55.40	43.76	78.61	76.40
40	52.29	55.79	44.16	78.68	76.71
50	52.47	55.57	44.46	78.50	76.55
60	52.45	55.75	44.00	78.83	76.51
70	52.27	55.71	43.98	77.92	76.63
Mean	52.36	55.68	44.07	78.41	76.57

Table 21: Entropy of activation ($\Delta^{\neq}S$) of acid phosphatase in different soils

Temperature ^{o}C	$\Delta^{\neq}S$ cal $mole^{-1}deg^{-1}$				
	Soil I	Soil II	Soil III	Soil IV	Soil V
20	54.77	53.79	54.07	52.24	57.41
30	54.45	53.89	54.01	52.22	57.07
40	54.45	53.82	53.88	52.07	56.80
50	54.69	54.00	54.22	52.05	56.94
60	54.37	53.90	54.10	52.37	56.48
70	54.57	53.86	54.08	52.33	57.82
Mean	54.53	53.87	54.06	52.25	57.09

recorded among the soils and followed the order : soil V > soil I > soil III > soil II > soil IV. $\Delta^{\neq}$S values of alkaline phosphatase (Table 22) showed similar results as that of acid phosphatase with the soils and followed the order: soil III > soil IV > soil I > soil V > soil II.

4.6 EFFECT OF MICRONUTRIENT CATIONS viz ZINC, MANGANESE, COPPER AND IRON ON SOIL UREASE AND PHOSPHOMONOESTERASES

The experiment was conducted to study the effect of four metallic cations viz Zinc, Manganese, Copper and Iron on soil urease and phosphomonoesterases in five different soils.

4.6.1 Urease

4.6.1.1 Zinc: In all the five soils, an increase in zinc concentration from 0 - 10 u M g^{-1} soil resulted in a progressive decrease in soil urease activity (Table 23). The decreases recorded in soils I to V were from 10.4 to 6.6, 8.8 to 5.9, 7.5 to 4.8, 1.4 to 0.5 and 2.4 to 1.2 μg of NH_4^+ released g^{-1} soil h^{-1}, respectively. The rate of decrease was more pronounced at the intial levels of zinc added and was significant between two successive levels upto 6 μ M g^{-1} soil. Further increase in zinc concentration did not bring significant differences in urease activity.

Table 22: Entropy of activation ($\overset{\neq}{\Delta}S$) of alkaline phosphatase in different soils

Temperature °C	S cal mole^{-1} deg^{-1}				
	Soil I	Soil II	Soil III	Soil IV	Soil V
20	60.15	54.49	60.43	59.77	56.71
30	59.95	54.68	60.21	60.29	57.01
40	59.90	54.91	59.62	60.54	57.12
50	59.38	55.07	59.52	60.30	57.20
60	59.14	54.87	59.26	59.12	56.90
70	60.83	54.59	61.05	59.92	56.76
Mean	59.89	54.17	60.02	59.99	56.95

Table 23: Effect of zinc on soil urease

Conc. of zinc ($\mu M\ g^{-1}$ soil)	Urease activity (μg of NH_4 released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
0	10.4	8.8	7.5	1.4	2.4	6.1
2.0	9.4 (9.6)	7.4 (15.9)	6.7 (10.7)	1.3 (7.1)	2.0 (16.6)	5.4
4.0	7.5 (27.9)	6.7 (23.9)	5.8 (29.3)	1.0 (28.6)	1.8 (25.0)	4.5
6.0	7.0 (32.7)	6.2 (29.5)	5.1 (32.0)	0.6 (57.1)	1.4 (41.7)	4.1
8.0	6.8 (34.6)	6.0 (31.8)	4.9 (34.6)	0.5 (64.8)	1.2 (50.0)	3.9
10.0	6.6 (36.5)	5.9 (32.9)	4.8 (36.0)	0.5 (64.3)	1.2 (50.0)	3.8
Mean	8.0	6.8	5.7	0.9	1.6	

(Figures in parentheses indicate the per cent decrease over control)

C.D. at 5 per cent

Zinc levels : 0.43
Soils : 0.39
Zinc levels x soils : 0.96

Wide variations were observed in the per cent reduction of urease activity among the soils (Fig. 14). The order of reduction was as follows: Soil II < soil III < soil I < soil V < soil IV.

4.6.1.2 Manganese: The results on effect of manganese showed similar trend as that of zinc on soil urease activity. A progressive decrease in enzyme activity was recorded with increasing concentration in all the five soils. The decreases recorded, thus, were from 10.3 to 6.4, 8.7 to 5.8, 7.5 to 4.8, 1.4 to 0.7 and 2.4 to 1.2 μg ofNH_4^+ released g^{-1} soil h^{-1} in soils I to V, respectively (Table 24). The rate of decrease was more at the initial levels of manganese added and was significant between two successive levels upto 6 μ M g^{-1} soil (Fig. 15). The decreas at further levels was non significant.

The decrease in urease activity (%) varied with the type of soil and followed the order: soil II < soil III < soil I < soil IV and V.

4.6.1.3 Iron: Similar to the effect of zinc and manganese, increase in iron concentration decreased the soil urease activity. The decreases recorded in soils I to V were from 10.3 to 6.6, 8.8 to 5.6, 7.6 to 4.8, 1.4 to 0.8 and 2.3 to 1.3 μg of NH_4^+ released g^{-1} soil h^{-1},

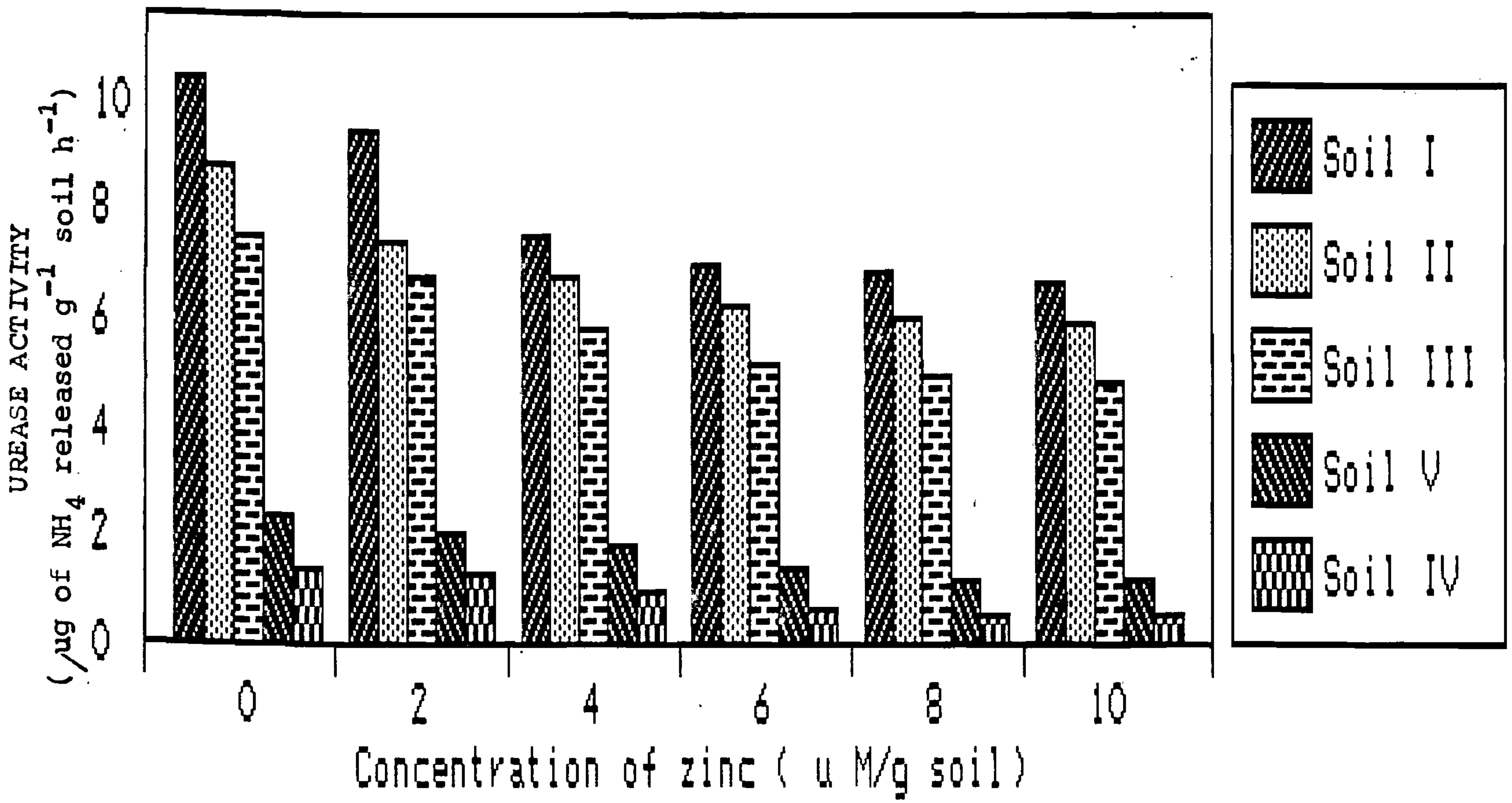


FIG.14 : EFFECT OF ZINC ON SOIL UREASE

Table 24: Effect of Manganese on soil urease activity

Conc. of manganese ($\mu M\ g^{-1}$ soil)	Urease activity (μg of NH_4 released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
0	10.3	8.7	7.5	1.4	2.4	6.1
2.0	9.2 (10.7)	7.7 (11.5)	6.8 (9.3)	1.3 (7.1)	2.0 (16.6)	5.4
4.0	7.7 (25.4)	6.9 (20.7)	5.5 (26.5)	1.0 (28.3)	1.8 (25.0)	4.6
6.0	6.7 (35.0)	6.2 (28.7)	5.0 (33.3)	0.7 (50.0)	1.4 (41.6)	4.0
8.0	6.5 (36.9)	5.9 (32.2)	4.9 (34.6)	0.7 (50.0)	1.3 (45.8)	3.8
10.0	6.4 (37.9)	5.8 (33.8)	4.8 (34.6)	0.6 (57.1)	1.2 (50.0)	3.8
	7.8	6.8	5.7	0.9	1.7	

(Figures in parentheses indicate the per cent decrease over control)

C.D. at 5 per cent :
Manganese levels : 0.34
Soils : 0.31
Manganese levels x soils : 0.77

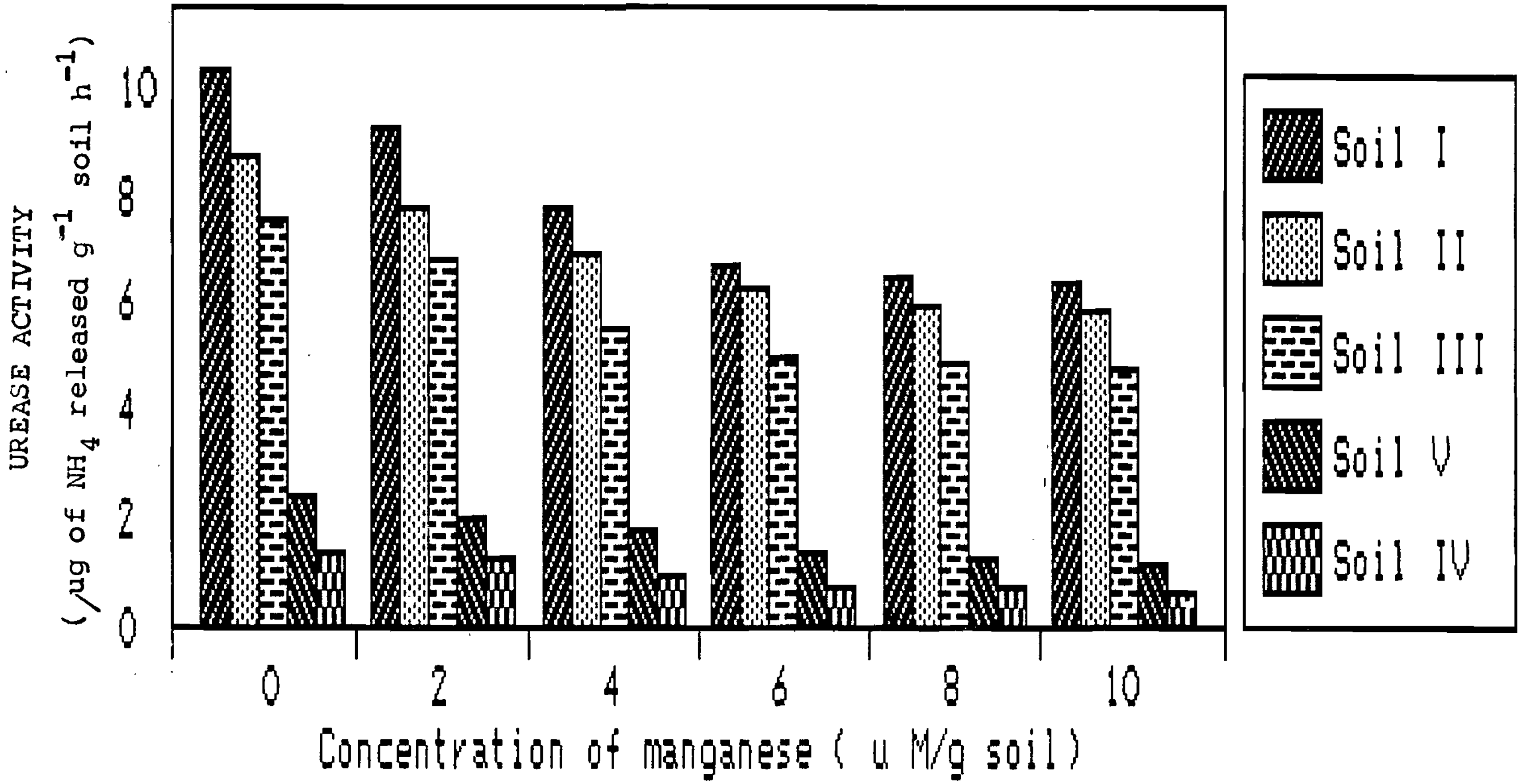


FIG. 15 : EFFECT OF MANGANESE ON SOIL UREASE ACTIVITY

respectively (Table 25). The differences were significant between two successive levels upto 6 μ moles/g soil and further additions of iron did not result in significant decreases. The order of per cent decrease in soils was soil I < soil II < soil III < soil IV < soil V (Fig. 16).

4.6.1.4 Copper: The effect of copper on soil urease activity (Table 26) showed similar trend as were observed in the other three cations studied but with higher magnitude.

In any of the five soils studied, an increase in copper concentration decreased the soil urease activity and the decrease recorded in Soils I to V were from 10.5 to 5.5, 8.8 to 4.4, 7.6 to 3.8, 1.4 to 0.6 and 2.3 to 0.9 μg of NH_4^+ released g^{-1} soil h^{-1}, respectively. The rate of decrease (Fig. 17) was significant between two succesive levels upto 6 μ M g^{-1} soil and further addition of copper did not result in significant decrease in urease activity in soils. The decrease of soil urease activity (%) varied with type of soil and followed the order: Soil I < Soil II and III < Soil IV < Soil V.

4.6.2 Phosphomonoestarases

The study on effect of four metallic cations on soil phosphomonoestarases showed similar trend of results as they were observed on soil urease.

Table 25: Effect of iron on soil urease activity

Conc. of iron ($\mu M\ g^{-1}$ soil)	Urease activity (μg of NH_4 released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
0	10.3	8.8	7.6	1.4	2.3	6.1
2.0	9.7 (5.8)	8.5 (3.4)	7.2 (5.3)	1.2 (14.3)	2.1 (8.6)	5.7
4.0	8.5 (17.5)	7.5 (14.8)	5.9 (22.4)	1.1 (24.4)	1.7 (26.1)	4.9
6.0	7.0 (32.0)	5.8 (34.1)	5.0 (34.2)	0.9 (35.7)	1.4 (39.1)	4.0
8.0	6.7 (34.9)	5.7 (35.2)	4.8 (36.8)	0.8 (42.8)	1.3 (43.5)	3.9
10.0	6.6 (35.8)	5.6 (36.4)	4.8 (36.8)	0.8 (42.8)	1.3 (43.5)	3.8
Mean	8.1	7.0	5.9	1.0	1.7	

(Figures in parentheses indicate the per cent decrease over control)

C.D. at 5 per cent :
Iron levels : 0.22
Soils : 0.20
Iron levels x soils : 0.50

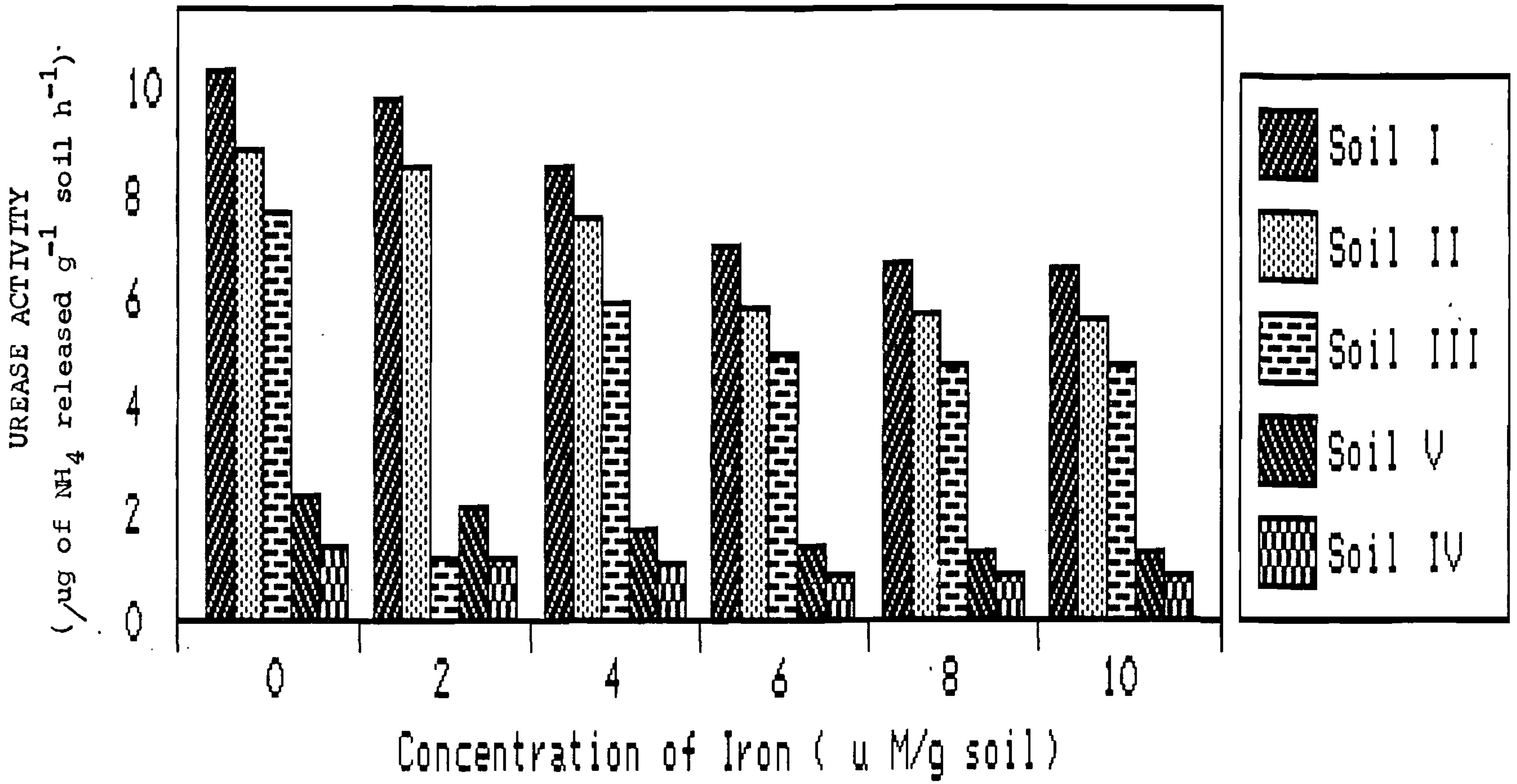


FIG. 16 : EFFECT OF IRON ON SOIL UREASE ACTIVITY

Table 26: Effect of copper on soil urease activity

Conc. of copper ($\mu M\ g^{-1}$ soil)	Urease activity (μg of NH_4 released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
0	10.5	8.8	7.6	1.4	2.3	6.1
2.0	8.9 (15.2)	8.5 (3.4)	6.2 (15.9)	1.0 (28.6)	2.0 (13.0)	5.4
4.0	6.6 (37.1)	6.3 (28.4)	5.4 (28.9)	0.9 (35.7)	1.5 (34.8)	4.1
6.0	6.0 (42.6)	4.6 (47.7)	4.1 (46.0)	0.7 (50.0)	1.0 (56.5)	3.3
8.0	5.5 (47.6)	4.4 (50.0)	4.0 (47.4)	0.6 (57.1)	0.9 (60.8)	3.1
10.0	5.5 (47.6)	4.4 (50.0)	3.8 (50.0)	0.6 (57.1)	0.9 (60.8)	3.0
Mean	7.2	6.2	5.2	0.8	1.4	

(Figures in parentheses indicate the per cent decrease over control)

C.D. at 5 per cent :
Copper levels : 0.36
Soils : 0.33
Copper levels x soils : 0.81

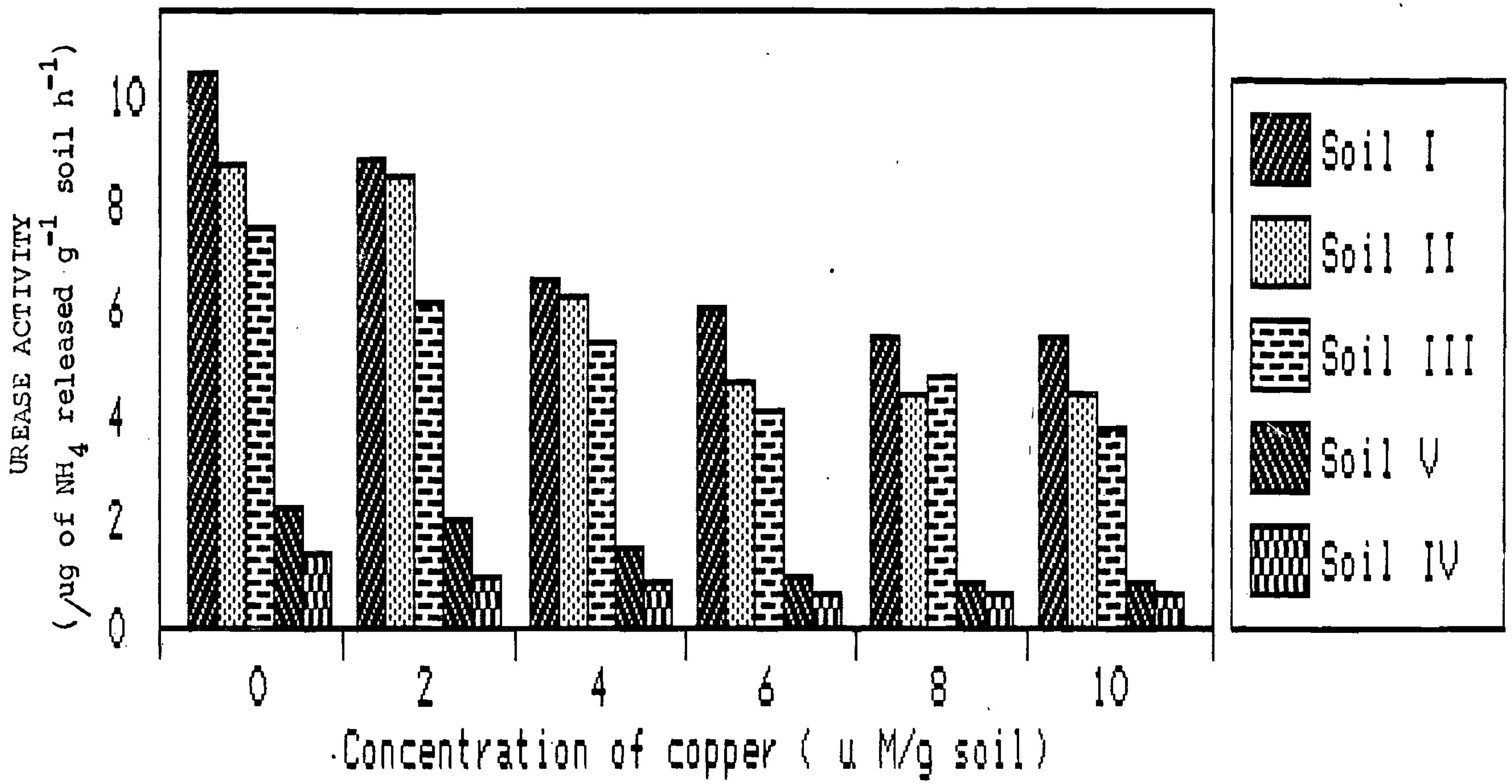


FIG.17 : EFFECT OF COPPER ON SOIL UREASE ACTIVITY

4.6.3 Acid phosphatase

4.6.3.1 Zinc: Results pertaining to the effect of zinc on soil acid phosphatase (Table 27) showed that activity of the enzyme in soil decreased with increase in zinc concentration. The decreases of the enzyme activity in soils were from 38.3 to 23.3, 31.3 to 20.3, 32.0 to 20.2, 21.1 to 12.6 and 42.9 to 25.2 μg of 4-nitrophenol released g^{-1} soil h^{-1} for soils I to V, respectively. The decrease was more at the initial levels of added zinc and significant between two successive levels upto 8 μ M g^{-1} soil (Fig. 18). The per cent inhibition varied with type of soil and decreased in the order soil II < soil III < Soil I < soil IV < soil V.

4.6.3.2 Manganese: The results are presented in Table 28 and are depicted in Fig. 19. Acid phosphatase activity in the soil decreased with increase in manganese concentration. The decreases of enzyme activity in five soils were 38.1 to 28.4, 30.6 to 23.2, 30.8 to 23.6, 21.1 to 15.8 and 43.0 to 32.1 μg of 4-nitrophenol released g^{-1} soil h^{-1}, respectively. The decrease was more at the initial levels and differences between two successive levels were significant upto 6 μ M g^{-1} soil. The per cent inhibition remained almost the same in all the five soils with the order: soil III < soil II < soil I < soil IV < soil V.

Table 27: Effect of zinc on soil acid phosphatase

Conc. of zinc (μM g^{-1} soil)	Acid phosphatase (μg of 4-nitrophenol released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
0	38.3	31.3	32.0	21.1	42.9	33.1
2.0	35.5 (7.3)	28.7 (6.8)	29.5 (7.8)	19.6 (7.1)	37.9 (11.6)	30.2
4.0	30.5 (20.4)	25.1 (19.8)	26.1 (18.4)	15.9 (24.6)	32.8 (23.5)	26.1
6.0	26.1 (31.9)	22.9 (26.8)	23.0 (28.1)	14.7 (30.3)	29.2 (31.9)	23.2
8.0	24.1 (37.0)	21.1 (32.6)	21,0 (34.4)	13.3 (36.9)	26.3 (38.7)	21.2
10.0	23.3 (39.1)	20.3 (35.1)	20.2 (36.8)	12.6 (40.2)	25.2 (41.2)	20.3
Mean	29.6	24.9	25.3	16.2	32.4	

(Figures in parentheses indicatesthe per cent decrease over control)

C.D. at 5 per cent

Zinc levels : 1.37
Soils : 1.25
Zinc levels x soils : 3.08

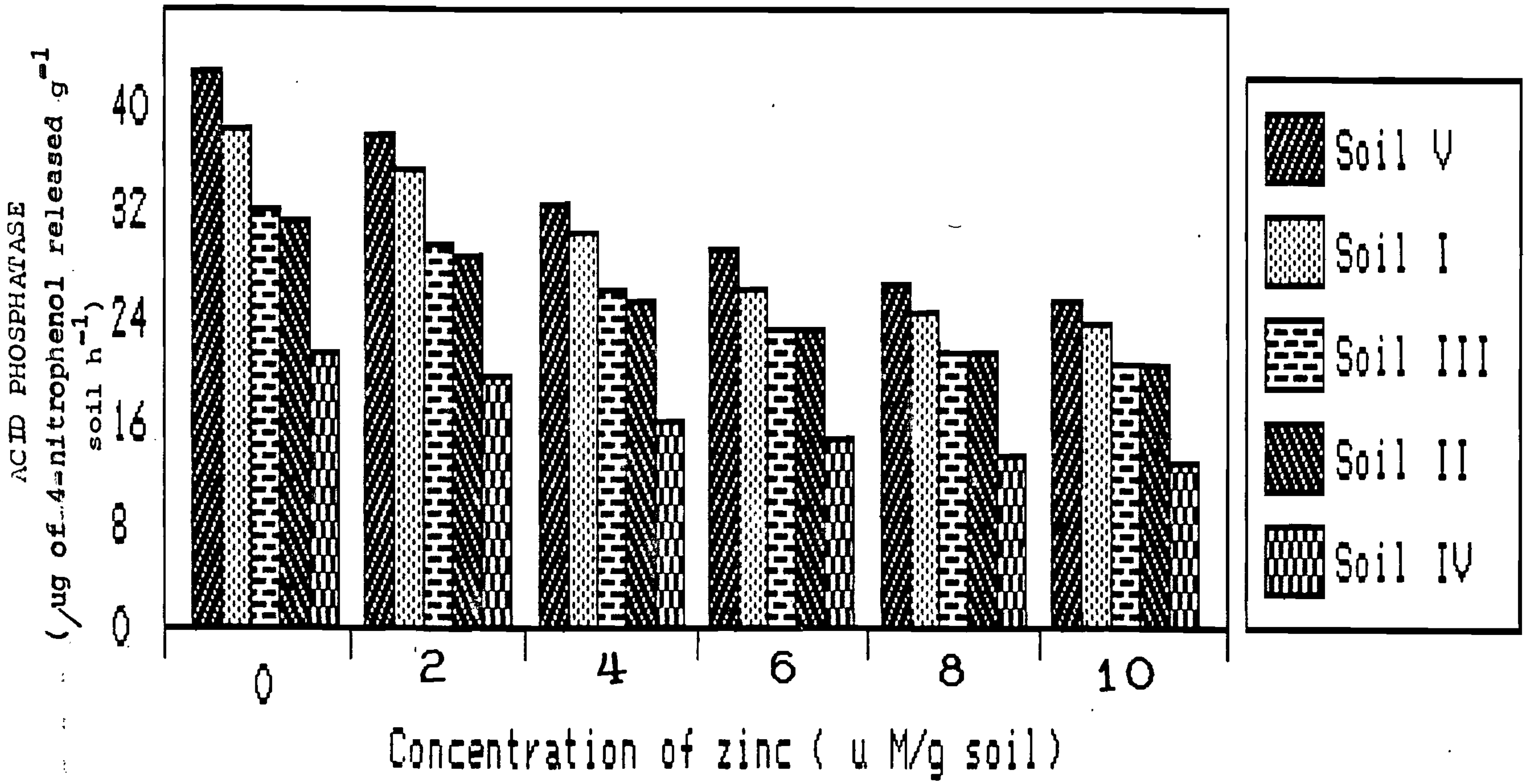


FIG. 18 : EFFECT OF ZINC ON SOIL ACID PHOSPHATASE

Table 28: Effect of manganese on soil acid phosphatase

Conc. of manganese (μM g^{-1}soil)	Acid phosphatase (μg of 4-nitrophenol released g^{-1}soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
0	38.1	30.6	30.8	21.1	43.0	32.7
2.0	34.9 (8.4)	28.3 (7.5)	28.1 (8.7)	19.8 (6.2)	39.6 (7.9)	30.1
4.0	32.0 (16.0)	26.0 (15.0)	26.6 (13.6)	18.1 (14.2)	36.1 (16.0)	27.8
6.0	30.6 (19.7)	24.5 (19.9)	25.9 (15.9)	16.9 (19.9)	34.2 (20.4)	26.4
8.0	29.1 (23.6)	23.9 (21.9)	24.3 (21.1)	16.2 (23.2)	32.8 (23.7)	25.3
10.0	28.4 (25.5)	23.2 (24.2)	23.6 (23.4)	15.8 (25.1)	32.1 (25.3)	24.6
Mean	32.2	26.1	26.5	18.0	36.4	

(Figures in parentheses indicate the per cent decrease over control)

C.D. at 5 per cent		
	Manganese levels	1.40
	Soils	1.25
	Manganese levels x soils	3.83

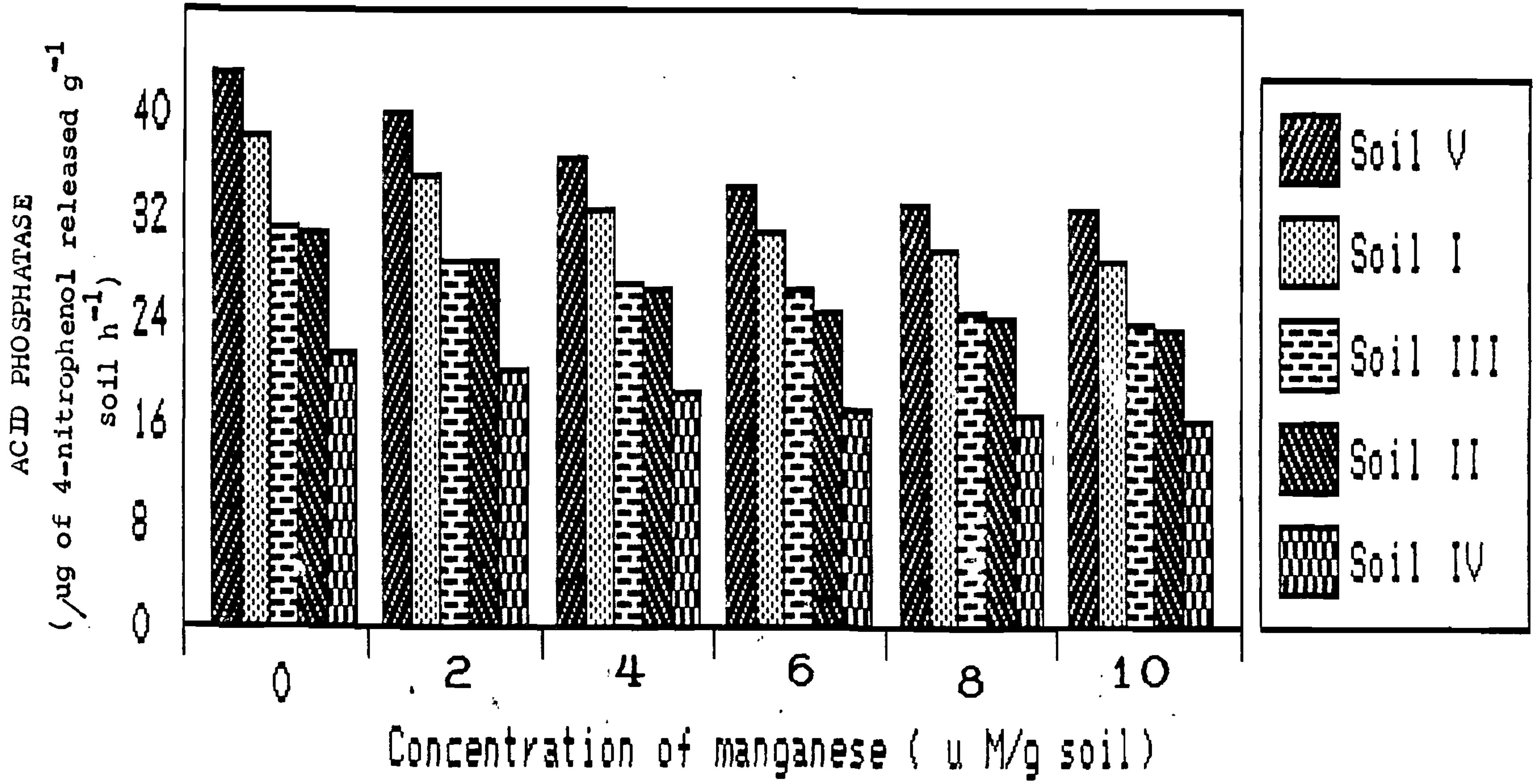


FIG.19 : EFFECT OF MANGANESE ON SOIL ACID PHOSPHATASE

4.6.3.3 Iron: Acid phosphatase activity of the soil decreased with increase in iron concentration (Table 29 and Fig. 20). The decreases in the enzyme activity in five soils were from 38.3 to 29.5, 31.4 to 25.2, 31.6 to 24.9, 21.1 to 16.3 and 43.1 to 34.1 μg of 4-nitrophenol released g^{-1} soil h^{-1}, respectively. The decrease was more at the initial levels of added iron and difference between two successive levels was significant upto 6 μ M g^{-1} soil. The decrease in soil acid phosphatase activity showed little differences following the order: soil II < soil V < soil III < soil IV < soil I.

4.6.3.4 Copper: The effect of copper on acid phosphatase activity in soil decreased with increasing copper concentration (Table 30). The decrease in enzyme activity in five soils were from 38.4 to 24.6, 31.1 to 22.5, 30.7 to 20.3, 21.0 to 14.9 and 43.3 to 23.4 μg of 4-nitrophenol released g^{-1} soil h^{-1}, respectively. The decrease in enzyme activity was more and significant between two successive levels upto 8 μ M g^{-1} soil. The per cent inhibition varied with the type of soil following the order: soil II < soil IV < soil III < soil I < soil V (Fig.21).

Table 29: Effect of iron on soil acid phosphatase

Conc. of iron (µM g^{-1} soil)	Acid phosphatase (µg of 4-nitrophenol released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
0	38.3	31.4	31.6	21.1	43.1	33.1
2.0	35.5 (7.3)	29.2 (7.0)	29.1 (7.9)	19.2 (9.0)	40.0 (7.2)	30.6
4.0	32.9 (14.1)	27.4 (12.7)	27.1 (14.2)	18.1 (14.2)	37.1 (13.9)	28.5
6.0	31.2 (18.5)	26.3 (16.2)	26.0 (17.7)	17.4 (17.5)	36.0 (16.5)	27.4
8.0	29.8 (22.2)	25.7 (18.2)	25.4 (19.6)	16.8 (20.4)	34.4 (20.2)	26.4
10.0	29.5 (22.9)	25.2 (19.7)	24.9 (21.2)	16.3 (22.7)	34.1 (20.8)	26.0
Mean	32.8	27.5	27.4	18.1	37.4	

(Figures in parentheses indicate the per cent decrease over control)

C.D. at 5 per cent

Iron levels : 1.39
Soils : 1.27
Iron levels x soils : 3.11

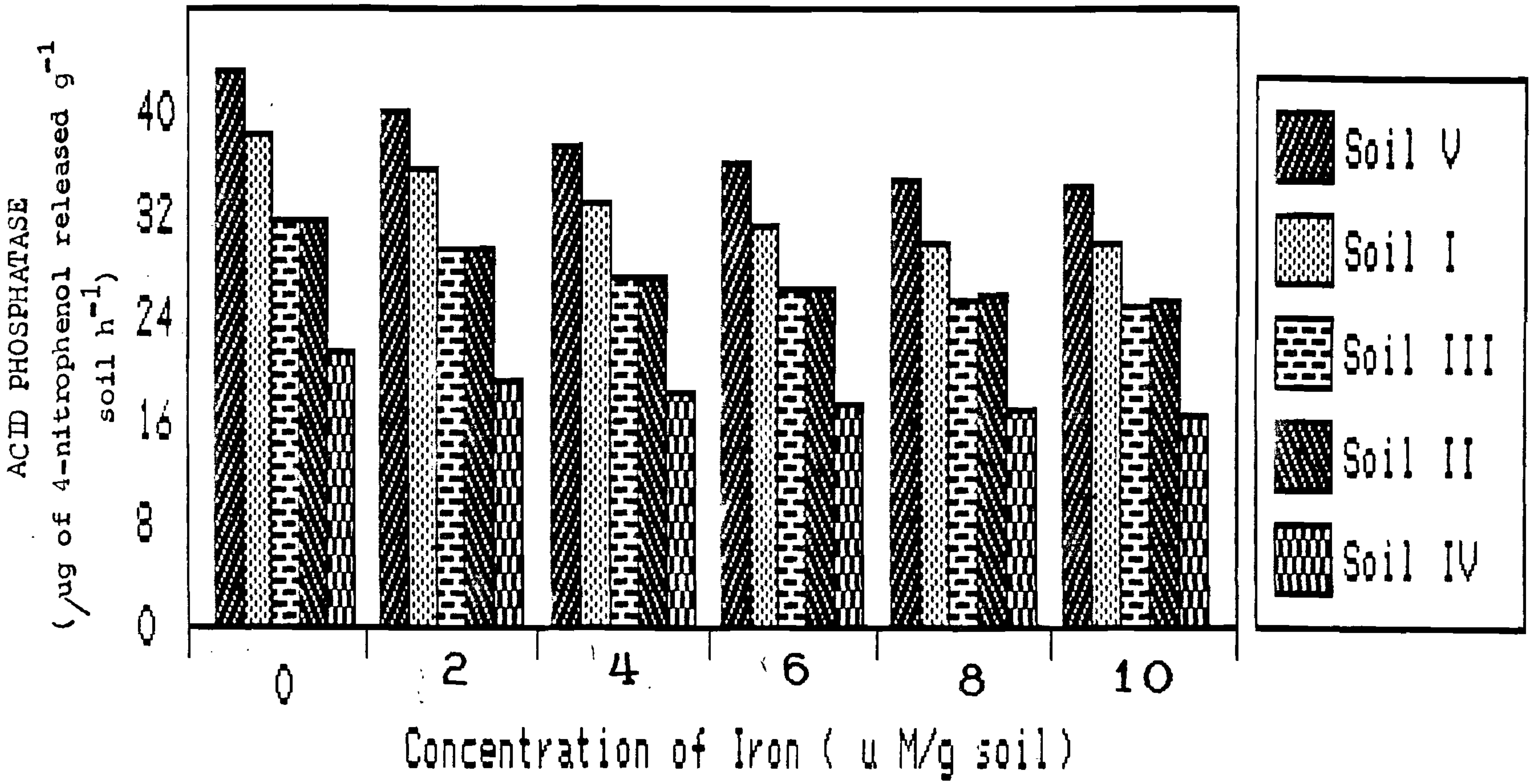


FIG.20 : EFFECT OF IRON ON SOIL ACID PHOSPHATASE

Table 30: Effect of copper on soil acid phosphatase

Conc. of copper ($\mu M\ g^{-1}$ soil)	Acid phosphatase (μg of 4-nitrophenol released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
0	38.4	31.1	30.7	21.0	43.3	32.9
2.0	33.1 (13.3)	28.1 (9.6)	27.2 (11.4)	19.8 (5.7)	35.5 (18.0)	28.8
4.0	27.9 (27.3)	25.5 (18.0)	25.0 (18.6)	17.3 (17.6)	28.6 (33.9)	24.9
6.0	26.3 (31.5)	23.8 (23.5)	23.0 (25.1)	15.9 (24.3)	24.8 (42.7)	22.8
8.0	25.0 (34.9)	22.1 (28.9)	20.8 (32.2)	14.6 (30.4)	22.7 (47.5)	21.0
10.0	24.6 (35.9)	22.0 (29.2)	20.3 (33.8)	14.9 (29.0)	23.4 (45.9)	21.0
Mean	29.2	25.4	24.5	17.3	29.7	

(Figures in parentheses indicate the per cent decrease over control)

C.D. at 5 per cent
Copper levels : 1.24
Soils : 1.13
Copper levels x soils : 2.77

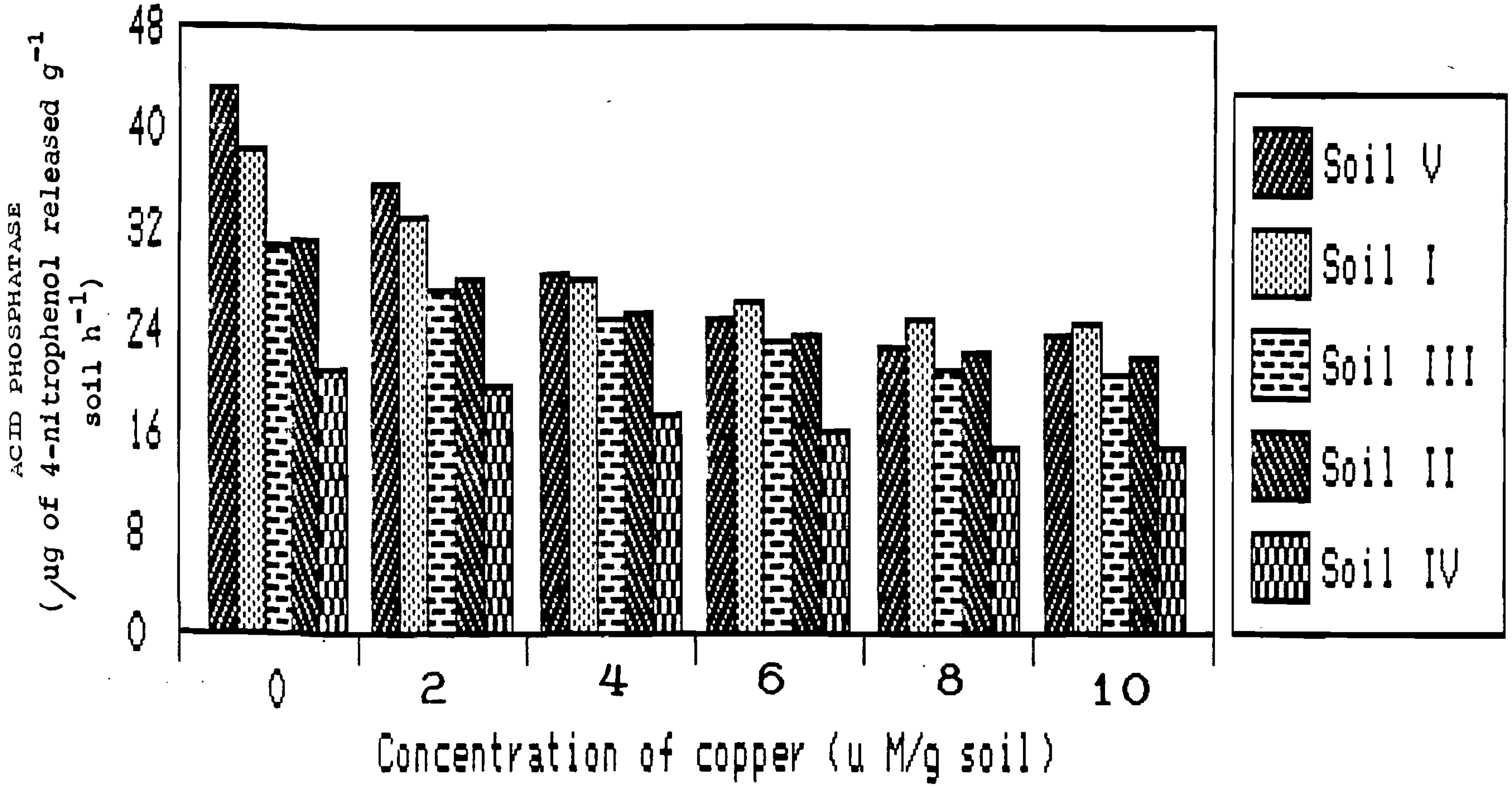


FIG. 21 : EFFECT OF COPPER ON SOIL ACID PHOSPHATASE

4.6.4 Alkaline phosphatase

4.6.4.1 Zinc: Alkaline phosphatase activity in soils decreased with increase in zinc concentration (Table 31). The decrease in enzyme activity in soils I to V were from 65.2 to 41.4, 73.1 to 44.1, 66.82 to 44.8, 19.4 to 12.3 and 13.1 to 7.9 ug of 4-nitrophenol released g^{-1} soil h^{-1}, respectively. For each addition of 2 μ M g^{-1} soil, there was decrease in enzyme activity which was significant between two successive levles upto 8 μ M g^{-1} soil. The per cent inhibition varied with the soil (Fig. 22) following order: soil III < soil IV < soil II < soil I < soil V.

4.6.4.2 Manganese: The results are presented in Table 32 and are depicted in Fig. 23.

Alkaline phosphatase activity of the soil decreased with increase in manganese concentration. The decreases in the enzyme activity in soils I to V were from 66.2 to 48.3, 72.7 to 56.6, 64.2 to 48.5, 19.0 to 15.2 and 12.7 to 10.3 μg of 4-nitrophenol released g^{-1} soil h^{-1}, respectively. The decrease was more in the initial levels of added manganese and the difference between two successive levels was significant upto 6 μ M g^{-1} soil. The per cent inhibition varied with type of soil following the order: soil V < soil IV < soil II < soil III < soil I.

Table 31: Effect of zinc on soil alkaline phosphatase

Conc. of zinc (μM g^{-1} soil)	Alkaline phosphatase (μg of 4-nitrophenol released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
0	65.2	73.1	66.8	19.4	13.1	47.5
2.0	60.3 (7.5)	67.1 (8.2)	60.9 (8.8)	11.3 (7.7)	11.3 (13.7)	43.5
4.0	51.3 (21.3)	58.7 (19.7)	53.9 (19.3)	15.2 (21.6)	9.9 (24.4)	37.8
6.0	46.1 (29.3)	50.3 (28.4)	48.8 (26.9)	13.7 (29.4)	8.9 (32.1)	34.0
8.0	42.4 (34.9)	46.1 (36.9)	45.8 (31.4)	13.1 (32.5)	8.4 (35.8)	31.2
10.0	41.4 (36.9)	44.1 (39.7)	44.8 (32.9)	12.3 (36.6)	7.9 (39.7)	30.1
Mean	51.1	56.9	53.5	15.3	9.9	

(Figures in parentheses indicate the per cent decrease over control)

C.D. at 5 per cent		
	Zinc levels	: 1.34
	Soils	: 1.22
	Zinc levels x soils	: 3.00

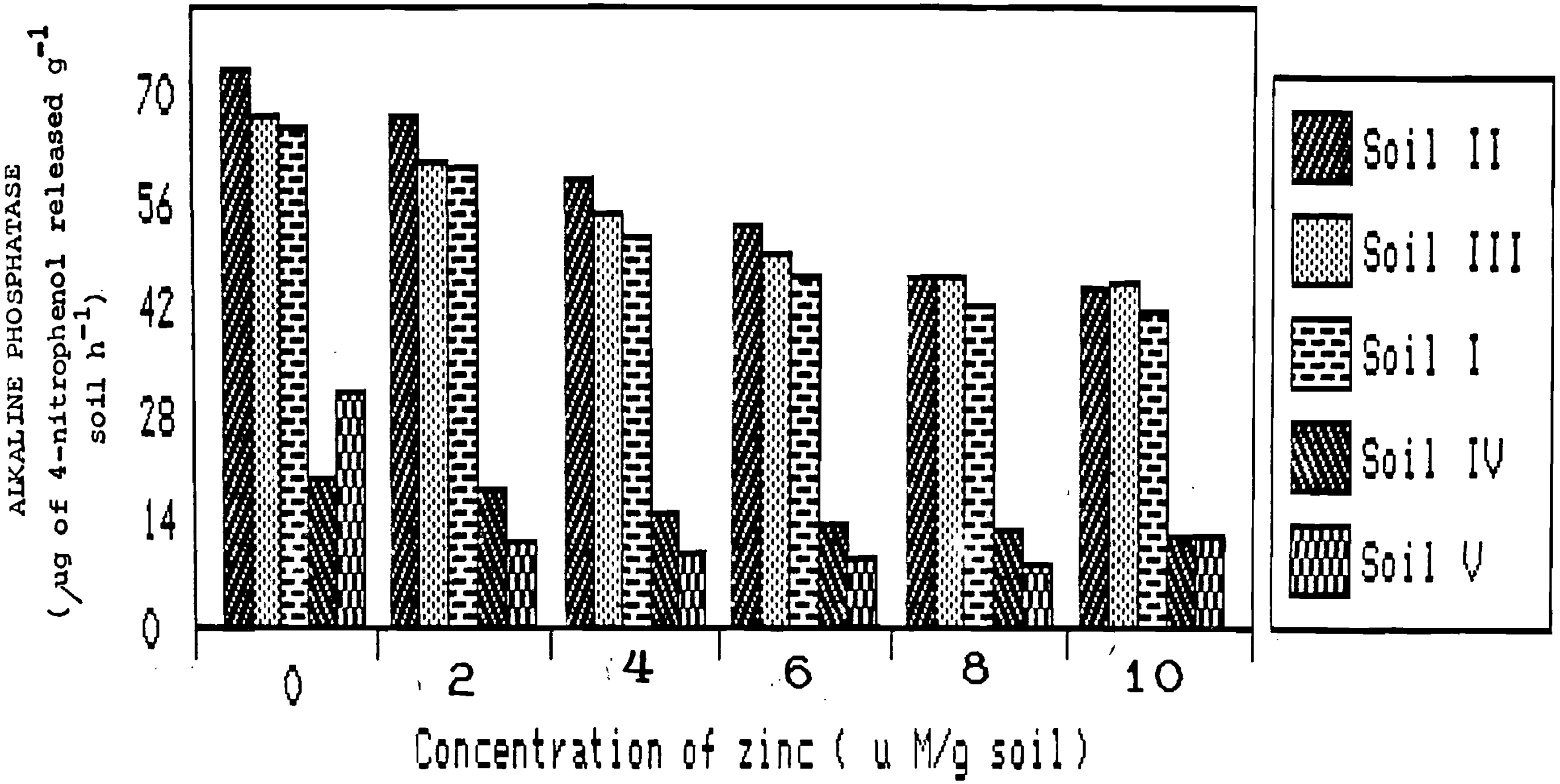


FIG.22 : EFFECT OF ZINC ON SOIL ALKALINE PHOSPHATASE

Table 32: Effect of manganese on soil alkaline phosphatase

Conc. of manganese (μ M g^{-1} soil)	Alkaline phosphatase (μg of 4-nitrophenol released g^{-1}soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
0	66.2	72.7	64.2	19.0	12.7	47.0
2.0	61.5 (7.1)	67.5 (7.2)	61.5 (4.2)	17.6 (7.4)	11.9 (6.3)	44.0
4.0	55.2 (16.6)	61.2 (15.8)	56.6 (11.8)	16.6 (12.6)	11.2 (11.8)	40.2
6.0	50.6 (23.6)	58.5 (19.5)	51.3 (20.1)	16.2 (14.7)	10.9 (14.2)	37.5
8.0	49.1 (25.8)	57.1 (21.5)	49.3 (23.2)	15.9 (16.3)	10.5 (17.3)	36.4
10.0	48.3 (27.0)	56.6 (22.1)	48.5 (24.5)	15.2 (20.0)	10.3 (18.9)	35.8
Mean	55.2	62.3	55.2	16.8	11.3	

(Figures in parentheses indicate the per cent decrease over control)

C.D. at 5 per cent
Manganese levels : 1.61
Soils : 1.47
Manganese levels x soils : 3.59

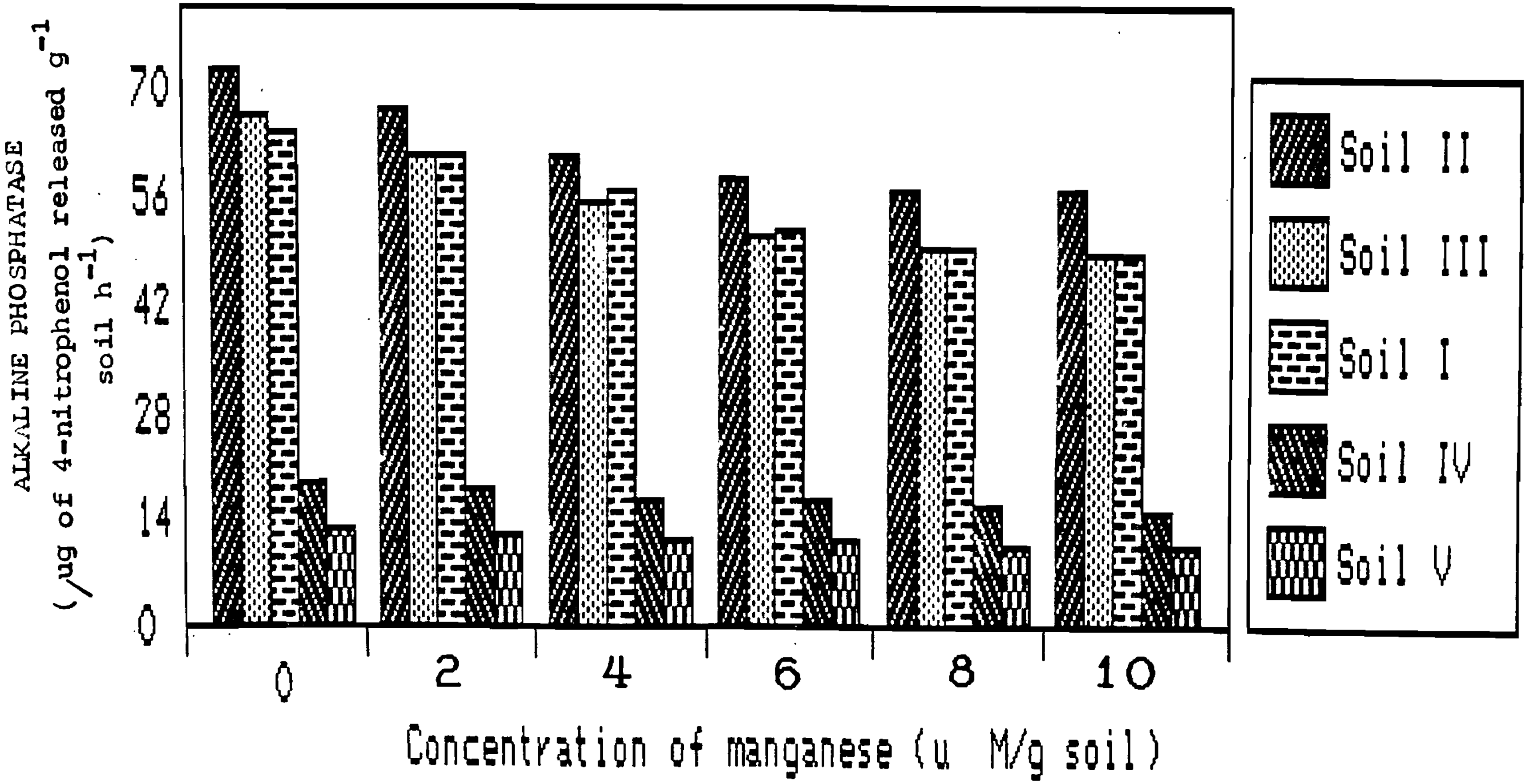


FIG. 23 : EFFECT OF MANGANESE ON SOIL ALKALINE PHOSPHATASE

4.6.4.3 Iron: The results are presented in Table 33 and are depicted in Fig. 24.

Alkaline phosphatase activity of the soil decreased with increase in iron concentration. The decreases in the enzyme activity were from 64.9 to 51.1, 72.8 to 55.6, 66.8 to 53.6, 19.5 to 15.3 and 13.0 to 9.8 μg of 4-nitrophenol released g^{-1} soil h^{-1} for soil I to V, respectively. The decrease was more at the initial levels of added iron and-the differences between two sucessive levels were significant upto the level of 6 μM g^{-1} soil. The per cent inhibition varied with the type of soil following the order: soil III < soil I < soil IV < soil II < soil V.

4.6.4.4 Copper: The results are presented in Table 34 and are depicted in Fig. 25.

Alkaline phosphatase activity in soils I to V were from 65.1 to 39.6, 73.6 to 48.4, 66.1 to 44.0, 19.2 to 12.2 and 12.6 to 7.1 μg of 4-nitrophenol released g^{-1} soil h^{-1}, respectively. At each level of copper added, there was a significant decrease in enzyme activity upto 8 μ M g^{-1} soil and the differences recorded thereafter were statistically non significant. The percent inhibition varied with the type of soil following the order: soil V > soil I > soil IV > soil II > soil III.

Table 33: Effect of Iron on soil alkaline phosphatase

Conc. of iron (μ M g^{-1} soil)	Alkaline phosphatase (μg of 4-nitrophenol released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
0	64.9	72.8	66.8	19.5	13.0	47.4
2.0	60.9 (6.2)	67.1 (7.8)	61.3 (8.2)	17.9 (8.2)	11.9 (8.5)	43.8
4.0	56.0 (13.7)	60.3 (17.2)	56.9 (14.8)	16.6 (14.9)	11.2 (13.8)	40.2
6.0	53.0 (18.3)	58.0 (20.3)	54.7 (18.1)	15.9 (18.5)	10.2 (21.5)	38.4
8.0	51.9 (20.0)	56.2 (22.8)	54.1 (19.0)	15.5 (20.5)	10.0 (23.1)	37.5
10.0	51.1 (21.3)	55.6 (23.6)	53.6 (19.8)	15.3 (21.5)	9.8 (24.6)	37.2
Mean	56.3	61.7	57.9	16.8	11.0	

(Figures in parentheses indicate the percent decrease over control)

C.D. at 5 per cent
Iron levels : 1.53
Soils : 1.39
Iron levels x soils : 3.42

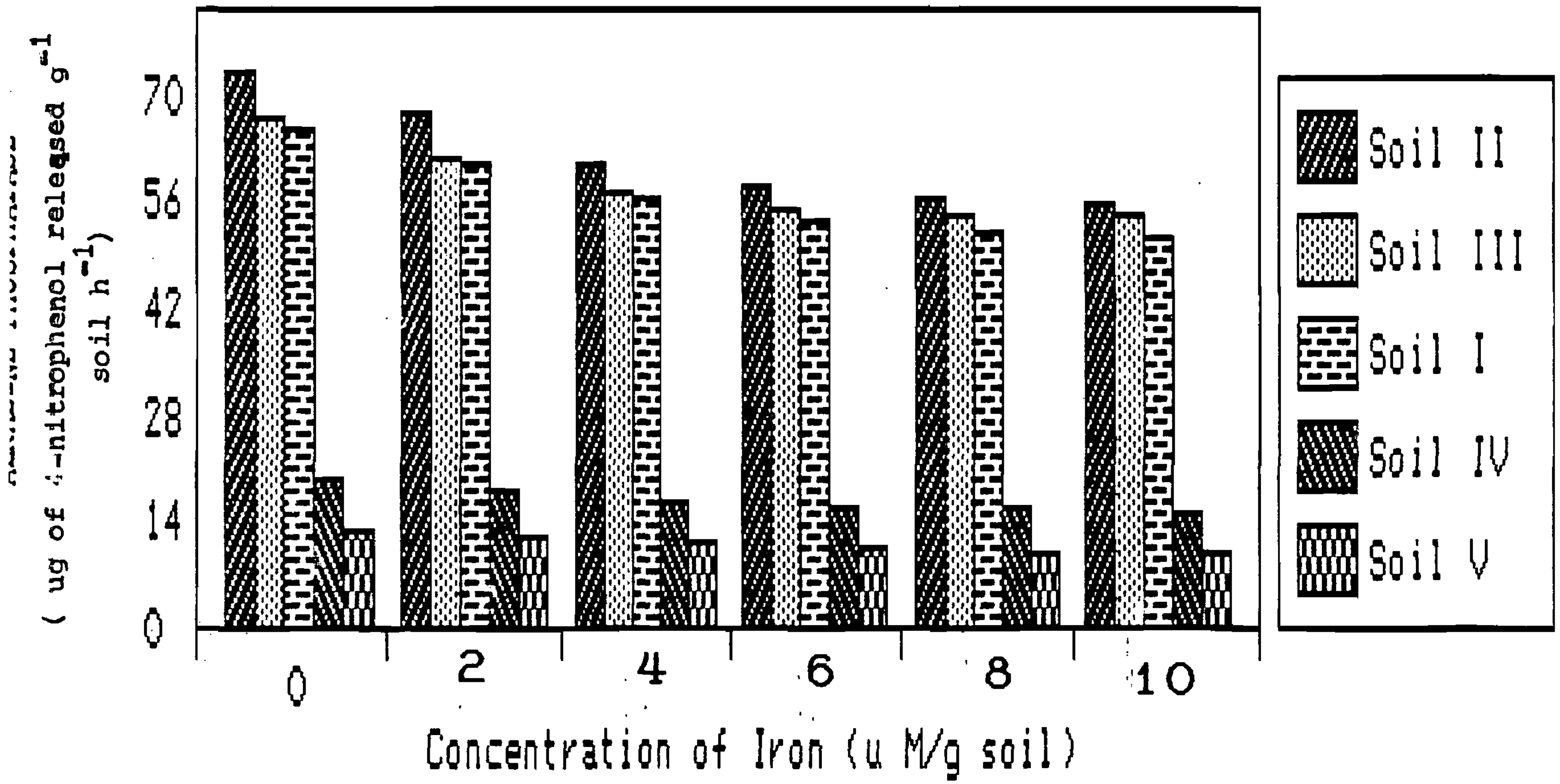


FIG.24 : EFFECT OF IRON ON SOIL ALKALINE PHOSPHATASE

Table 34: Effect of Copper on soil alkaline phosphatase

Conc. of copper ($\mu M\ g^{-1}$ soil)	Alkaline phosphatase (μg of 4-nitrophenol released g^{-1} soil h^{-1})					
	Soil I	Soil II	Soil III	Soil IV	Soil V	mean
0	65.1	73.6	66.1	19.2	12.5	47.3
2.0	58.3 (10.4)	64.9 (11.8)	61.0 (7.7)	17.6 (8.3)	9.9 (21.4)	42.4
4.0	50.3 (22.7)	51.3 (30.3)	52.1 (21.1)	15.2 (20.8)	8.4 (33.3)	35.5
6.0	44.6 (31.5)	49.8 (32.3)	46.3 (29.9)	13.4 (30.2)	7.6 (39.6)	32.3
8.0	40.6 (37.6)	48.9 (33.5)	44.4 (32.8)	12.7 (33.8)	7.1 (43.6)	30.8
10.0	39.6 (39.2)	48.4 (34.2)	44.0 (33.4)	12.2 (36.4)	7.1 (43.6)	30.3
Mean	49.8	56.1	52.3	15.0	8.8	

(Figures in parentheses indicate the percent decrease over control)

C.D. at 5 per cent

Copper levels : 1.37
Soils : 1.25
Copper levels x soils : 3.06

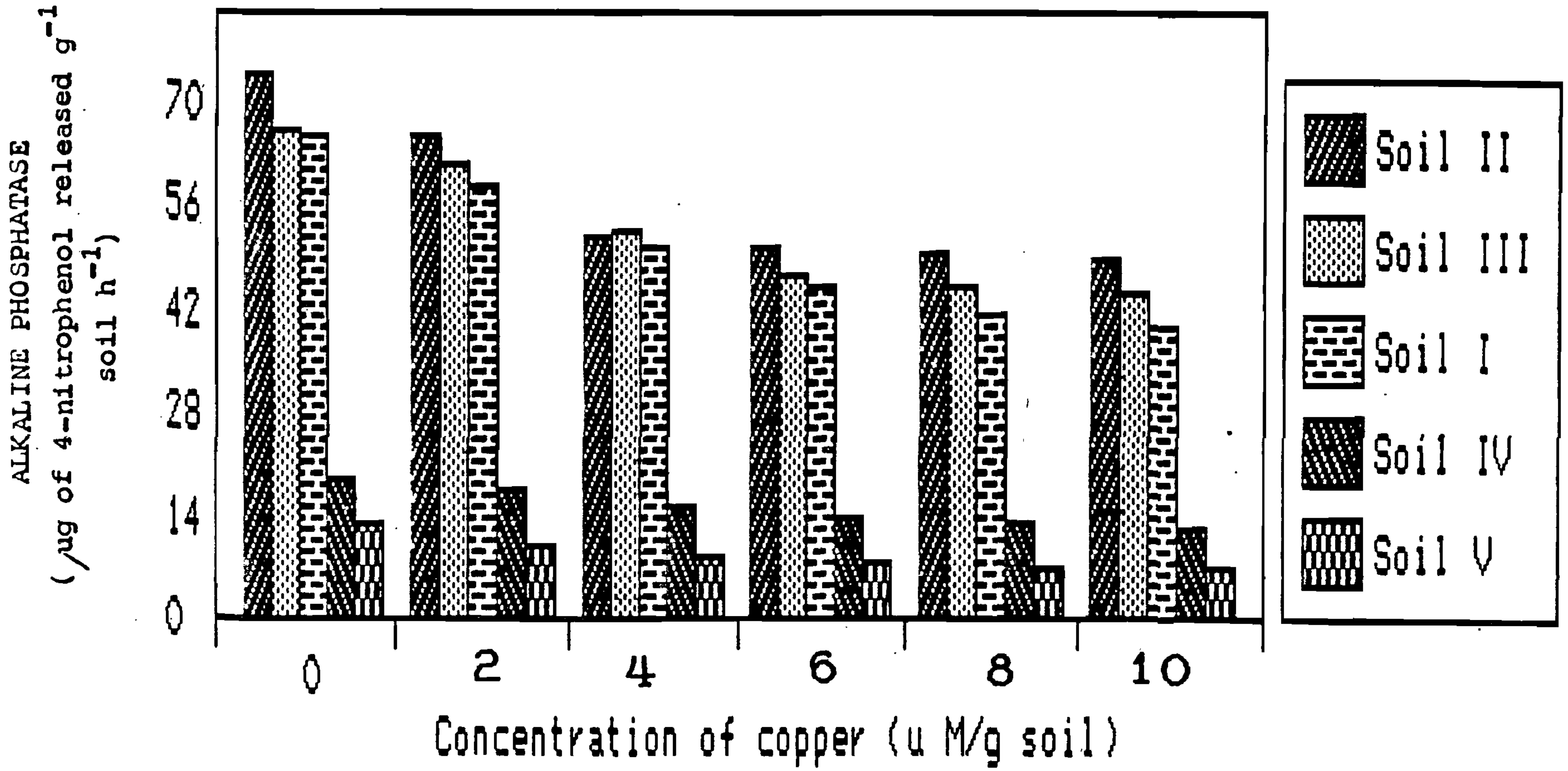


FIG.25 : EFFECT OF COPPER ON SOIL ALKALINE PHOSPHATASE

4.7 EFFECT OF CLAYS ON UREASE ACTIVITY

The concentration of enzymes in the equilibrium solution and the amount adsorbed on the clay were represented in terms of the activity of the enzyme in mg of NH_4^+ released h^{-1}.

Aliquots varying from 1 to 8 ml from the stock solution of urease obtained by dissolving one tablet of enzyme in 100 ml and the enzyme activity was assayed. A plot of enzyme concentration vs activity is shown in Fig. 26.

As seen, there was an excellent linearity in the graph and it ensured that the aliquots of the enzyme solution added were truely representative of the activity and, hence, the amount of enzyme added.

The amounts of enzyme adsorbed by different clays at different concentrations of the urease added are given in Tables 35 and 36.

It is observed from the results that the amount of enzyme adsorbed in all the clays increased with the increase in the enzyme concentration. The increases varied from 2.91 to 13.44, 1.66 to 13.48, 1.42 to 6.89 and 2.76 to 13.76 mg of NH_4^+ released $100mg^{-1}$ clay in montmorillonite, bentonite, kaolinite and illite, respectively.

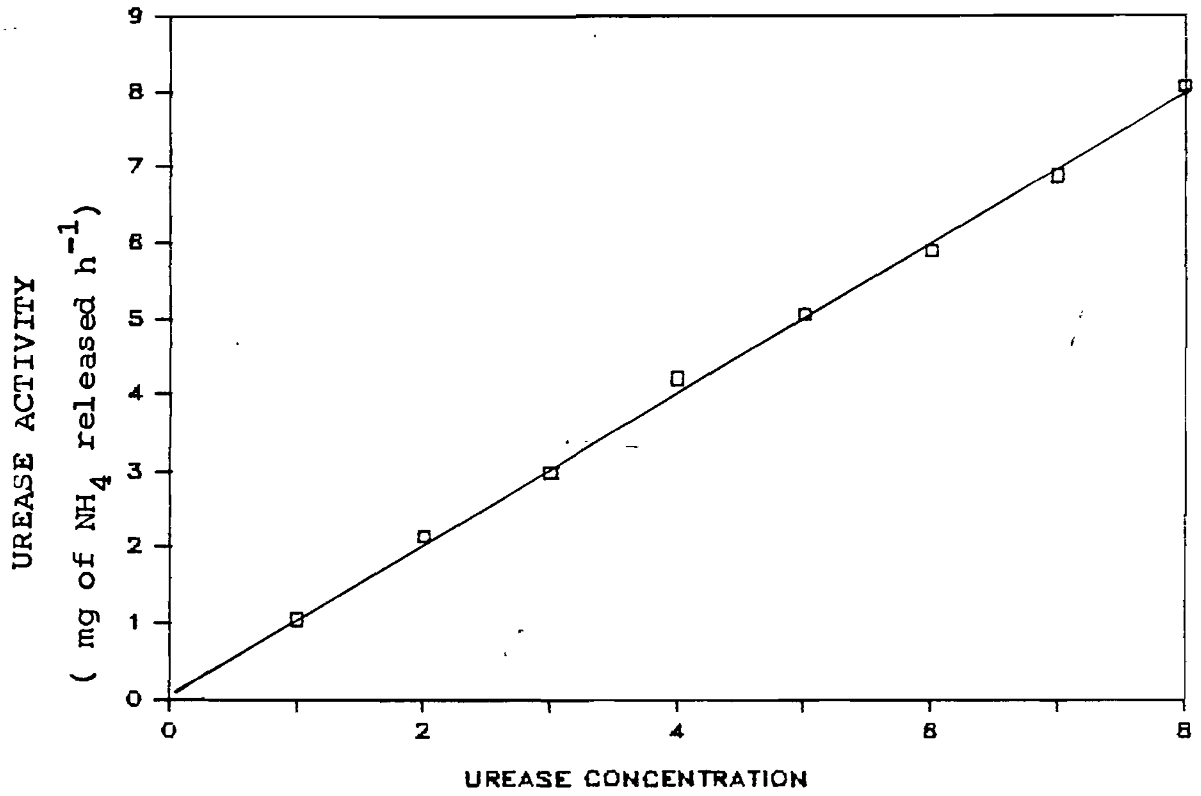


FIG. 26 : LINEAR RELATIONSHIP OF UREASE CONCENTRATION AND ACTIVITY

Table 35 : Effect of Montmorillonite and Kaolinite on urease activity

Enzyme activity added (mg of NH_4 released h^{-1})	Montmorillonite						Kaolinite					
	Suspension			Supernatant			Suspension			Supernatant		
	A.E.A	Enzyme Inhibition (%)	A.A	A.E.A	Enzyme Inhibition (%)	A.A	A.E.A	Enzyme Inhibition (%)	A.A	A.E.A	Enzyme Inhibition (%)	A.A
1.05	0.13	12.4	2.91	0.17	16.2	2.76	0.32	30.5	1.42	0.32	30.4	1.42
2.15	0.75	34.8	4.16	0.85	39.5	3.83	1.08	50.2	1.93	1.06	49.3	1.96
3.00	1.36	45.3	5.46	1.47	49.0	5.10	1.67	55.6	2.78	1.77	59.0	2.56
4.20	1.92	45.7	6.93	2.13	50.7	6.23	2.36	56.2	3.41	2.38	56.6	3.37
5.06	2.45	48.4	8.50	2.60	51.4	8.00	3.08	60.1	4.01	2.99	59.1	4.19
5.90	2.95	50.0	10.16	3.10	52.5	9.66	3.54	60.0	5.12	3.65	61.8	4.89
6.90	3.40	49.2	12.00	3.62	52.5	11.26	4.28	62.0	5.66	4.23	61.3	5.77
8.07	3.97	49.2	13.44	3.96	49.0	13.46	4.69	58.2	6.87	4.79	59.4	6.68

A.E.A. = Activity of enzyme at equilibrium concentration (mg of NH_4 released h^{-1})

A.A = Activity of enzyme adsorbed (mg of NH_4 released h^{-1} 100 mg^{-1}clay)

Table 36: Effect of Bentonite and Illite on urease activity

Enzyme activity added (mg of NH_4 released h^{-1})	Bentonite						Illite					
	Suspension			Supernatant			Suspension			Supernatant		
	A.E.A	Enzyme Inhibition (%)	A.A	A.E.A	Enzyme Inhibition (%)	A.A	A.E.A	Enzyme Inhibition (%)	A.A	A.E.A	Enzyme Inhibition (%)	A.A
1.00	0.41	39.0	1.66	0.37	35.2	1.75	0.13	12.3	2.63	0.18	17.1	2.48
2.15	0.84	39.0	3.24	0.76	35.3	3.44	0.66	31.7	4.06	0.55	25.6	4.39
3.00	1.12	37.3	5.17	1.13	37.6	5.19	1.23	41.0	5.35	1.20	40.0	5.45
4.20	1.59	37.8	6.76	1.55	36.9	6.80	1.88	44.8	6.66	1.80	44.7	6.66
5.06	1.94	38.3	8.58	1.92	37.9	8.55	2.36	46.6	7.99	2.24	44.0	8.36
5.90	2.32	39.3	10.32	2.38	40.3	10.05	2.88	48.8	9.44	2.74	46.4	9.88
6.90	2.84	41.2	11.65	2.72	39.4	11.88	3.33	48.2	11.13	3.11	45.1	11.78
8.07	3.19	39.5	13.48	3.15	39.0	13.47	3.46	42.8	13.76	3.64	45.1	13.21

A.E.A. = Activity of enzyme at equilibrium concentration (mg of NH_4 released h^{-1})

A.A = Activity of enzyme adsorbed (mg of NH_4 released h^{-1} 100 mg^{-1} clay)

In all the clays, the isotherms were 'S' shaped i.e. slow increase in adsorption in the beginning followed by a much sharper increase in adsorption with slight change in concentration (Fig. 27). The complete isotherms could not be drawn because there was deviation in the linearity of the activity vs. concentration as determined by the dilution of the stock solution. Kaolinite, which is a 1:1 type of clay, exhibited lower adsorption when compared with montmorillonite and bentonite. The adsorption isotherms of bentonite, illite and montmorillonite are comparable.

In all the clays, the activities were assayed in suspension, supernatant and in the clay sediment. The activity of the enzyme adsorbed on the clay was predominantly nil showing that the enzyme was completely deactivated consequent to its adsorption on the clay. Thus, activity of the suspension in all the clays was nearly equal to the activity in the supernatant. This also confirmed that urease was deactivated due to the adsorption on the clay and the clay suspension did not interfere with the activity of the supernatant.

4.8 EFFECT OF DIFFERENT CATIONS SATURATED CLAYS ON UREASE ACTIVITY

The effect of different cations on the adsorption of urease was studied using hamoionic

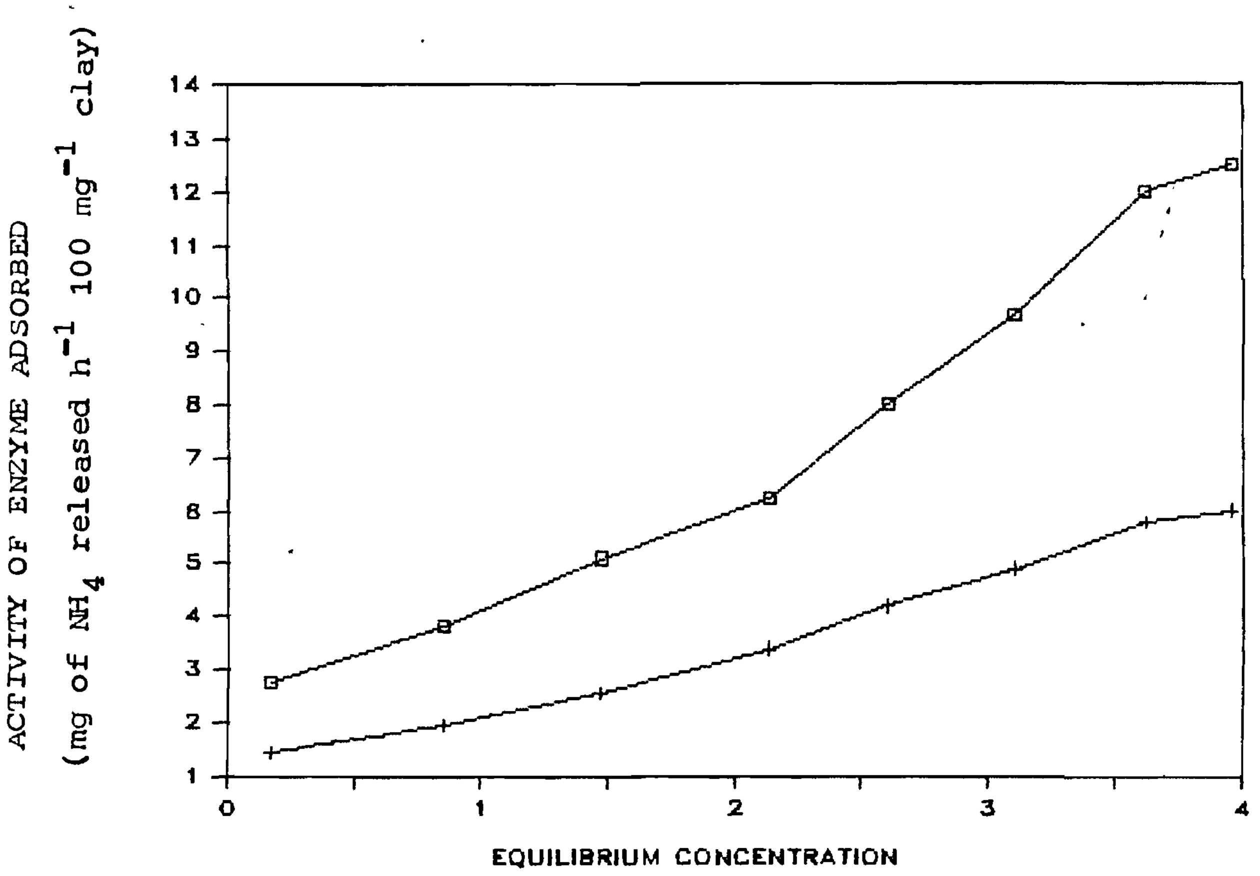


FIG.27 : EFFECT OF MONTMORILLONITE AND KAOLINITE ON UREASE ACTIVITY

FIG.27 (CONTD..)

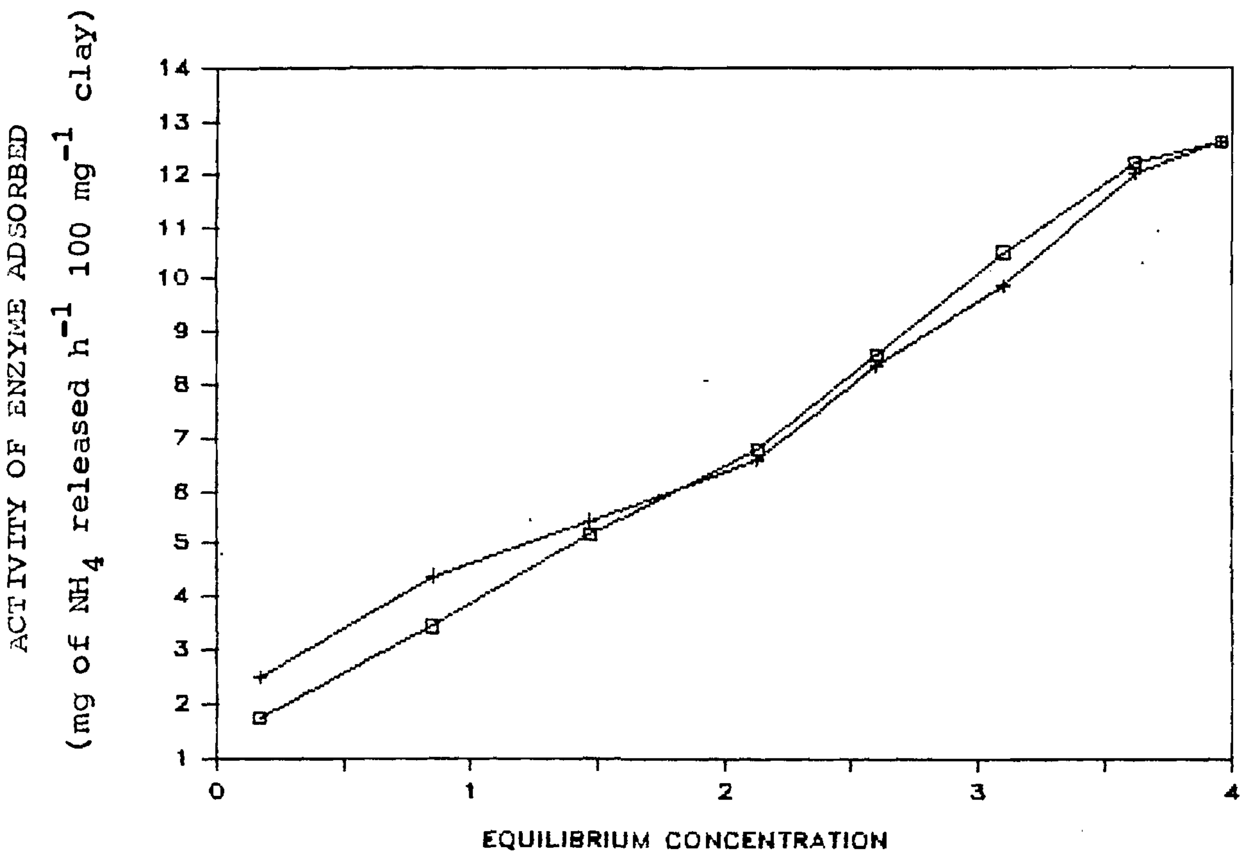


FIG.27 a : EFFECT OF BENTONITE AND ILLITE ON UREASE ACTIVITY bentonites. The amounts adsorbed by different clays from an initial concentration of enzyme corresponding to 5.06 mg of NH_4^+ liberated h^{-1} are given in Table 37.

It is observed from the results that there are clear differences among different homoionic bentonites in their capacity for adsorption of the enzyme. The alkali metal cations i.e., Li, K and Cs showed similar capacity of enzyme adsorption. The divalent cations Ca^{++} and Mg^{++} adsorbded higher amounts of enzyme. The effect of transition metal cations on adsorption was significantly more. It was three times more than alkali and two times more than that of alkaline earth cations. The order of adsorption was Zn > Cu > Co > Mg > Ca > Cs > Na > K > Li.

4.9 EFFECT OF SOME HERBICIDES ON SOIL UREASE ACTIVITY

Use of herbicides has became a widely adopted practice in modern day agricultural production and an understanding of their interaction in soil biochemical properties is important. Urea and triazine herbicides have fairly long persistance and hence, their role in hydraulysis of urea by urease was considered worth investigating. This aspect has so far received little attention. Hence a pot culture experiment was conducted

Table 37 : Effect of Ion saturated bentonite on urease activity

Ions saturated	Activity of urease added (mg of NH_4 released h^{-1})	Activity in the equilibrium solution (ug of NH_4 released h^{-1})	Activity adsorbed (mg of NH_4 released 100 mg^{-1} clay)
Zn	5.06	0.02	8.40
Cu	5.06	0.14	8.20
Co	5.06	0.35	7.85
Ca	5.06	2.68	3.96
Mg	5.06	2.03	5.06
Na	5.06	3.34	2.80
K	5.06	3.53	2.55
Cs	5.06	3.34	2.86
li	5.06	3.66	2.33

to study the effect of four herbicides on soil urease activity in a clay loam soil using maize as test crop.

4.9.1 Atrazine

The results pertaining to the effect of atrazine on soil urease activity are presented in Table 38 and are shown graphically in the Fig. 28.

There was an increase in soil urease activity from stage I to IV of the crop growth irrespective of the treatment of soil with atrazine. The increase with stage, however, was less during the first two stages of the crop growth with significant increase at later stages.

Soil urease activity decreased with atrazine concentration at any stage of the crop growth. The decrease was steep from control to 1 ppm level during the first two stages of the crop. The per cent inhibition with concentration stage I to V were 18.2 to 36.6, 12.8 to 39.5, 11.4 to 34.8 and 2.6 to 23.6 respectively.

4.9.2 Simazine

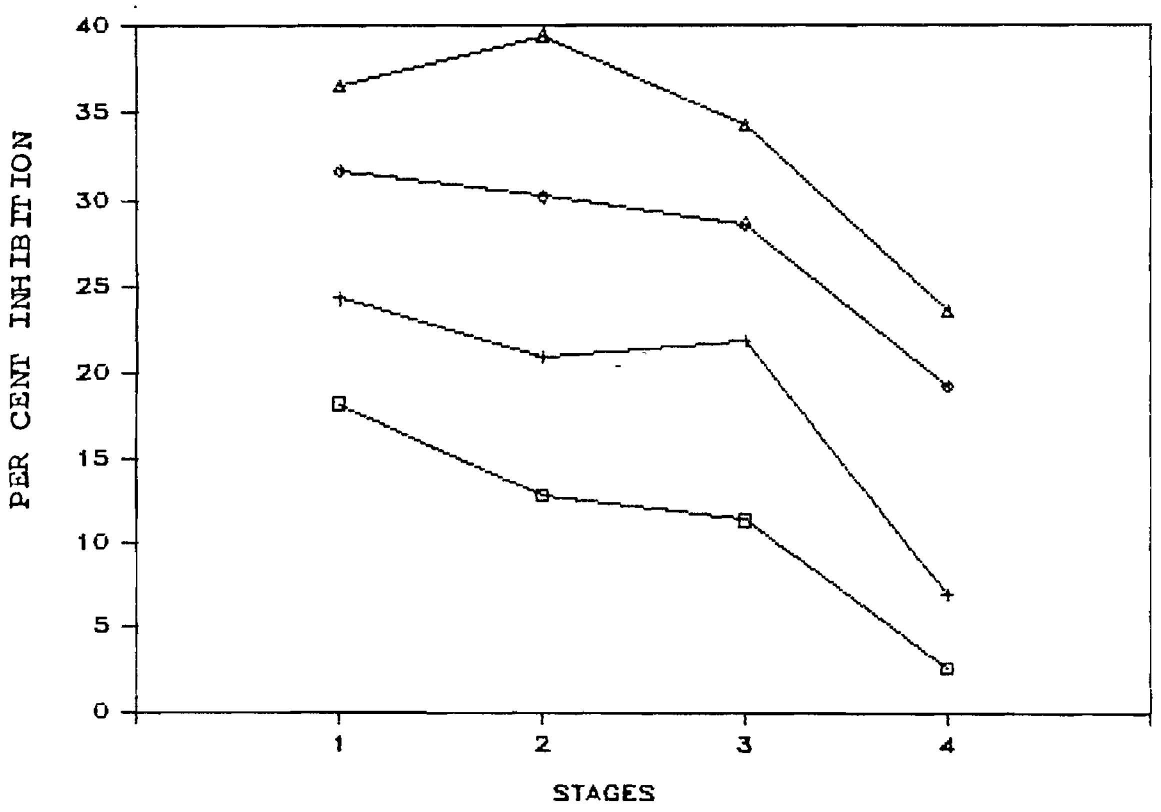
The results pertaining to the effect of simazine on soil urease activity are presented in Table 39 and are depicted graphically in the Fig. 29.

Table 38: Effect of Atrazine on soil urease activity

Atrazine added to soil(mg Kg^{-1})	Urease activity (μg of NH_4 released g^{-1} soil h^{-1})				
	Stage I	Stage II	Stage III	Stage IV	mean
0	8.2	8.6	10.5	11.4	9.7
1	6.7 (18.2)	7.5 (12.8)	9.3 (11.4)	11.1 (2.6)	8.7
2	6.2 (24.3)	6.8 (20.9)	8.2 (21.9)	10.6 (7.0)	8.0
3	5.6 (31.7)	6.0 (30.2)	7.5 (28.6)	9.2 (19.2)	7.1
4	5.2 (36.6)	5.2 (39.5)	6.7 (34.3)	8.7 (23.6)	6.5
Mean	6.43	6.83	8.40	10.16	

(Figures in parentheses indicate the per cent decrease over control)

C.D. at 5 per cent
Atrazine levels : 1.13
Stages : 1.01
Atrazine levels x stages : 2.26



△ 1 mg kg^{-1} ◇ 2 mg kg^{-1} + 3 mg kg^{-1} □ 4 mg kg^{-1}

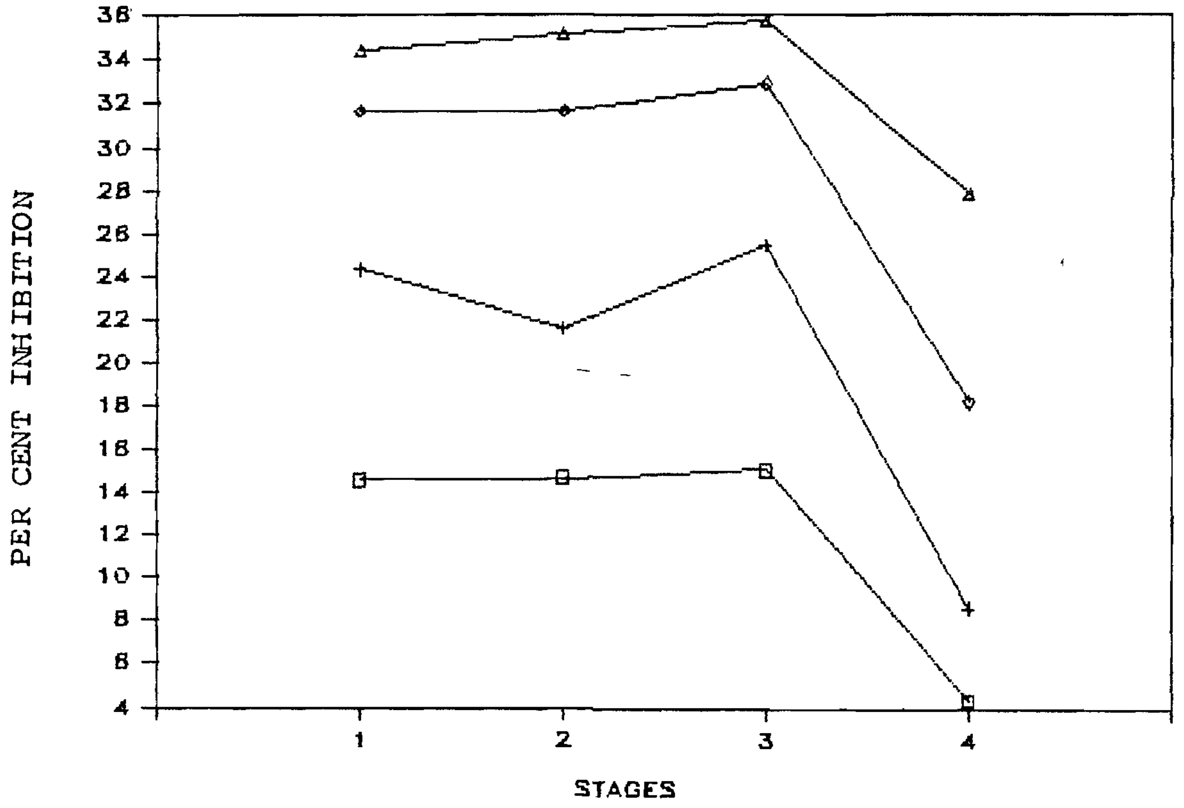
FIG.28 :EFFECT OF ATRAZINE ON SOIL UREASE ACTIVITY

Table 39: Effect of Simazine on soil urease activity

Simazine added to soil(mg Kg^{-1})	Urease activity (μg of NH_4 released g^{-1} soil h^{-1}) Stage I	Stage II	Stage III	Stage IV	mean
0	8.2	8.8	10.6	11.5	9.8
1	7.0 (14.6)	7.5 (14.7)	9.0 (15.1)	11.0 (4.3)	8.6
2	6.2 (24.3)	6.9 (21.6)	7.9 (25.5)	10.5 (8.6)	7.8
3	5.6 (31.7)	6.2 (31.7)	7.1 (33.0)	9.4 (18.2)	7.1
4	5.3 (35.4)	5.7 (35.2)	6.8 (35.8)	8.3 (27.8)	6.5
Mean	6.45	7.02	8.28	10.12	

(Figures in parentheses indicate the per cent decrease over control)

C.D. at 5 per cent			
	Simazine levels	:	1.44
	Stages	:	1.29
	Simazine levels x stages	:	2.88



△ 1 mg kg^{-1} ◇ 2 mg kg^{-1} + 3 mg kg^{-1} □ 4 mg kg^{-1}

FIG.29 : EFFECT OF SIMAZINE ON SOIL UREASE ACTIVITY

Simazine showed similar trend of results as was observed in atrazine. Application of simazine at the rates of 1, 2, 3 and 4 mg Kg^{-1} decreased soil urease activity and the decrease was more pronounced at the earlier stages of crop growth with significant increases at later stages.

Increase in herbicide concentration decreased soil urease activity irrespective of the stage of the crop. The per cent decrease recorded with respect to control at stages I to IV were 14.6 to 35.4, 14.7 to 35.2, 15.1 to 35.8 and 4.3 to 27.8, respectively. On the other hand, with the advancement of age of the crop, there was an increase in urease activity at all the levels of herbicide. The increase was slow at the initial stages with relatively greater increase at the last stage. Also, the high per cent decreases recorded in enzyme activity during early stages of the crop growth were narrowed down from 14.6 to 4.3, 24.3 to 8.6, 31.7 to 18.2 and 35.4 to 27.8 at 1, 2, 3 and 4 mg Kg^{-1}, respectively.

4.9.3 Methabenzthiazuron

The results pertaining to the effect of methabenzthiazuron are presented in Table 40 and are shown graphically in the Fig. 30.

Table 40: Effect of Mathabenzthiazuron on soil urease activity

Methabenzthiazuron added to soil(mg Kg^{-1})	Urease activity (µg of NH_4 released g^{-1} soil h^{-1})				
	Stage I	Stage II	Stage III	Stage IV	mean
0	8.2	8.8	10.4	11.6	9.8
1	7.0 (14.6)	7.4 (15.9)	8.0 (23.1)	11.0 (5.1)	8.4
2	6.5 (20.7)	7.0 (20.5)	7.5 (27.8)	10.0 (13.8)	7.8
3	5.3 (35.4)	5.9 (32.9)	7.0 (32.6)	8.7 (25.0)	6.7
4	4.8 (41.5)	4.9 (44.3)	6.4 (38.5)	7.5 (35.3)	5.9
Mean	6.37	6.80	7.86	9.76	

(Figures in parentheses indicate the per cent decrease over control)

C.D.at 5 per cent

Methabenzthiazuron levels : 1.03
Stages : 0.92
Mathabenzthiazuron levels x stages : 2.06

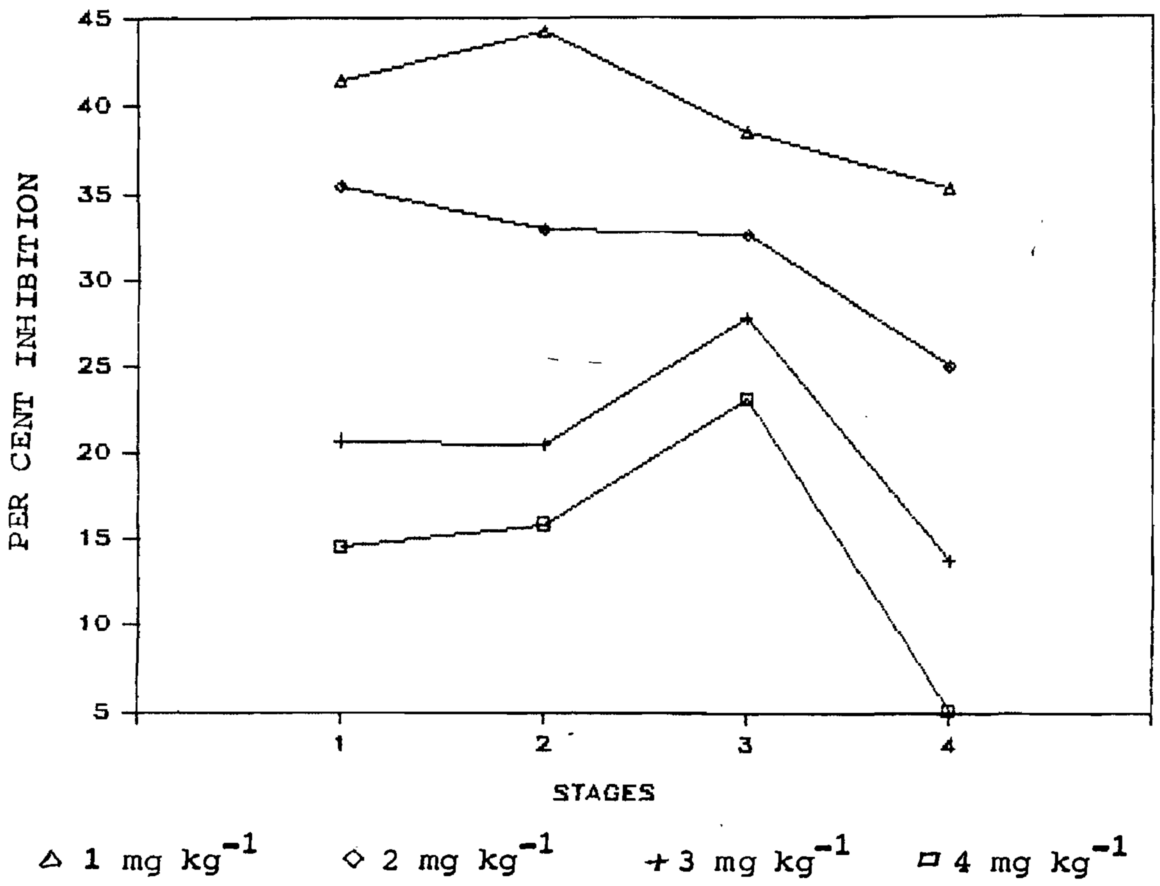


FIG.30 : EFFECT OF MATHABENZTHIAZURON ON SOIL UREASE ACTIVITY

Application of methabenzthiazuron decreased soil urease activity in general and the level of inhibition was relatively more compared to the other group of herbicides used in this study. The decrease in the activity was significantly more at added levels of herbicide. In any of the stages, the per cent decrease showed an increase with herbicide concentration and varied from 14.6 to 41.5, 15.9 to 44.3, 23.1 to 38.5 and 5.1 to 35.3, with respect to control respectively.

On the other hand, with advancement in the age of the crop, there was an increase in soil enzyme activity. The increase remained more or less same in the first two stages of the crop growth showing greater increase at the later stages. However, in stage III, though there was an increase in soil urease activity, the per cent inhibition by herbicide remained almost same as in the case of earlier two stages. However, the high per cent decrease recorded during initial stages of the crop growth at added levels of herbicide were narrowed down in the last stage from 14.6 to 5.1, 20.7 to 13.8, 35.4 to 25.0 and 41.5 to 35.3, respectively.

4.9.4 Metoxuron

The results related to effect of metoxuron on urease activity at different stages of the crop growth are presented in Table 41 and are depicted graphically in the Fig. 31.

Application of metoxuron decreased soil urease activity and the decreases recorded were significantly different at all the levels of added herbicide. In any of the four stages studied, decrease in the activity of the enzyme increased with increase in herbicide concentration. The per cent decrease with concentration at four different stages were 14.5 to 37.3, 17.2 to 39.0, 18.4 to 35.9 and 4.3 to 35.9, respectively.

On the other hand with the advancement of the age of the crop, not much differences were recorded in soil urease activity during first two stages. Though there was slight increase in enzyme activity, the per cent inhibition remained the same at added levels of herbicide. At later stages, increase in soil urease activity was correspondingly more and the per cent inhibition was narrowed down.

4.10 INFLUENCE OF PLANT COVER ON SOIL UREASE ACTIVITY

Some reports exhist about the contribution of plant crops to soil enzyme activity through endoenzymes in roots or through extracellular enzymes secreted by the roots. The type of crop as well as stage of growth

Table 41 : Effect of Metoxuron on soil urease activity

Metoxuron added to soil(mg Kg^{-1})	Urease activity (μg of NH_4 released g^{-1} soil h^{-1})				
	Stage I	Stage II	Stage III	Stage IV	mean
0	8.3	8.7	10.3	11.4	9.7
1	7.1 (14.5)	7.2 (17.2)	8.4 (18.4)	10.9 (4.3)	8.4
2	6.5 (21.7)	6.9 (20.6)	7.9 (23.3)	9.9 (13.2)	7.8
3	6.0 (27.7)	6.2 (28.7)	7.0 (32.0)	8.5 (25.4)	6.9
4	5.2 (37.3)	5.3 (39.0)	6.6 (35.9)	7.3 (35.9)	6.1
Mean	6.62	6.86	8.04	9.60	

(Figures in parentheses indicate the per cent decrease over control)

C.D. at 5 per cent

Metoxuron levels : 0.95
Stages : 0.85
Metoxuron levels x stages : 1.90

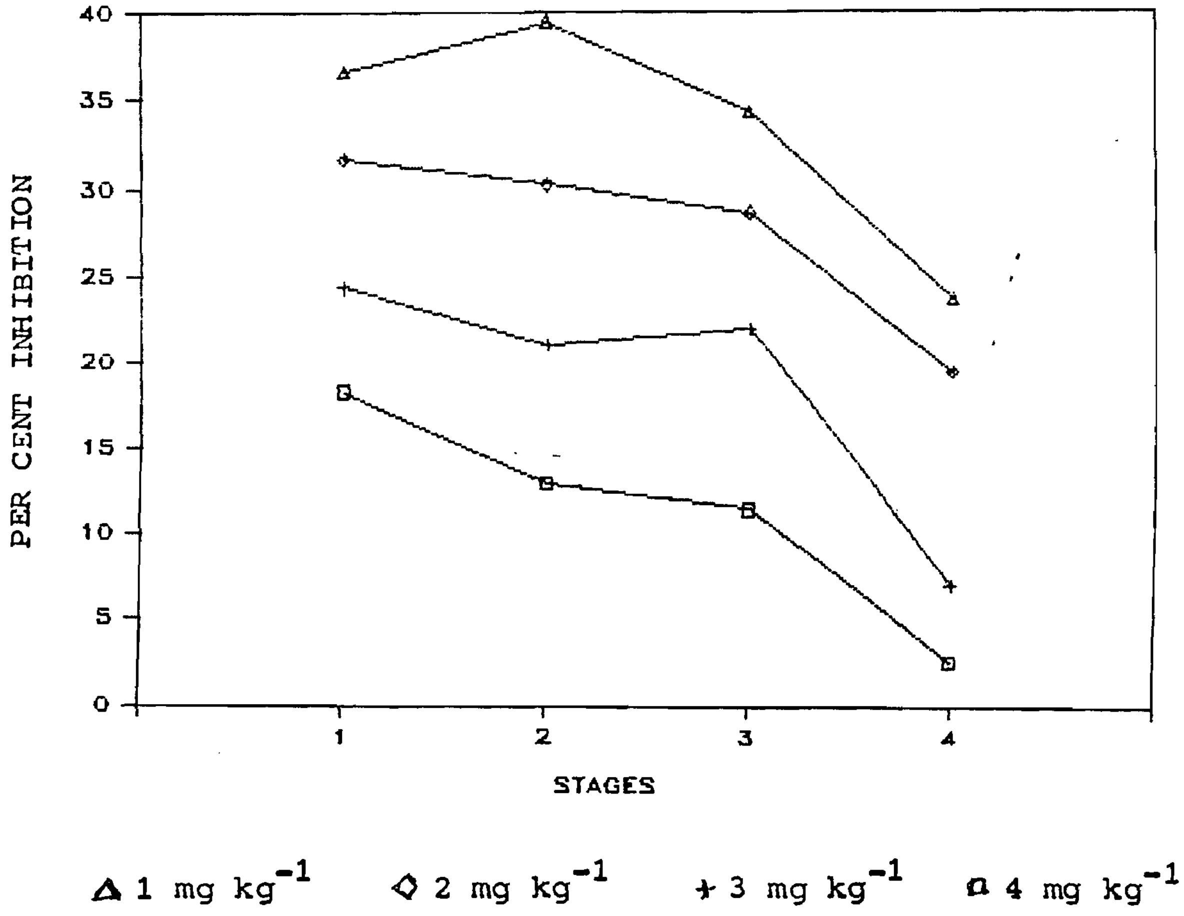


FIG.31 : EFFECT OF METOXURON ON SOIL UREASE ACTIVITY

may influence enzyme activity in soil. To test these an experiment was conducted to monitor the changes in urease activity with four different rainfed crop species viz., sorghum, sunflower, groundnut and redgram grown in sole and mixed cropping conditions and at different stages of crop growth.

4.10.1 Sole crops

The results obtained with regard to the effect of four principal crops of the area on soil urease activity when grown as sole crops are presented in Table 42. The crops were grown for two successive years.

Urease activity in the soils collected under four different crops varied with the crops grown. The enzyme activity was consistantly high in the soils covered with groundnut followed by sunflower, sorghum and redgram. However, a similar comparison between two varieties of sorghum did not show much differences with respect to urease.

Enzyme activity in the soil increased with plant age and the increase recorded at each stage of the crop was significantly higher over the other upto IVth stage (80 days after sowing). Though soil urease activity increased at later stages of the crop, the

Table 42: Influence of crop cover on soil urease activity (sole crops)

Treatment	Urease activity (μg of NH_4 released g^{-1} soil h^{-1})											
	Kharif 1986						Kharif 1987					
	Stages						Stages					
	I	II	III	IV	V	Mean	I	II	III	IV	V	Mean
T1 Sorghum var. CSH6	9.40	10.55	14.62	15.54	15.60	13.14	8.68	10.45	12.92	14.99	14.80	12.70
T2 Sorghum var. CSH9	8.78	10.34	13.72	14.88	15.34	12.61	8.27	10.90	12.03	13.87	14.06	11.82
T3 Groundnut var. PGN2	9.76	12.25	17.04	20.02	20.40	15.98	9.89	11.92	16.95	18.83	18.30	15.18
T4 Sunflower var. Ec68414	9.04	12.24	16.42	17.18	17.52	14.48	9.78	11.78	14.64	16.25	17.11	13.91
T5 Redgram var. C11	8.21	9.06	11.24	13.14	14.16	11.16	7.89	8.82	10.28	12.00	12.19	10.24
Mean	9.04	10.89	14.61	16.15	16.60		8.90	10.77	13.36	15.19	15.29	

C.D. at 5 per cent :
Treatments : 1.21
Stages : 0.87
Treatments x stages : 2.14

C.D. at 5 per cent :
Treatments : 1.45
Stages : 1.12
Treatments x stages : 2.86

differences were not significant. The increase ranged from 9.40 to 15.6 in sorghum (CSH 6), 8.78 to 15.34 in sorghum (CSH 9), 9.76 to 20.40 in groundnut, 9.04 to 17.50 in sunflower and 8.21 to 14.16 μg of NH_4^+ released g^{-1} soil h^{-1} in redgram. The increase in soil enzyme activity with the age of the crop varied with the plant species grown.

Similar trend of results was also obtained during *kharif* 87 with slightly lower values. Enzyme activity of the soils under different crop coverages during *kharif* 87 followed the same order as was observed during *kharif* 86 i.e., groundnut > sunflower > sorghum > redgram. The increases in enzyme activity with the age of the crop were 8.68 to 14.80 in sorghum (CSH 6), 8.27 to 14.06 for sorghum (CSH 9) 9.89 to 18.30 in groundnut, 9.78 to 17.11 in sunflower and 7.89 to 12.19 μg of NH_4^+ released g^{-1} soil h^{-1} in redgram.

4.10.2 Mixed crops

The pattern of soil urease activity when sorghum was grown as the base crop under mixed cropping with redgram, groundnut and sunflower are given in Table 43.

Comparison of enzyme activity in the pure and mixed stands revealed that in general the enzyme

Table 43 : Influence of crop cover on soil urease activity (mixed crops)

Treatment		Urease activity (μg of NH_4 released g^{-1} soil h^{-1})											
		Kharif 1986						Kharif 1987					
		Stages						Stages					
		I	II	III	IV	V	Mean	I	II	III	IV	V	Mean
T1 Sorghum CSH6 + Redgram	(1:1 row)	9.05	10.76	11.62	14.72	15.84	12.40	8.70	9.59	12.42	14.56	14.22	11.89
T2 Sorghum CSH9 + Redgram	(1:1 row)	8.98	9.24	12.02	14.68	16.54	12.29	8.28	9.68	12.92	14.47	14.67	12.00
T3 Sorghum + Redgram	(3:3 rows)	8.24	9.76	11.38	13.68	14.49	11.51	8.11	9.54	12.08	13.55	14.29	11.51
T4 Sorghum + Groundnut	(3:.3 rows)	10.48	14.92	20.46	23.24	23.44	18.51	10.20	14.71	20.25	21.94	21.69	17.76
T5 Sorghum + Sunflower	(3:3 rows)	9.68	12.89	15.56	18.38	19.56	15.21	9.26	11.78	15.60	18.00	18.49	14.62
T6 Sorghum + Redgram	(4:2 rows)	8.56	9.32	12.08	13.52	14.85	11.66	8.02	9.48	11.92	12.41	13.76	11.12
T7 Sorghum + Groundnut	(4:2 rows)	10.24	14.02	16.95	19.52	21.24	16.55	9.76	14.56	16.85	19.36	19.80	16.06
T8 Sorghum + Sunflower	(4:2 rows)	9.64	11.63	14.21	16.75	18.21	14.08	9.03	11.43	14.33	16.06	18.09	13.78
Mean		9.36	11.66	14.28	16.81	18.02		8.92	11.34	14.55	16.33	16.89	

C.D. at 5 per cent :		C.D. at 5 per cent :	
Treatments	: 1.64	Treatments	: 1.82
Stages	: 1.54	Stages	: 1.34
Treatments x stages	: 2.78	Treatments x stages	: 2.62

activity of the soil was more in the mixed crop cover with sorghum as base crop than that under the pure stand. Results obtained during both the years revealed that under mixed cropping, urease activity was more in the soils of sorghum grown with groundnut followed by sorghum with sunflower and sorghum with redgram. A similar comparison between two varieties of sorghum in combination with redgram grown at 1:1 ratio did not show much difference in the soil urease activity. When grown in different proportions, urease activity in the soils of mixed crops in 3:3 rows was slightly higher than with crops in the same combination with 4:2 rows (4 rows base crop and 2 rows mixed crop). The mean soil urease activity values recorded in the experiment conducted during *kharif* 86 were 11.51, 18.51 and 15.21 μg of NH_4^+ released g^{-1} soil h^{-1} when redgram, groundnut and sunflower, respectively were grown with sorghum as base crop in 3:3 ratio while the corresponding activity values were 11.66, 16.55 and 14.08 respectively when they were grown in 4:2 ratio. Similar trend of results was obtained during *kharif* 87.

Soil urease activity increased with the age of the crop in the field. The increase recorded at each stage of the crop was significantly high over the other till fourth stage (80 days after sowing), however, the small increase recorded thereafter was statistically non significant.

4.11 EFFECT OF LEVELS AND SOURCES OF SULPHUR ON SOIL UREASE ACTIVITY AT DIFFERENT STAGES OF GINGELLY GROWN ON A SANDY SOIL

The results of the experiment conducted for two successive years to study the effect of levels of sulphur in two different forms on soil urease activity in a sandy soil using gingelly as test crop are presented in the Table 44.

Experimental data of both the years showed that addition of sulphur to soil had no significant effect on soil urease activity. Though the soil urease activity was slightly more when gypsum was used as the source of sulphur over the elemental sulphur, the differences were not significant.

Soil urease activity increased with the age of the crop. The increase recorded was low during the initial stages but was significantly high at later stages of crop growth.

****

Table 44 : Effect of sulphur and gypsum on urease activity in a gingelly grown soil

Sulphur added (kg S ha^{-1})		Urease activity (μg of NH_4 released g^{-1} soil h^{-1})											
		Kharif 1986						Kharif 1987					
		Stages						Stages					
		I	II	III	IV	V	Mean	I	II	III	IV	V	Mean
T1	0	4.06	4.17	5.42	6.88	7.06	5.52	3.82	4.02	5.12	6.44	6.60	5.20
ELEMENTAL SULPHUR													
T2	10	3.66	4.08	5.66	6.61	7.10	5.42	3.54	3.80	4.64	6.12	6.40	4.90
T3	20	3.61	3.86	5.88	6.21	6.80	5.27	3.72	3.84	4.76	6.24	6.80	5.07
T4	30	3.53	3.58	5.42	6.33	6.40	5.05	3.44	3.67	4.60	6.30	6.72	4.95
T5	40	3.44	3.97	5.53	6.05	6.20	5.04	3.20	3.56	4.24	6.14	6.65	4.76
T6	50	3.10	3.90	5.67	6.30	6.50	5.09	3.08	3.48	4.30	6.38	6.24	4.70
GYPSUM													
T7	10	3.78	4.57	6.25	7.67	7.80	6.01	4.14	4.30	5.88	7.24	7.80	5.88
T8	20	3.43	4.11	6.00	7.50	7.90	5.79	4.08	4.45	5.76	6.98	7.60	5.77
T9	30	3.12	3.84	6.67	7.64	7.88	5.83	3.98	4.24	5.68	7.02	7.54	5.69
T10	40	3.07	3.68	6.20	7.05	7.23	5.45	4.12	4.34	5.24	6.86	7.36	5.58
T11	50	3.00	4.04	6.02	6.83	7.08	5.39	4.00	4.62	5.84	7.21	7.78	5.89
		3.44	3.98	5.88	6.82	7.08		3.74	4.03	5.09	6.63	7.04	

C.D. at 5 per cent :
Sulphur levels : N.S.
Stages : 0.88
Sulphur levels x stages : N.S.

C.D. at 5 per cent :
Sulphur levels : N.S.
Stages : 1.04
Sulphur levels x stages : N.S.

CHAPTER V

DISCUSSION

Soil urease and phosphatase are the most important enzymes as they are involved in the mineralization of the organically bound fractions of nitrogen and phosphorus respectively, which are not immediately and directly available to the plants. The activity of these enzymes are dependent on a number of soil and environmental factors like pH, temperature, moisture, ionic strength, nature and extent of clays and organic matter present in the soil. In addition to this, the level of micronutrient cations, presence of agricultural chemicals like herbicides, fertilizers like sulphur, stage of crop growth and nature of crop cover also affect the level of enzyme activity in soils. The results on some of these aspects obtained through experiments detailed in the earlier chapter are discussed here under appropriate heads.

5.1 ASSAY, LEVELS AND KINETICS OF SOIL UREASE AND PHOSPHOMONOESTERASES

As rightly opined by Burns (1978), many of the primary obstacles impeding advance in soil enzymology are associated with methodology because, as with any evolving scientific d scipline, these methods have yet to stabilize and throw up a cogent series of experimental techniques.

Unlike classical enzymology, soil enzyme methodology is beset with a number of constraints and compromises due to the very nature, heterogeneity and complexity of the system. Thus, a continuous updating and improving of existing methodologies is required added to these inherent problems, lack of appreciation, of the rigours of enzyme assay by some earlier workers in the field has made it difficult for meaningful comparisons to be made between the results of different workers. Hence, caution needs to be exercised in choosing proper assay procedures.

Though the first report on soil urease dates back to 1940 (Conrad), evaluation of proper assay precedures were not available till 1960. The methods reported for the assay of urease activity, involve the estimation of ammonium ion released on incubation of toluene-treated/untreated soil with buffered or non buffered urea solution (Mc Garity and Myers, 1967, Tabatabai and Bremner, 1972). Other methods involve the estimation of urea decomposed or the carbondioxide released on incubation of soil with urea (Douglas and Bremner, 1972). Some workers have also used buffers (Simpson, 1968, Zantua and Bremner, 1975). Of these the non buffer method described by Zantua and Bremner (1975) and the buffer method of Tabatabai and Bremner (1972) have been thoroughly evaluated. The use of buffers in the assay of urease has been debated on the basis of some results obtained. Although both methods gave precise and consistent values, the buffer method give markedly higher values than the non buffer method and detects urease activity that did not occur when soils were treated with urea in the absence of buffer. The non buffer method provides a very good index of the activity of soils to hydrolyse urea under natural conditions and is thus, of more practical relevance than the buffer method which measures the total potential of the soil for urea hydrolysis. Further, the composition of the buffer should also be carefully chosen. Thus, other buffers (eg. phosphate, acetate and universal) give lower results of urease activity in soils (Malcolm, 1983). THAM buffer, being widely used for the assay of urease, should be prepared using H_2SO_4 because of the results obtained by Wall and Laidler (1953) showing that buffers prepared by treatment of THAM with HCl had an activation effect on hydrolysis of urea by Jackbean urease.

A major impediment in the assay of soil enzymes is the elimination of enzyme production by growing populations of soil micro organisms and assimilation of reaction products during the assay, because "Abiontic enzymes" (Skujins, 1967) do not include the emzymes accumulated from growing micro organisms. Among the several methods suggested to overcome this constraint, addition of toluene was widely propagated as almost a standard procedure in soil enzyme assays (Burns, 1978). Serious questioning of the use of toluene in soil enzyme assays started when inconsistent and haphazard results started showing up. Thus, the activity of urease was seen to increase (Skujins and Mc Laren, 1969) or decrease by the addition of toluene (Thente, 1970). Similar results were also reported for soil phosphatase (Ladd, 1978). Thus, it was realised that toluene may itself act as a carbon source for some fungi and bacteria. Further, toluene could neither sterilize soil, nor in some treatments eliminate completely dehydrogenase or respiratory activities and thus there may remain the potential for energy generation and enzyme synthesis. Also, toluene may affect the activities of accumulated enzymes by modes of action which cannot be unequivocally interpreted. Thus, Tabatabai (1983) from a thorough evaluation of the role of toluene came to the conclusion that the use of toluene creates more problems than it solves and is better avoided. Hence, in our assay of soil urease and phosphomonoesterases, we preferred not to use toluene and resorted to the better method of using short incubation periods to overcome the difficulty of controlling the proliferation of microbial cells during the period of assay.

The importance of the choice and concentration of the substrate is another point which has been overlooked even by the prolific and seasoned workers like Tabatabai and his group. This was pointed out by Malcolm (1983). Substrate concentration determines the velocity of an enzyme reaction. The amount of substrate present in the assay is also important, especially when low (rate limiting) substrate concentrations are used because substrate depletion can occur quickly affecting the assay. The substrate concentrations used for enzyme assays should wherever possible be sufficient for the saturation of the enzyme, so that the kinetics in the standard assay approaches zero order. The relation between substrate concentration and velocity of the enzyme reaction is given by the Michaelis-Menten equation

$$V = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

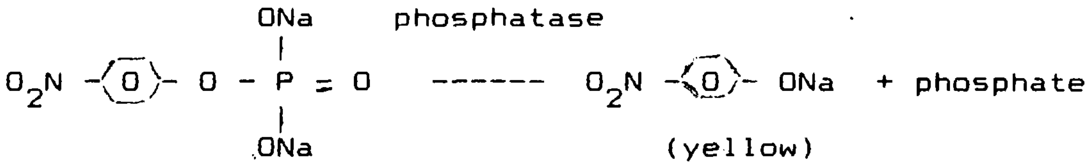
Where, V_{max} is the maximum velocity obtained when the substrate concentration is high enough to saturate the enzyme. K_m is the Michaelis constant for the enzyme and V is the velocity at substrate concentration S. When S >> K_m V ≈ V_{max} and is independent of substrate concentration and hence zero order kinetics applies.

The choice of incubation temperature is most important as it has a substantial effect on enzyme activity.

In the present study, precautions were taken to avoid all the pitfalls discussed above. The substrate concentration was maintained sufficiently high to ensure zero order kinetics through out the assay. The temperature was maintained at 37°C and the non buffer and no-toluene conditions were adopted. In addition to these as explained in the reults section, a modified indophenol blue method suggested by Dorich and Nelson (1983) was used for determining the concentration of NH_4^+ liberated during the urease assay. This method, which is a refinement of the earlier distillation method (Tabatabai and Bremner, 1972), could enhance the sensitivity of the assay by several fold. Thus, the estimable concentration of NH_4^+ could be brought down to 0.04 ug of N ml^{-1} as compared to the distillation method which corresponds to 70 ug ml^{-1} of 0.005 N acid. The end point detection is also indecisive with such low concentration of the acid. Further, this method could cut down the period of incubation to half an hour, thus eliminating the complications arising from proliferating micro organisms. In addition to this, there was the advantage of economy of time and chemicals and was particularly useful in carrying out large number of assays and also in the kinetic method for the determination of Michaelis-Menten constants. Errors due to the hydrolysis of urea during steam distillation can also be overcome by using this method.

Since it is imperative that any modified method should be properly evaluated before its adoption, the above method of urease assay was evaluated in twenty seven surface samples of soils of different physico-chemical properties. As seen from the results, excellent correlation was obtained between urease determined by the distillation method and indophenol method ($r = 0.99$). This confirms the validity of the method.

Unlike soil urease, which has a natural substrate in urea, phosphomonoesterases have to be assayed only with model substrates, purported to have a direct correlation with the actual activity of the enzyme in soil. This is because the organic phosphates, present in soil are complex and cannot be simulated. Among the several substrates suggested for phosphatase, phenyl phosphate (Kroll and Krammer, 1955), Naphthyl phosphate (Ramirez and Mc Laren, 1966) and 4-nitrophenyl phosphate (Tabatabai and Bremner, 1969) are most recent. 4-nitrophenyl phosphate was shown to be superior to others by Tabatabai and Bremner (1969) who evaluated it for the assay of acid and alkaline phosphatases. This consists of incubating the soil with 4-nitrophenyl phosphate in modified universal buffer at pH 6.5 for assay of acid phosphatase and at pH 11.0 for assay of alkaline phosphatase, adding aqueous $CaCl_2$ and NaOH to stop the reaction and estimating the liberated 4-nitrophenol colourimetrically. The reaction can be written as



The addition of NaOH is necessary to extract the 4-nitrophenol from the soil because 4-nitrophenol is adsorbed by soil under neutral or slightly acidic conditions (Pettit *et al.*, 1977). Saratchandra and Perrott (1984) recommended extraction of 4-nitrophenol with diethyl ether for highly organic soils. This was not necessary for any of the soils used in this study.

Just as in the assay of urease, phosphatase assay is also plagued with uncertainties when the buffer system is changed. Thus, it is found that phosphatase is inhibited when phosphate buffers are used (Abramyan and Galstyan, 1975). Geller and Ginzburg (1979) showed that borate and acetate buffers were inhibitory while tris buffer was stimulating. Fauvel and Rouquerol (1970), Tarafdar and Chhonkar (1978) used acetate buffer.

Since the capacity of a buffer to hold the pH of the reaction mixture against the buffering capacity of the soil is important, polybasic buffer system like the modified universal buffer which has a wide range of pKa values and is effective over a wide range of pH 3-11 is superior to others. Hence, we have chosen to use the modified universal buffer of Tabatabai and Bremner (1969).

Urease activity (μg of NH_4^+ released g^{-1} soil h^{-1}) of the surface soils collected from various parts of Andhra Pradesh varied from 3.8 to 26.5 with an average value of 10.5. The activity (μg of 4-nitrophenol released g^{-1} soil h^{-1}) of acid phosphatase determined using 4-nitrophenyl phosphate showed variation from 6.4 to 142.7 with an average of 34.8 while alkaline phosphatase varied from 10.3 to 155.2 with a mean of 67.1. The ranges of urease, acid and alkaline phosphatases found in our study fall in line with those reported by other Indian workers (Ananthanarayana and Mithyantha, 1970; Sahrawat, 1983; Beri and Brar, 1987; Tarafdar and Chhonkar, 1981). An approximate range of 0 to 113 μg of urea hydrolysed g^{-1} soil h^{-1} has been reported in soils of North America (Zantua *et al.*, 1977). There is no merit in comparing soil enzyme activities in soils from widely different locations, environments and ecology as these have a pronounced effect on the enzyme activity. Added to these are the uncertainties and non-uniformities in the assay procedures. For example, pretreatment of soil and storing (Burns, 1978) alter enzyme activity. Thus, soil enzyme levels are more useful when compared under more uniform conditions and extrapolative inferences need to be avoided.

Correlation coefficients of soil properties showed that urease and phosphomonoesterases correlated highly significantly with organic carbon and clay content of the soil. Soil urease also correlated significantly with pH and silt content of the soil while acid phosphatase correlated significantly with available phosphorus determined by Olsen's method. Similar results were also reported by a number of investigators (Mc Garity and Myers, 1967; Tabatabai, 1977 and Zantua *et al.*, 1977). Chhonkar and Tarafdar (1984) showed a significant positive correlation of phosphatases with soil organic carbon, organic phosphorus and bacterial population and a negative correlation with pH and no correlation with clay and actinomycetes. Multiple regression analysis of the data showed that most of the variation in analysis in urease activity observed in the soils studied could be accounted for, by organic matter content. This is in harmony with the conclusion from other studies that organic soil constituents contribute significantly to the protection of native soil enzymes.

The two kinetic constants of the enzymes i.e. maximum enzyme reaction velocity (V_{max}) and the Michaelis constant (K_m) of soil urease and phosphomonoesterases varied considerably within the five soils studied. V_{max} values varied from 0.04 mM to 0.43 mM of urea hydrolysed g^{-1} soil h^{-1} for urease, from 21.10 to 51.10 for acid phosphatase and from 19.00 to 85.67 µg of 4-nitrophenol released g^{-1} soil h^{-1} for alkaline phosphatase. The K_m values for the soil ranged from 0.45×10^{-3} to 3.00×10^{-3} M for urease, 0.68×10^{-3} to 1.42×10^{-3} M for acid phosphatase and 0.35×10^{-3} to 4.82×10^{-3} M for alkaline phosphatase.

Similar results were reported by Aradakani *et al.* (1975) for soil urease and Eivazi and Tabatabai (1977) and Tabatabai and Bremner (1971) for phosphomonoesterases.

K_m values of soil enzymes are higher than pure enzymes which suggest that the soil enzyme substrate complexes are formed to a much less degree than the pure enzymes. Several reasons have been putforth for this:

1. soil enzymes being immobilized may have more rigid conformational requirement than pure enzymes, and

2. the diffusion of the substrate to the active site may have some constraint although for a small substrate like urea, this is not limiting.

Irving and Cosgrove (1976) attributed the higher K_m value for soil acid phosphatase to the adsorption of the substrate 4-nitrophenyl phosphate to the soil. This could, in effect, lower the concentration of substrate in the solution and lead to an apparent increase in K_m value. Cervelli *et al* (1973) calculated K_m value for acid phosphatase making allowance for adsorption using Freundlich adsorption isotherm. These calculated values of K_m were similar to that of the pure enzyme. But such a possibility cannot be seen in the case of urease because urea is not highly adsorbed especially in soil with low organic matter. Soil enzymes *per se* are different from pure enzymes because of their genesis, immobility, stabilization, location, etc. It is thus, clear that soil enzymes, one is dealing with a system of enzymes widely varying in structure and conformations, etc. and hence too much of emphasis should not be given to compare them with pure enzymes, which act completely in homogenous systems. The main point of significance is that soil enzymes from widely varying sources seem to be comparable.

V_{max} consists of two factors, viz k_3, decomposition constant of an enzyme complex to products and the enzyme concentration $[E]$. Variation in either of these will manifest in a variation in V_{max}. It is more likely that soils of different origin and under different environmental conditions show wide variation in the amount of enzyme and hence $[E]$ is likely to be the most relevant factor to explain V_{max}. At the same time, variations in k_3 are also entirely possible because of differences in the soil enzyme *per se* which arise from differences in nature of the enzyme and its binding to soil constituents. This eventually will lead to difference in the binding property of the enzyme substrate complex and its dissociation.

5.2 EFFECT OF pH AND MOISTURE ON SOIL UREASE

The pH activity profile of soil urease showed that enzyme activity increased with an increase in pH from 3.0 to about 7.0, reached a plateau and then decreased sharply upto a pH 12.0. Similar results for soil urease were reported by a number of workers (Tabatabai and Bremner, 1972; May and Douglas, 1976 and Frankenberger and Johnson, 1982). There are two significant points to note regarding the effect of pH on soil urease: 1. the pH at which soil urease in general exhibits maximum activity is one or two pH units higher than that observed for urease from microbial and plant sources. For example, the pH optima of Jackbean urease varied from 6.5 to 7.0 (Fishbein, 1969). The upward trend in the pH stability of soil enzymes is attributed to the predominently negatively charged surface of the soil colloids which leads to a lowering of pH of about two units near their vicinity (Weetall and Suziki, 1975 and Theng, 1979). 2. the optimum pH of soil urease is found to vary with soils and locations. Thus there are small differences in the pH range over which soil urease exhibits maximum activity. This can best be explained by the inherent differences in the enzymes from different sources their location, mode of attachment of the enzyme to the soil constitutents and the charge on the enzyme and its binder. The effect of pH on soil urease is perfectly logical because enzymes are amphoteric globular proteins composed of many acidic and basic groups of amino acid side chains which impart varying charge densities at different pH (Wharton and Eisenthal, 1981).

When soil enzymes are exposed to extreme acid or alkaline conditions, the catalytic activity of the enzyme protein decreases probably because of the disruption of the overall three dimensional structure of the protein itself. Denaturation results when the ordered structure of a globular protein is altered into randomly non functional disordered arrangement of peptide chains. Exposure to high H^+ ion concentration or OH^- ion concentration tends to disrupt the ionic and hydrogen bonds needed to maintain the active conformation of the enzyme protein resulting in loss of biological activity.

Urease activity in the soils was not significantly affected when moisture level was varied from 20 to 100 per cent. Similar results were also reported by Gould *et al.* (1973) Zantua and Bremner (1977). Thus, moisture is not a constraint in well irrigated fields. Recently Sahrawat (1984) showed that urease activity increased with increase in moisture from air dried to field capacity and recorded no change with further increase in moisture. Dalal (1975) also reported similar effects. Thus, the effect of moisture on soil urease may be crucial in dryland agriculture where moisture is, more often, less than field capacity. Under these conditions the urease activity may be too low to have a substantial quantity of NH_4+ liberated from the mineralization of urea. At the same time this may also be exploited to one's advantage. Thus in summer months, in dryland agriculture, urea may be applied along with the seed without waiting for the first shower. Since the moisture content is low, most of the urea remains intact without being hydrolysed.

5.3 EFFECT OF TEMPERATURE ON SOIL UREASE AND PHOSPHOMONOESTERASES

Soil urease activity increased with increase in temperature from 20-70°C and then decreased with further increase in temperature in all the soils except in soil IV where the activity kept on increasing till 90°C (Tables 14 to 16). Similar results were also reported by Zantua and Bremner (1977) and by Sahrawat (1984) using non buffer and buffer methods, respectively. Results of this study differ from those recently reported for some Indian soils by Dash *et al.* (1981) who found that urease activity increased with increase in temperature from 17°C to 47°C and then decreased with increase in temperature upto 57°C. Pal and Chhonkar (1979) demonstrated that urease activity was unaffected by temperature between 40-70°C but completely inactivated at 80°C.

Temperature of inactivation of phosphomonoesterases showed that soil phosphatases like soil urease are extraordinarily stable to heat treatment. With increase in temperature, enzyme activities increased upto 70°C and then decreased with further increase in temperature except in soil I and III where alkaline phosphatase activity started decreasing after 60°C. Similar results were reported by Fauvel and Rouquerol (1970) while measuring soil alkaline phosphatase activity with incubation temperatures ranging from 30-100°C. The activity was maximum at 60°C and then decreased rapidly at 100°C but was almost as active as at 30°C.

As seen above significant differences have been reported in the relative tolerence of soil enzymes from different sources to thermal mechanism. This can be attributed to the variation in the nature and source of enzyme *per se* or to the manner they are localised in soil.

O'Toole and Morgan (1984) in their study demonstrated variation in thermal inactiviation with soils and attributed their results to the existence of a number of types of organic matter-urease complexes in soils each possessing distinct characteristics including thermal stability (Nanniperi *et al.*, 1978; Pettit *et* al., 1976) and also to the differences in pH and adsorptive properties of the soil.

Results of this study along with evidence reported by the authors mentioned, indicate that urease activity in soil is stable over a much higher temperature range as compared to pure enzymes from other sources. Enzymes in general are sensitive to temperature and are denatured beyond 50°C. For example urease (Jackbean) was completely deactivated at 70°C. The stability of soil enzymes to high temperatures shows that soil urease is protected from inactivation by some mechanism even at high temperatures. The greater stability of enzyme in soil can be attributed to its adsorption on clay colloids or complexing of urease by organic colloids which offer protection against heat denaturation (Pink *et al.*, 1954; Ambroz, 1966; Pettit *et al.*, 1976 and Zantua and Bremner. 1977).

The arguments forwarded in favour of the behaviour of soil urease with respect to temperature holds for soil phosphatase also. However, nonuniform incubation is a factor which may preclude just comparison of the data reported by other workers. Thus, longer time of incubation may lead to higher inactivation even at comparatively lower temperatures. Thus the absence of knowledge about the exact nature of the enzymes, their mode of stabilisation and added to this, the non uniformity of experimental conditions, make all arguments more of a possible conjecture.

Temperature coefficient (Q_{10}) values were calculated for urease and phosphomonoestarases in the temperature range of 20-70°C. These values depended on type of soil ranging from 1.46 to 2.06 for urease, 1.92 to 2.08 for acid phosphatase and 1.77 to 2.09 for alkaline phosphatase. The variation of these values in the soils may be due to the heterogeneity in the composition and state of enzyme. At temperatures above 60-70°C, the Q_{10} values are less than one indicating that temperature inactivation of the enzyme sets in at that temperature.

Energy of activation (E_a) calculated over the entire range of temperature i.e. 20-70°C varied considerably with soils ranging from 16.40 to 6.76 for urease, 14.00 to 13.00 for acid phosphatase and from 14.00 to 9.75 for alkaline phosphatase. Similar results were also reported by Khaziev (1975) and Gould *et al.* (1973).

Enthalpy of activation ($\Delta^{\neq}H$) of soil urease varied greatly with the soils, ranging from 6.3 to 13.8 kcal $mole^{-1}$. Phosphomonoesterases showed less variation i.e. 12.0 to 13.9 kcal mole-1 for acid phosphatase and 10.9 to 12.5 kcal mole^{-1} for alkaline phosphatase.

Entropy of activation ($\Delta^{\neq}S$) of the enzyme catalysed reactions did not show much differences in the soil at the given temperatures, but varied with the soils, ranging from -52.1 to -78.8 cal deg^{-1} mole^{-1} for soil urease, from -52.0 to 57.4 cal deg^{-1} mole^{-1} for acid phosphatase and from -54.2 to -60.0 cal deg^{-1} mole^{-1} for alkaline phosphatase. Similar results were also reported by Kumar and Wagenet (1984) but with slightly lower values.

Very little information is avilable on the activation parameters of soil enzymes in general. One point that has consistently emerged from soil enzyme kinetics is that the energy of activation/entropy of activation/Q_{10} are much smaller than uncatalysed chemical reactions. This is in conformity with the behaviour of enzymes in general. The only difference as discussed earlier, is that soil enzymes are active over a much higher range of temperature as compared to free enzymes. The thermodynamic activation parameters throw important light on the natue of the activated complex and can be of immense help in understanding the mechanism of reaction. The nagative values of the entropy of activation for enzyme catalysed reactions confirm that a different pathway is negotiated when enzymes are active. The consistent negative entropy of activation in all the soils and for all the three enzymes point to a highly ordered activated complex. This is entirely logical because enzymes require rigid spatial criteria to be fulfilled and can only act when the lock and key type compatibility is ensured between the enzyme and the substrate.

5.4 SOME INHIBITORY EFFECTS ON SOIL UREASE AND PHOSPHOMONOESTERASES

Urease and phosphomonoesterase activities decreased with the addition of micronutrient cations. The decreae in the activity was more with increasing concentration of cation but the rate of decrease narrowed down at higher levels. The decrease of the enzyme activity varied with the type of soil used and cation added to the soil. For example, the comparison of the per cent inhibition at highest concentration level of the four micronutrient cations showed the order soil IV > Soil V > Soil I > Soil III > Soil II for zinc and manganese while iron and copper followed the order Soil V > Soil IV > Soil III > Soil II > Soil I. Inhibition of phosphomonoesterases in the five soils did not conform to any regular pattern. Among the four cations used in this study inhibition by copper was more than the other three ions. Similar results were also reported by Shaw (1954), Juma and Tabatabai (1977), Tylor (1976) and Tabatabai (1977). The decrease in enzyme activity by metal ions may be due to the complexing of these metal ions with substrate or with the substrate-enzyme complex. The relative effectiveness of trace elements as inhibitors of enzyme activities varied with the soil, the concentration and form of the added element and the enzyme assayed. This may be attributed to the variation in physico-chemical properties of the soils used in the present investigations. Bremner and Douglas (1971) found that the amounts of Ag^+ and Hg^+ and other heavy metal ions needed to effect substantial inhibition of soil urease were much greater than the amounts needed for equivalent inhibition of Jackbean urease. They also found variations in the inhibitory effect of metallic cations. Similar results were also reported by Tabatabai (1977) who opined that metal ions inhibition was non competitive and postulated that the inhibition involved the reaction of metal ions with sulphahydryl groups in the catalytic site of urease. Hughes *et al.* (1969) reported that copper ion which forms a more stable sulphide is a considerably more powerful inhibitor than zinc ion.

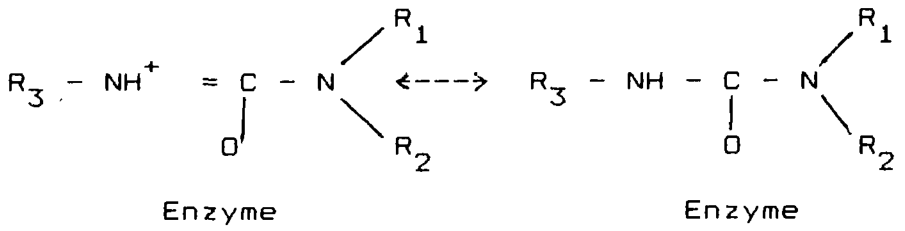
Studies on the effect of metal ions on soil biochemical activity are gaining importance in the use of sewage sludge and industrial effluents in agriculture. However, caution needs to be exercised in correlating the results because, studies performed with water soluble metallic salts may not permit to draw valid conclusions concerning the effects of heavy applications of sewage sludges containing water insoluble forms of heavy metals. Zantua and Bremner (1977) and Frankenberger *et al.* (1983) reported that urease activity in soils was not affected by addition of larger amounts of heavy metals in the form of sewage sludge. This may be due to the fact that most of the metal ions in sewage sludge remain insoluble and thus unavailable.

Clays, one of the major components of soil, shown to be correlated with enzyme activity in soils (Theng, 1979). In order to understand the manner in which they might interact with soil urease, the effect of the addition of practically pure samples of four different types of calys to Jackbeen urease was taken up. The results indicated that all the four clays reduced urease activity over a wide range of enzyme concentration from 1-8 mg of NH_4^+ released $hour^{-1}$. These results are in conformity with those observed by several other workers (Ross and Mc Neilly, 1972; and Dick and Tabatabai, 1987). Thus, it seems to be a general observation that the addition of clay minerals to homogenous enzyme solutions decreases their activity. The effect of the nature of clay on enzyme activity reveals that expansible clays like smectites inhibit enzyme activity to a greater extent than the non expansible clays like kaolinite and illite. Thus, Dick and Tabatabai (1987) reported that the per cent inhibition is more in montmorillonite followed by illite and kaolinite. The results of this study also followed the same sequence of inhibition except for some negligible variations at some concentrations. Given the experimental constraints, not much importance can be attached to these minor differences. The more important finding is that 2:1 type minerals consistently bring out greater inhibition of the enzyme than kaolinite, a 1:1 mineral. When the rate of decrease in enzyme activity was considered, except kaolinite, all the three clays showed a steep inhibition in enzyme activity when the clay to enzyme ratio was high. Similar results were observed by other workers also (Mortland and Gieseking, 1952; Kiss, 1958). Theng (1979) reviewed extensively the interaction of proteins and enzymes with clays. It is not possible to refer to all the cases individually except that the behaviour of urease seen in this study is in conformity with those reported for other enzymes.

As given in the results, the amount of enzyme corresponding to the initial activity minus the total enzyme activity at equilibrium is tentatively assumed to be the amount adsorbed and adsorption isotherms were drawn (Fig. 27). The isotherms in all the cases are essentially linear for most part except that a slight tendency for 'S' shape existed in the begining. Similar results were reported earlier by Chhonkar and Rao (1985) and Boyd and Mortland (1986). The activity of urease measured in the clay urease suspension and in the supernatant after the removal of the clay by centrifugation were similar within the experimental precision. This would mean 1. either all the enzyme that is adsorbed on clay is inactivated 2. or part of the enzyme in the supernatant might have suffered deactivation due to conformational changes. It is generally believed that change in protein conformation in solution does not take place; hence the first event is more likely. Although deactivation of enzymes adsorbed on clays is considered more probable, the reasons for this have not been properly understood. The earlier view, that there would be a drastic conformation changes due to adsorption is now discounted (Theng, 1979). Enzymes are mostly globular in nature organised into several levels of structural complexity. Hence, the deactiviation on adsorption can only be explained due to the masking/blocking of active groups involved in the complexing of the enzyme with the substrate. It is, however, clear, that enzymes in adsorbed state encounter drastic structural constraints as they have to express themselves from within a rigid frame work. This leads to the lowering of K_m and V_{max} of immobilised enzymes. Thus, the seeming inactivity of the adsorbed urease on clays might in most cases be atleast due to the inability to detect enzyme activity in the time span normally given to the assay of the pure enzyme in the supernatant. Further, as seen from the inhibition percentage, the degree of inhibition is lowered as more enzyme is loaded on the clay. Similar behaviour was also observed by Morgan and Corke (1975) for glucose-oxidase adsorbed on different clay minerals. At lower levels of uptake of enzyme, activity was remarkably suppressed where as, at higher levels of uptake, the specific activity increased until it approached that of the free enzyme. In this context, it is relevant to note that Boyd and Mortland (1986) observed that montmorillonite can adsorb 40 per cent of its weight of urease when it is rendered hydrophobic by exchange for cetyl, trimethyl ammonium cation. Similarly, an initial clay-enzyme base may facilitate expression of the subsequently adsorbed enzyme. This is an important concept because it suggests that the stabilization of soil enzymes may actually be due to the interaction with clay-organo sufaces. In addition to the other forces like adsorption, entrapment hydrophobic or any other form of attachment like covalent, ionic or hydrogen bonding with the soil organic polycondensate colloids.

The use of agrochemicals have become a standard practice in modern agriculture. Although the environmental pollution aspects of these chemicals have received immense interest in recent times, their effects on soil biochemical properties have barely been touched. Except one or two detailed reports (Cervelli *et al.*, 1975; Lethebridge and Burns, 1976) the others are more superficial. Hence it was considered important to initiate studies on this aspect. The effect of atrazine, simazine (S-triazine herbicides) and methabenzthiazuron and metoxuron (substituted urea herbicides) added to soil at 0-4 mg Kg^{-1} concentrations has been detailed in Section 4.9. As seen from Tables 38 to 41 consistent increase in urease activity was observed from 20-80 days but the increase was inhibited at all stages and at all concentrations of the herbicides compared to the control. The consolidated pattern of inhibition by the herbicide shows that the per cent inhibition was almost similar till 60 days but there was sharp decrease in inhibition at 80 days. Also increase in the herbicide concentration gave rise to increase in inhibition, with a steep fall from control to 1 ppm herbicide level followed by more or less constant rate of reduction in urease activity. Similar results have been reported by Balasubramanyan *et al.* (1970) for simazine, Karanth *et al.* (1975) for dexon and Voets *et al.* (1974) for atrazine and Cervelli *et al.* (1975) for substituted urea herbicides. Lethebridge and Burns (1976) reported time effect of phosphate pesticides on soils which are very similar to the results of our study. They found that, in contrast to soil urease, the Jackbean urease was totally inactivated in a very short time by these chemicals. Thus, the soil enzymes are much better protected against the inhibitory action of these agrochemicals as compared to urease from plant and microbial sources. This can best be accounted for by the stabilization of soil enzymes through their immobilization on colloids in soil. Lethebridge and Burns (1976) could show that part of the soil urease, which was extracellular, was deactiviated more quickly than the part which was an integral part of the soil humus complex.

The inhibition by herbicides and pesticides can be due to a direct effect of herbicide on soil urease activity due to competitive and non competitive inhibition. Cervelli *et al.* (1975) suggested such a complex formation between Jackbean urease and urea herbicides.



Ruggiro and Radegna (1985) also showed that inhibition of humus-laccase, extracted from soil by 2,4-D, was non competitive. Apart from these direct effects, indirect effects of these chemicals are also possible. Thus the tapering of inhibitory effect of soil urease with time and the recovery during the later stages may be due to different reasons. For example 1. the herbicide on the microbial population may get stabilized after some time. 2. the herbicides or pesticides themselves are adsorbed increasingly irreversibly on the soil colloid with increase in time and this may result in decreased inhibition. 3. the partial degradation of these herbicides with time in the soil is also another factor for decrease in the inhibition. The recovery from inhibition at later stages may also be due to enzymes secreted by the plant roots themselves. However, lot more data need to be generated before understanding and rationalising the effect of agrochemicals on soil enzymes. Whatever be the mechanism of action, it is necessary that the actual effects be documented in the interest of improving agronomic practices.

5.5 INFLUENCE OF CROP COVER ON SOIL UREASE

The effect of the nature of vegetation on soil polysaccharidases like amylase and cellulase was studied by several workers and has been reviewed ably by Kiss *et al.* (1978). Various factors like rhizosphere effect, age of vegetation, nature of present and past crops and weeds influence the level of polysaccharidases in soil. Since plants are atleast partial sources for most enzymes, it was thought relevant to initiate some preliminary investigation on the effect of a few crops on soil urease. The crops chosen and their effect on soil urease at various stages of growth have been given in Section 4.10. As discussed earlier the crops were all raised under identical fertilizer and moisture regimes. Significant differences in urease levels were observed depending on the nature of the crops grown. The soil enzyme activity was more for groundnut followed by sunflower, sorghum and redgram. Further, there was a steep increase in urease activity during earlier stages followed by a levelling off at later stages.

Reports on the effect of type of vegetation on soil urease activity are limited to only a few important references. The most important and thorough investigation was done by Pancholy and Rice (1973) who tested widely different vegetation types like tallgrass prairie, post oak-blackjack, oak forest and oak pine forest and suggested that the differences in enzyme activity were mainly due to the type of vegetation. Ross (1966) also conclusively showed that it is actually the type of vegetation that influences the soil urease activity.

The influence of the crop species on the levels of urease activity in the upper zones of the soils could be a reflection on the nature and amount of litter return and rate of decomposition which inturn would influence the size and composition of the microbial population (Hoult and Mc Garity, 1986).

In addition to this, different quantities of urease secreted by the plant roots themselves and also by micro organisms associated with that rhizosphere will also contribute to the soil enzyme activity. Thus, a large and complex number of factors are prevalent during any crop growth and one may not be able to seperate out these factors. It may also be relevant to mention here that Bolton *et al.* (1985) observed higher urease activity in a farm management system comprising of leguminous green manure rotation with winter wheat. In our experiment maximum urease activity was found under groundnut cover.

One experiment on mixed cropping was also conducted with sorghum as main crop and others as intercrops. Results were similar to those observed for sole crops with groundnut producing highest enzyme activity.

As seen from the results no significant differences in urease activity emanated at any stage of crop growth with gingelly when fertilized with sulphur in the form of elemental sulphur or gypsum. No earlier reference of this kind of work could be traced. However, the differences in urease levels triggered by nitrogen fertilization in the form of anhydrous ammonia applied to and winter wheat were reported by Bolton *et al.* (1985) had inspired us to check if similar results could be seen with different forms of sulphur fertilizers also. It is, however, too premature to draw any conclusions about this treatment. Extensive investigation over long periods and with different crops are warranted to draw specific conclusions.

The major conclusions that can be drawn from the present investigation are summarised below:

1. Urease activity in sixty four surface soils collected from various parts of Andhra Pradesh ranged from 3.8 to 26.5 with an average of 10.5 μg of NH_4^+ released g^{-1} soil h^{-1}. Acid phosphatase ranged from 6.4 to 142.7, with an average 34.8 while alkaline phosphatase varied from 10.3 to 155.2, with an average of 67.1 μg of 4-nitrophenol released g^{-1} soil h^{-1}. All these enzymes correlated highly significantly with organic carbon and clay content of the soil.

2. Assay of urease activity by modified indophenol blue method gave comparable results with that of distillation method.

3. The two fundamental kinetic constants viz V_{max} and K_m varied with the type of soil from 0.04 to 0.43 mM urea hydrolysed g^{-1} soil h^{-1} for soil urease, from 21.09 to 51.09 for acid phosphatase and from 19.00 to 85.67 μg of 4-nitrophenol released g^{-1} soil h^{-1} for alkaline phosphatase. Michaelis-Menten constant (K_m) also varied with the type of soil from 0.45 to

3.00x10-3 M for soil urease, 0.68 to 1.42x10-3M for acid phosphatase and 0.35 to 4.82×10^{-3} M for alkaline phosphatase.

4. Increase in temperature from 20 to 60-70°C increased urease and phosphomonoesterase activities in soil. The thermodynamic activation parameters (enthalphy $\Delta^{\neq}H$ and entropy $\Delta^{\neq}S$) of these enzymes varied with the type of soil.

5. The pH activity profile of soil urease increased with increase in pH from 3 to 7 observed a plateau for 2 or 3 units and then decreased with further increase in pH. The pH optima of soil urease was higher than that of pure enzyme.

6. Moisture level varying from 20-100 per cent did not show significant effect on soil urease activity.

7. Micronutrient cations viz zinc, manganese, iron and copper inhibited soil urease and phosphatase activities. Inhibition of the enzyme increased with concentration. The inhibition due to copper was more compared to

other cations. Inhibition of enzyme activity by these metal ions varied with the type of soil.

8. The addtion of clay minerals to homogenous enzyme solutions decreased their activity. The effect was more with the expandable clays than with kaolinite. The inhibition of enzyme activity was more when the clay to enzyme ratio was high. Clays saturated with transitional metal ions decreased enzyme activity more then that by saturated with divalent cations. The order of decrease with ion saturated clays was Zn > Cu > Co > Mg > Ca > Cs > Na > K > Li.

9. Addition of atrazine, simazine, methabenz-thiazuron and metoxuron urea decreased soil urease activity. The inhibition was persistent till about 60 days of crop growth.

10. Urease activity increased in the soil when four different crops were grown. The increase was more with groundnut followed by sunflower, sorghum and redgram. Enzyme activity also followed the same pattern in the mixed cropping system with sorghum as main and other three as mixed crops.

11. Sulphur added in the form of elemental sulpher and gypsum did not record any difference in soil urease activity.

SUMMARY

SUMMARY

Present research in soil enzymology has several facets. One seeks basic understanding of the activity and characteristics of the enzymes in the heterogenous soil matrix and their stabilization mechanism associated with the soil particulate matter. The other aspect is to determine their involvement in soil organic matter turn over and in plant nutrition. Other pertinent questions concern the interactions of abiontic enzymes with various metallic ions and agrochemicals. The influence of vegetation on enzymatic activities are inconclusive. The methodology of soil enzyme assays has often been accorded less attention than it deserves. For a better understanding of the role of soil enzymes in soil fertility and crop production, each of these components should be studied individually, its source determined, its state specified and importance interms of the total soil environmental system described.

Laboratory, pot and field experiments were conducted to study the effect of pH, moisture, temperature, metal ions, clay, sulphur and influence of plant cover on soil urease and phosphomonoesterases. The results obtained in the present investigation are summarised below:

1. Enzyme (urease and phosphomonoesterases) activities were assayed using standard conditions. Urease was assayed by modified indophenol blue method under natural conditions without adding buffer and toluene. Phosphomonoesterases activities were assayed using 4-nitrophenyl phosphate as substrate. Urease activity in sixty four surface samples widely varying in physico-chemical properties collected from various parts of Andhra Pradesh ranged from 3.8 to 26.5 with an average value of 10.5 ug of NH_4^+ released g^{-1} soil h^{-1}. Acid phosphatase activity (ug of 4-nitrophenol released g^{-1} soil h^{-1}) ranged from 6.4 to 142.7 with an average of 34.8 while alkaline phosphatase showed variation from 10.3 to 155.2 with an average of 67.1.

2. Statistical studies of the relationship between soil urease, phosphomonoesterases and other soil properties have shown that all the enzymes correlated highly significantly with organic carbon and clay content of the soil. Urease also show significant correlation with pH and silt content of the soil while acid phosphatase exhibited significant raltionship with available Olsen's phosphorus.

3. In order to characterise enzyme activities in soil, maximum enzyme reaction velocity (V_{max}) and Michaelis constant (K_m) were determined using Michaelis-

Menten equation similar to those determined in homogenous system.

V_{max} values varied with the type of soil from 0.04 to 0.43 mM of urea hydrolysed g^{-1} soil h^{-1} for soil urease, from 21.09 to 51.09 for acid phosphatase and from 19.00 to 85.67 ug of 4-nitrophenol released g^{-1} soil h^{-1} for alkaline phosphatase.

Michaelis constant (K_m) which indicates the affinity of the enzyme also varied with type of soil from 0.45 to 3.00 x 10^{-3} M for soil urease, from 0.68 to 1.42 x 10^{-3} M for acid phosphatase, and from 0.35 to 4.82 x 10^{-3} M for alkaline phosphatase.

4. The effect of temperature on enzyme activities was studied. Increase in temperature of incubation mixture from 20 to 60-70° C increased soil urease and phosphomonoesterase activities but decreased sharply with further increase in temperature. The temperature coefficient values (Q_{10}) calculated were less than one when the temperatures exceeded 70°C indicating the deactivation of the enzyme.

Energy of activation (Ea) varied considerably with the soils ranging from 16.40 to 6.76 for soil urease from 14.00 to 13.00 for acid phosphatase and from 14.00 to 9.75 for alkaline phosphatase, respectively.

Enthalpy of activation (H) of soil urease varied greatly with the soil urease ranging from 6.3 to 13.8 K cal mole^{-1}. Phosphomonoesterases showed less variations i.e., 12.1 to 13.9 for acid phosphatase and 10.9 to 12.6 K cal mole^{-1} for alkaline phosphatase.

Entropy of activation (S) was consistently negative indicating the highly ordered activated complex with the values ranging from -52.1 to -78.8 for soil urease, -52.1 to -57.4 for acid phosphatase and -54.5 to -60.8 cal deg^{-1} mole^{-1} for alkaline phosphatase.

5. Modified universal buffer with a pH range from 3-12 was used to study the effect of pH on soil urease activity. Urease activity increased with increase in pH from 3 to 7, reached a plateau for one or two units and again decreased sharply upto pH 12.0. The pH optima of soil urease is higher than that of pure enzyme.

6. Effect of moisture on urease activity was studied. Urease activity in the soils was not affected when moisture level was varied from 20-100 per cent.

7. Enzyme activity decreased with concentration of micronutrient cation. The decrease due to copper was more compared to other ions. The per cent inhibition

due to these metal ions also varied with the type of soil.

8. Enzyme activity with a concentration ranging from 1 to 8 mg h^{-1} was added to clay suspension containing 40 mg clay. All the four clays reduced urease activity when it was added to clays. Among the clays studied 2:1 type minerals caused more inhibition than kaolinite. When the rate of decrease in enzyme activity was considered, except kaolinite, the other three clays showed steep inhibition when the clay enzyme ratio was high. The isotherems drawn in all the clays were essentially linear for most part except that a slight tendency for 'S' shape in the beginning.

Clays saturated with different ions inhibited urease activity. The order of inhibition was Zn > Cu > Co > Mg > Ca > Cs > Na > K > Li.

9. The effect of herbicides viz., atrazine, simazine, methabenzthiazuron and metoxuron urea on soil urease activity was studied in a pot culture experiment with maize as test crop grown in black clayey soil. There was a consistent increase in enzyme activity from 20 to 80 days of crop growth but the increase was inhibited at all stages and at all concentrations of the herbicide. The inhibition was similar till 60 days with sharp decrease at 80 days of crop growth.

10. Enzyme activity was more in the soils planted to groundnut followed by sunflower, sorghum and redgram. There was a steep increase in urease activity during earlier stages of crop growth followed by a levelling off at later stages. One experiment on mixed cropping was also conducted with sorghum as main crop and others as intercrops. The results are similar to those observed for sole crops.

11. Urease activity in the soil did not show significant variation with either of the sulphur forms at any stage of the crop growth.

***

LITERATURE CITED

LITERATURE CITED

Abramyan S A and Galstyan A SH 1975 Effect of the nature of buffer solutions on the biological activity of soil enzymes. *Biologicheskei Zhurmal Armenu* 28; 25-31 Chemical Abstracts 8250, 1976.

*Ambroz Z 1966 Adsorption of two proteases occurring frequently in soil. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Ananthanarayana R and Mithyantha M S 1970 Urease activity of the soils of M R S Hebbal. Mysore Journal of Agricultural Science 4: 109-111.

Anderson P and Bengtsson M 1975 Effects of copper and zinc on urease and phosphatase activity of spruce needle. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Ardakani J R, Volz M G and Mc Laren A D 1975 Consecutive study state reactions of urea ammonium and nitrate nitrogen in soil. Canadian Journal of Soil Science 55: 83-91.

*Badr EL-DIN S M S, Moawad H, Mahamoud S A Z, Gamal R F and Enany M A 1985 Urease inhibition by metals and its relation to nitrogen transformation. *Leitschrift. fur Pflanzenernahrung und Batenkunde* 148(5): 551-558 Soils and Fertilizer Abstracts 1215,1986.

Balasubramanian A, Bagyaraj D J and Rangaswami G 1970 Studies on the influence of foliar application of chemicals on the microflora and enzyme activities in the rhizosphere. Plant and Soil 32: 198-206.

Bartholomew W V 1965 Mineralisation and immobilization of nitrogen in the decomposition of plant and animal residues. In Soil nitrogen (ed W V Bartholomew and F E Clark) American Society of Agronomy Inc, Madison, USA.

Baruah M and Mishra R R 1986 Effect of herbicide butachlor 2, 4-D and oxyfluorfen on enzyme activities and Co_2 evolution in submerged paddy field soil. Plant and Soil 96: 287-291.

Beri V and Brar S S 1987 Urease activity in subtropical alkaline soils of India. Soil Science 126: 330-335.

Bernhard K 1965 The dependence of microbial cellulase activity in the near rhizosphere and rhizoplane on species of cultivated and wild plants. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Bolton Jr H, Elliott L F, Papendick R I and Bezdicek D F 1985 Some microbial biomass and selected soil enzyme activities Effect of fertilization and cropping practices. Soil Biology and Biochemistry 17(3): 297-302.

Boyd S A and Mortland M M 1986 Selective effects of smectite-organic complexes on the activities of immobilized enzymes. Journal of Molecular Catalyst 34: 1-8.

Bremner J M and Douglas L A 1971 Decomposition of urea phosphate in soils. Soil Science Society of America Proceedings 35: 575-578.

Bremner J M and Mulvaney R L 1978 Urease activity in soils. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Burns R G 1977 Soil enzymology. Science Progress (Oxford) 64: 275-285.

Burns R G 1978 Enzyme activity in soil - Some theoritical and practival considerations. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Calero B J, Arcia F J and Chang 1984 Influence of atrazine and ametryne on the enzymatic activity of a plastic dark gleyish soil. *Ciencias de la Agriculture* 18: 115-121. Soils and Fertlizer Astracts 6958,1985.

*Cameron F K and Bell J M 1905 The mineral constituents of the soil solution USDA Bureau of Soils Bulletin 30 Washington D C. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Cerevelli S, Nannipieri P, Ceccanti B and Sequi P 1973 Mechaelis constants of soil acid phosphatase. Soil Biology and Biochemistry 5: 841-845.

Cerevelli S, Nannipieri P, Giovannini G and Perna A 1975 Jackbean urease inhibition by substituted ureas. Pestcide Biochemistry and Physiology 5: 221-225.

*Cerevelli S, Nannipieri P and Sequi P 1978 Interactions between agrochemicals and soil enzymes. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Chhonkar P K and Rao Y S S 1985 Adsorption of cellulase derived from Trichoderma reesi by Kaolinite and Bentonite. Journal Indian Society of Soil Science 33(2): 265-271.

Chhonkar P K and Tarafdar J C 1984 Accumulation of phosphatases in soils. Journal Indian Society of Soil Science 32(2): 266-272.

Conrad J P 1940 Catalytic activity causing the hydrolysis of urea in soil as influenced by several agronomic factors. Soil Science Society of America Proceedings 5: 238-241.

Conrad J P 1941 The catalytic activity causing the hydrolysis of urea in soils as influenced by several agronomic factors. Soil Science Society of America Proceedings 5: 238-241.

Conrad J P 1942 The occurrence and origin of urease like activities in soils. Soil Science 54: 367-380.

Conrad J P 1944 Some effects of developing alkalinitics and other factors upon urease-like activities in soils. Soil Science Society of America Proceedings 8: 171-174.

Conrad J P and Adams C N 1940 Retention by soils of the nitrogen of urea and some related phenomena. Journal American Society of Agronomy 32: 48-54.

Dalal R C 1975 Urease activity in some trinidad soils. Soil Biology and Biochemistry 7: 5-8.

Dalal R C 1982 Effect of plant growth and addition of plant residues on the phosphatase activity in soil. Plant and Soil 66(2): 265-269.

Dash M C, Mishra P C, Mohanty R K and Bhatt N 1981 Effects of specific conductance and temperature on urease activity in some Indian soils. Soil Biology and Biochemistry 13: 73-74.

Dick W A and Tabatabai M A 1978 Inorganic pyrophosphatase activity of soils. Soil Biology and Biochemistry 10: 59-65.

Dick W A and Tabatabai M A 1984 Kinetic parameters of phosphatases in soils and organic waste materials. Soil Science 137(1): 7-15.

Dick W A and Tabatabai M A 1987 Kinetics and activities of phosphatase - clay complexes. Soil Science 143(1): 5-15.

Doelman P and Haanstra L 1986 Short and long term effects of heavy metals on urease activity in soils. Biology and Fertility of Soils 2(4): 213-218.

Doran J W 1980 Soil microbial and biochemical associated with reduced tillage. Soil Science Society of America Journal 44: 765-771.

Dorich R A and Nelson D W 1983 Direct colorimetric measurement of Ammonium in potassium chloride extracts of soils. Soil Science Society of America Journal 47(4): 833-836.

Dormaar J F Johnston A and Smoliak S 1984 Seasonal changes in carbon content and dehydragenase phosphatase and urease activities in mixed prairie and fescua grassland horizons. Journal of Range Management 37(1): 31-35.

Douglas L A and Bremner J M 1971 A rapid method of evaluating different compounds as inhibitors of urease activity in soils. Soil Biology and Biochemistry 3: 309-315.

Dowd J E and Riggs D S 1965 A comparison of estimates of Michaelis-Menten Kinetic constants from various linear transformations. Journal of Biological Chemistry 240: 863-869.

*Durand G 1966 Ph.D. Thesis submitted to Univ Toulouse. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Eivazi F and Tabatabai M A 1977 Phosphatases in soils. Soil Biology and Biochemistry 9: 167-172.

*Fauvel B and Rouquerol T 1970 The phosphatase test considered as an index of soil activity and evolution. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Fishbein W N 1969 Urease catalysis III Stiochiometry Kinetics and inhibitory properties of a third substrate dihydroxyurea. Journal of Biological Chemistry 244: 1188-1193.

*Foster R C and Martin J K 1981 In soil Biochemistry (ed E A Paul and J N Ladd) Marcel Deller, New York.

Frankenberger W T Jr and Johanson J B 1982 Effect of pH on enzyme stability in soils. Soil Biology and Biochemistry 14: 433-437.

Frankenberger W T Jr, Johanson J B and Nelson C O 1983 Urease activity in sewage sludge - Amended soils. Soil Biology and Biochemistry 15(5): 543-549.

Frankenberger W T Jr and Tabatabai M A 1982 Amidase and urease activities in plants. Plant and Soil 64: 153-166.

*Gadkeria D 1984 Influence of the herbicide goltix on extra cellular urease and phosphatase in suspended soil. *Zentralblati for Mikrobiologic* 139: 415-424. Soils and Fertilizer Astracts 1333,1985.

*Galstyan A Sh 1965 Effect of temperature on activity of soil enzymes. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Geller I T and Ginzburg K Ye 1979 Determination of the phosphatase activity of various soil groups. Soviet Soil Science 11: 44-51. soils and fertilizer Abstracts 9983, 1980.

Goskowska K and Furezak J 1981 Biological activity of soil under monocultures with applied herbicides. Polish Journal of Soil Science 14 (2): 151-158.

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

Harrison A F 1983 Relationship between intensity of phophatase activity and physico-chemical properties in woodland soils. Soil Biology and Biochemistry 15(1): 93-99.

Harrison A F and Pearce T 1979 Seasonal variation of phosphatase activity in Woodland soils. Soil Biology and Biochemistry 11: 405-410.

*Hoffmann-Ostenhof O 1963 Enzyme inhibition by quinones. In Metabolic inhibitors (ed R M Hochster and J H Quastel) Academic Press, New York.

*Haunova T and Hubarova S 1984 Changes in the ability of dehydrogenase, invertase and urease in alluvial meadow soil and Smolritza caused by linuron and metobromuron. In (ed J Szegi) Soil Biology and conservation of the biosphere.

*Hoffmann E and Hoffmann G 1955 The enzyme system of our arable soils IV Amylase. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Hoffmann E and Hoffmann G 1966 Determination of soil biological activity with enzymatic methods. Advances in enzymology 28: 365-390.

Hoffmann E and Schmidt W 1953 The enzyme system of our arable soils II. Urease. Biochemistry Journal 324: 125-127.

Hoult E H and Mc Garity J W 1986 The measurement and distribution of urease activity in a pasture system. Plant and Soil 93: 359-366.

Hughes R B, Katz S A and Stubbins S E 1969 Inhibition of urease by metal ions. Enzymologia 36: 332-334.

Irving G C and Cosgrove D J 1976 The kinetics of soil acid phosphatase. Soil Biology and Biochemistry 8: 335-340.

Jackson M L 1956 Soil chemical analysis - An advanced course. University of Wisconsin, Madison, U S A.

Jackson M L 1967 Soil chemical analysis. Prentice Hall of India, New Delhi.

Juma N J and Tabatabai M A 1977 Effects of trace elements on phosphatase activity in soils. Soil Science Society of America Journal 41: 343-346.

Kaplan D L and Hartenstein R 1979 Problems with toluene and the determination of extracellular enzyme activity in soils. Soil Biology and Biochemistry 11: 335-338.

Karanth N G, Chitra K C and Vasantharajan V N 1975 Effect of fungicide dexon on the activities of some soil enzymes. Indian Journal of Experimental Biology 13: 52-54.

Khan S U 1970 Enzymatic activity in a grey wooded soil as influened by cropping systems and fertilizers. Soil Biology and Biochemistry 2: 137-139.

*Khaziev F Kh 1975 Thermodynamic characteristics of enzymic reactions in soil. *Biol Nauki* 10: 121-127. Chemistry Abstracts 42394,1976.

*Khaziev F Kh and Burangulova M N 1965 Activity of enzymes which dephosphorylate organic phosphorus compounds of soil. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Kishk F N, El-Eassawai T Abdel Ghafar S and Abou-Donia M B 1976 Hydrolysis of methylparathion in soils. Journal Agriculture and Food Chemistry 24: 305-307.

Kiss S 1958 Invertase activity in clay mineral - soil mixtures. Nature (London) 182: 203-204.

Kiss S, Dragan-Bularda M and Radulescu D 1975 Biological significance of enzymes accumulated in soil. Advances in Agronomy 27: 25-87.

*Kiss S, Dragan-Bularda M and Radulescu D 1978 Soil polysaccharidases Activity and Agricultural Importance. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Kiss S, Stefanic G and Dragan-Bulgarda M 1974 Soil enzymology in Romania II. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Koning J, Hasenbaumer J and Coppenrath E 1906 Several new properties of cultivated soils. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Koning J, Hasenbaumer J and Coppenrath E 1907 Relationship between properties of soil and the nutrient uptake by plants. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Kozlov K A, Kislitsina V and Markova Yu 1973 Enzymic activity and humus content in Eastern Siberian soils. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Kozlov K A and Mikhailova E N 1965 Dehydrogenase activity of some soils of Eastern Siberia. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Kroll L and Kramer M 1955 Effect of clay minerals on the enzyme activity soil phosphatase. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Kumar V and Wagenet R J 1984 Urease activity and kinetics of urea transformation in soils. Soil Science 137(4) : 263-269.

*Kuprevich V F 1958 Problems of soil enzymology. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Ladd J N 1978 Origin and range of enzymes in soil. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Ladd J N and Butler J H A 1969a Inhibitory effect of soil humic compounds on the proteolytic enzyme Pronase. Australian Journal of Soil Research 7: 241-251.

Ladd J N and Butler J H A 1969b Inhibition and stimulation of proteolytic enzyme activities by soil humic acids. Australian Journal of Soil Research 7: 253-261.

*Ladd J N and Butler J H A 1975 Humus-enzyme systems and synthetic organic polymer-enzyme analogs. In Soil Biochemistry (ed E A Paul and A Mc Laren) Marcel Deller, New York.

*Ladd J N and Jackson R B 1982 Biochemistry of ammonification. In Nitrogen in Agricultural Soils (ed F J Stevenson) Agronomy Monograph No 22 American Society of Agronomy, Madison, Wisconsin, U S A.

Lethbridge G and Burns R G 1976 Inhibition of soil urease by organophosphorus insecticides. Soil Biology and Biochemistry 8: 99-102.

Liao C F H and Raines S G 1985 Inhibition of soil urease activity by amido derivatives of phosphoric and thiophosphoric acids. Plant and Soil 85: 149-152.

Mahmoud S A Z and Attia R M 1970 Amylase adsorption in soils. United Arab Republic Journal of Soil Science 10: 143-153.

*Makboul H E and Ottow J C G 1984 Kinetic behaviour of alkaline phosphatase desorbed from different clay minerals. *Pflan and Boden* 147: 497-501. Soils and Fertilizer Abstracts 1331,1985.

Malcolm R E 1983 Assesment of phosphatase activity in soils. Soil Biology and Biochemistry 15(4): 403-408.

*Mamytov A M, Vovonov S I and Tynaevzh M 1974 Composition of phosphorus compounds and phosphatase activity of soils of the territory brought under cultivation in the Issyk-Kul Basin of Kirozia. *Dokl Vses Akad Sel'skokhoz Nauk* 3: 5-7 Chemistry Abstracts 144651,1975.

May P B and Douglas L A 1976 Assay for soil urease activity. Plant and Soil 45: 301-305.

Mayaudon J 1968 Stabilization biologique des proteines ^{14}C dansIc Soils In Isotopes and radiation in soil organic matter studies International Atomic Energy Agency, Vienna.

Mc Garity J W and Myers M G 1967 A survey of urease acticity in soils of northern New South Wales. Plant and Soil 27: 217-238.

*Mc Laren A D 1963 Enzyme activity in soils sterilized by ionizing radiation and some comments on micro-environments in nature. In Recent progress in Microbiology (ed N E Gibbons) University of Toronto Press, Toronto.

*Mc Laren A D and Packer E 1970 Some aspects of enzyme reactions in heterogeneous systems. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Miller R J Pinkham C Overman A R and Dumfor S W 1968 Temperature effects on the acid hydrolysis of glucose - 1 - phosphate and urease hydrolysis of urea. Biochemistry and Biophysics Acta 167: 607-609..

Morgan H W and Corke C T 1975 Adsorption desorption and activity of glucose oxidase on selected clay species. Canadian Journal of Microbiology 22: 684-693.

Mortland M M and Gieseking J E 1952 The influence of clay minerals on the enzymatic hydrolysis of organic phosphorus compounds. Soil Science Society of America Proceedings 16: 10-13.

*Mulvaney R L and Bremner J M 1981 Control of urea transformations in soils. In Soil Biochemistry (ed E A Paul and J N Ladd) Marcel Deller, New York.

Myers M G and Mc Garity J W 1968 The urease activity in profiles of five great soil groups from northern New South Wales. Plant and Soil 28: 25-37.

*Nannipieri P, Cervelli S and Perna A 1973 Enzyme activities in some Italian Soils. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Nannipieri P, Cervelli S and Sequi P 1978 Stability and kinetic properties of humus-urease complexes. Soil Biology and Biochemistry 10: 143-147.

Neal J L 1982 Abiontic enzymes in aretic soils influence of predominent vegetation upon phosphomonoesterases and sulphatase activity. Communication in Soil Science and Plant Analysis 13(10) : 863-878.

Nor Y M 1982 Soil urease activity and Kinetics. Soil Biology and Biochemistry 14: 63-66.

O'Toole P and Morgan M A 1984 Thermal stabilities of urease enzymes in Irish soils. Soil Biology and Biochemistry 16(5): 471-474.

Pal S and Chhonkar P K 1979 Thermal sensitivity and kinetic properties of soil urease. Journal Indian Society of Soil Science 27(1): 43-47.

Pancholy S K and Rice E L 1973 Soil enzymes in relation to old field succession: amylase cellulase invertage dehydrogenase and urease. Soil Science Society of America Proceedings 33: 897-901.

*Parks F P 1974 The sorption of *Escherichia coli* alkaline phosphatase by selected clay minerals. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Paulson K N and Kurtz L T 1970 Michaelis constant of soil urease. Soil Science Society of America Journal 34: 70-72.

Perucci P, Scarponi L and Businelli M 1984 Enzyme activities in a clay loam soil amended with various crop residues. Plant and Soil 81: 345-351.

*Pettit N M, Gregory L J, Freeman R B and Burns R G 1977 Differential stabilities of soil enzymes- Assay and properties of phosphatase and arylsulphatase. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Pettit N M, Smith A R J, Freedman R B and Burns R G 1976 Soil urease activity stability and kinetic properties. Soil Biology and Biochemistry 8: 479-484.

Pinck L A, Dyal R S and Allison F E 1954 Protein-Montmorillonite complexes, their preparation and the effects of soil microorganisms on their decomposition. Soil Science 78: 109-118.

Piper C S 1950 Soil and Plant Analysis. Inter Science Publishers Inc, New York.

Quastel J H 1946 Soil Metabolism. The Royal Institute of chemistry of Great Britain and Ireland, London.

*Rachinskiy V V and Pel'tser A S 1967 Effect of temperature on rate of decomposition of urea in soil. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Ramirez-Martinez J R and Mc Leren A D 1966 Determination of phosphatase activity by fluorimetric technique. Enzymologia 30: 243-253.

hards L A 1954 Diagnosis and improvement of saline and alkaline soils. USDA Hand Book No 60.

jers H V, Pearson R W and Pierre W H 1941 Absorption of organic phosphorus by corn and tomato plants and the mineralising action of exoenzyme systems of growing roots. Soil Science Society of America Proceedings 5: 285-291.

gers H V, Pearson R W and Pierre W H 1942 The source and phosphatase activity of exoenzyme systems of corn and tomato roots. Soil Science 54: 353-366.

oss D J 1965 Effect of air-dry refrigerated and frozen storage on activities of enzymes hydrolysing sucrose and starch in soils. Journal of Soil Science 16: 86-94.

oss D J 1966 A survey of activities of enzymes hydrolysing sucrose and starch in soils under pasture. Journal of Soil Science 17: 1-15.

oss D J and Mc Neilly B A 1972 Some influences of different soils and clay minerals on the activity of glucose oxidase. Soil Biology and Biochemistry 4: 9-18.

Rotini O T 1933 *La Presenza e l'attivita delle Pirofosfatasi in alcuni substrati organici e nel terreno. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.*

uggiero P and Radegna V M 1985 Inhibition of soil Humus-Laccase complexes by some phenoxyacetic and S-Triazine herbicides. Soil Biology and Bichemistry 17: 309-312.

Rysavy P and Macura J 1972 The formation of B-galactosidase in soil. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Sahrawat K L 1983 Relationship between soil urease activity and other properties of some tropical wetland rice soils. Fertilizer Research 4(2): 145-150. →

Sahrawat K L 1984 Effects of temperature and moisture on urease activity in semi-arid tropical soils Plant and Soil. 78: 401-408.

Saksena V P and Kalyan Singh 1984 Effects of pesticides on urea nitrification. Indian Journal of Agricultural Chemistry 17(1): 53-57.

*Sanikidze G S, Gogorikidze N I and Shoniya N K 1973 Enzymatic activity of basic soil types of the subtropical zones of the western Georgian. Chemistry Abstracts 114397,1974.

Sarathchandra S U and Perrott K W 1984 Assay of B-glucose activity in soils. Soil Science 138:17-19.

*Segel I L 1975 In enzyme kinetics (ed W A Dick and M A Tabatabai) Wiley, New York.

Shaw W H R 1954 The inhibition of urease by various metal ions. Journal of American Chemical Society 76: 2160-2163.

Simpson J R 1968 Losses of urea nitrogen from the surface of pasture soils. Tans 9th International Congress of Soil Science. Adelaide 2: 459-466.

*Skujins J J 1967 Enzymes in soil. In Soil Biochemistry (ed A D Laren and G H Peterson) Marcel Deller Inc, New York.

*Skujins J J 1976 Extracellular enzymes in soil. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Skujins J J 1978 History of abiontic soil enzymes research. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Skujins J J and Mc Laren A D 1967 Enzyme reaction rates at limited water activities. Science. 158: 1569-1570.

Skujins J J and Mc Laren A D 1969 Assay of urease activity using ^{14}C-urea in stored geologically preserved and in irradiated soils. Soil Biology and Biochemistry 1: 89-99.

Skujins J J, Braal L and Mc Laren A D 1962 Characterization of phosphatase in a terrestrial soil sterilized with an electron beam. Enzymologia 25: 125-133.

*Simonart P and Mayaudon J 1961 *Humification des proteines ^{14}C dans Ie soil. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.*

**Speir T W 1977 Studies on a climosequence of soils in tussock grasslands II Urease phosphatase and sulphatase activities of top soils and their relationships with other properties includig plant available sulphur. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.*

Speir T W, Lee R, Pansier E A and Cairns A 1980 A comparison of sulfatase, urease and protease activity in planted and in fallow soils. Soil Biology and Biochemistry 12: 281-291.

**Speir T W and Ross D J 1978 Soil phosphatase and sulphatase. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.*

Tabatabai M A 1973 Michaelis constants of urease in soils and soil fractions. Soil Science Society of America Proceedings 37: 707-710.

Tabatabai M A 1977 Effects of trace elements on urease activity in soils. Soil Biology and Biochemistry 9: 9-13.

Tabatabai M A and Bremner J M 1969 Use of P-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biology and Biochemistry 1: 301-307.

Tabatabai M A and Bremner J M 1970 Factors affecting soil arylsulfatase activity. Soil Science Society of America Proceedings 34: 427-429.

Tabatabai M A and Bremner J M 1971 Michaelis constants of soil enzymes. Soil Biology and Biochemistry 3: 317-323.

Tabatabai M A and Bremner J M 1972 Assay of urease activity in soils. Soil Biology and Biochemistry 4: 479-487.

Tabatabai M A and Singh B B 1976 Rhodanese activity of soils. Soil Science Society of America Journal 40: 381-385.

Tabatabai M A and Singh B B 1979 Kinetic parameters of the rhodanese reaction in soils. Soil Biology and Biochemistry 11: 9-12.

*Tarafdar J C and Chhonkar P K 1978 Status of phosphatases in the root-soil interface of leguminous and nonleguminous crops. *Zeitschrift fur Pflanzeneraehr Bodenkunde* 141: 347-351.

Tarafdar J C and Roy A B 1981 Urease and phosphatase in the rhizosphere of different mesta genotypes. Journal of Indian Society of Soil Science 31: 469-472.

Theng B K G 1979 Formation and properties of claypolymer complexes. Elsevier Publishers. Amsterdam.

*Thente B 1970 Effects of toluene and high energy radiation on urease activity in soil. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Thornton J I and Mc Laren A D 1975 Enzymic characterisation of soil evidence. Journal of Forensic Science 20: 674-692.

Tyler G 1976 Heavy metal pollution phosphatase activity and mineralization of organic phosphorus in forest soils. Soil Biology and Biochemistry 8: 327-332.

Verma L, Martin J F and Haider H 1975 Decomposition of carbon-14 labelled proteins, peptides and aminoacids free and complexed with Humic Polymers. Soil Science Society of America Journal 39: 279-284.

*Verstraete W and Voets J P 1974 Impact in sugarbeet crops of some important pesticides treatment systems on the microbial and enzymatic constitution of the soil. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Vlasyuk P A, Dobrotvorskaya K M and Gordienko S A 1957 The intensity of the acidity of enzymes in the rhizosphere of individual crops. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Voets J P, Meerschman P and Verstraete W 1974 Soil microbiological and biochemical effects on long-term atrazine application. Soil Biology and Biochemistry 6: 149-152.

Vogel A I 1978 A text book of quantitative inorganic analysis. Longman Inc, New York.

*Wall M C and Laidler K J 1953 The molecular kinetics of the urea-urease system IV The reaction in an inert buffer. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Wang C H Tseng Y I and Puh Y S 1966 A study on the behaviour of urea in Taiwan soils. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Weetall H H and Suzuki S 1975 Immobilised enzyme technology research and applications. Plenum Press, New York.

*Wood A F 1899 The destruction of chlorophyll by oxidising enzymes. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Zantua M I and Bremner J M 1975 Comparison of methods of assaying urease activity in soils. Soil Biology and Biochemistry 7: 291-295.

*Zantua M I and Bremner J M 1977 Factors affecting urease activity in soils. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

Zantua M I Dumenil L C and Bremner J M 1977 Relationships between soil urease activity and other soil properties. Soil Science Society of America Journal 41: 350-352.

*Zubets I P 1973 Residual action of simazine and atrazine on the microflora and enzyme activity in Sod-Podzolic soil. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

*Zvyagintsev D G and Velikanov L L 1968 Influence of soils and clay minerals on the activity of glucose oxidase and invertase. In Soil enzymes (ed R G Burns) Academic Press Inc, New York.

* Original not seen