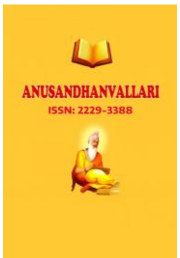

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Lipase Enzyme activity in Oryza sativa, Zea mays, Gossipium herbacium, Arachis hypogea soil strata of four agro ecosystems

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Abstract

The present study analysis the distribution of Lipase enzyme activity and microbial populations across different soil strata (A1, A2, B1, and B2 soil horizons) in four distinct agro-ecosystems: *Oryza sativa* (Paddy), *Zea mays* (Maize), *Gossipium herbacium* (Cotton), and *Arachis hypogea* (Groundnut). Soil samples were analyzed for physicochemical properties, microbial population, and enzymatic activity using spectrophotometric method. Results indicated that soil p^H and organic matter gradually decreased with depth, while electrical conductivity peaked at the B1 horizon. Bacterial and fungal populations were most abundant in the surface A horizons, showing a positive correlation with organic carbon. Interestingly, Lipase activity was found to be maximum in the B1 horizon (0.2–0.5 units), with *Zea mays* exhibiting the highest enzymatic potential. Activity became negligible below the B1 strata. These findings suggest that soil depth and crop-specific root exudates significantly influence the biochemical profile of agricultural soils, highlighting the need for organic amendments to maintain fertility in deeper soil layers.

Keywords: Lipase, Soil Horizons, Agro-ecosystems, Microbial Biomass, *Zea mays*, Soil Fertility.

Introduction

Microbial lipases are preferred for industrial use due to their high stability across wide pH and temperature ranges, rapid production, and cost-effectiveness. Common microbial sources include fungi such as *Aspergillus niger* and *Rhizopus* species, as well as bacteria like *Bacillus* species. These enzymes are also important components of the soil microbiome and are often found in high concentrations in lipid-contaminated environments, such as oil-polluted soils and dairy waste sites. In bioremediation, lipolytic microorganisms such as *Pseudomonas* and *Bacillus* species are used to degrade fats, oils, and grease in industrial waste. This process improves soil quality and aids in the breakdown of environmental pollutants. Soil lipases typically show optimal activity in neutral to alkaline pH ranges (7.0–11.0) and moderate temperatures (30°C–40°C), although some adapted species can function under extreme conditions. In plants, lipases play essential roles in metabolism and development. They are particularly important during seed germination, where they hydrolyze stored triglycerides in seeds, grains, and fruits to provide energy for early seedling growth. Plant-derived lipases, such as those from *Carica papaya* (papaya latex), sunflower, and castor seeds, have been studied for applications in food and pharmaceutical industries. Additionally, lipase production in plants can be induced by wounding or pathogen attack, contributing to defense mechanisms. Lipases are also involved in metabolic changes during fruit ripening, including chlorophyll degradation.

Material and Methods

Surface horizons are classified as 'A' horizons depending on their organic content in highly eroded areas where surface soil materials have been removed. Horizons that have accumulated constituents translocated from the horizon above are called 'B' horizons. Plants grow in A1 horizon 0 – 9 cm, A2 horizon 9- 25 cm, B1 horizon 25-39 cm, B2 horizon 39 -52 cm. Parent materials (Brady, 1990; Brady and Weil, 2000).

The present study was conducted with a primary objective of determining the influence of soil profile on microbial biomass and activities of some enzyme in crop fields these are belongs to Angiosperms site 1- Oryza sativa (family:Poaceae) common name: Paddy, Monocot, site 2 - Zea mays (family:Poaceae) common name: Corn Monocot, site 3 -Gossipium herbacium (family:Malvaceae) common name: Cotton, Dicot and site 4 - Arachis hypogea (family: Fabaceae) common name: Groundnut, Dicot cropping systems. Further, the relationship between microbial activities and relevant soil properties in different depths was also examined. The influence of different horizons in four agro-ecosystems showed considerable changes in the properties and enzyme activity. Certain important physical properties and quantities of accumulated enzymes in soil profiles were measured.

Preparation of Soil Enzyme : The soil solutions (1:5 soil and water) were made and filtered through Whatmann No. 42 filter paper and clear solution was used as soil enzyme extract. pH of the soil samples were recorded with the help of pH meter. Organic Matter Walkey and Black's wet digestion method.

Lipase (Sch tz et al., 1970) : Principle: Lipase hydrolyses triglycerides to release free fatty acids and glycerol. Triglycerides + H_2O Glycerol + Fatty acids. The quantity of fatty acids released in unit time is measured by the quantity of Sodium Hydroxide required to maintain pH constant. The milli equivalent of alkali consumed is taken as a measure of the enzyme. Reagents : 0.1M Tris Hydrogen Chloric Acid buffer (pH 7.5). Substrate solution: 10 seeds of ground nut macerated in pestle and mortar by adding of 25 ml of Distilled Water and the contents heated for 10 minutes and filtered. This filtered solution was taken as substrate solution. Acitic Acid (anhydrate) in 100 ml Distilled Water. Sodium Hydroxide (0.1 M), phenolphthalein indicator. Ten ml of soil enzyme and 4.5 ml Tris Hydro Chloric Acid buffer (pH 7.5) were taken in a clean conical flask and shaken well for 10 minutes. After, the above solution was added with 5 ml substrate solution and 1 ml of 0.1M Acitic Acid. Kept the contents for 45 minutes incubation at room temperature. After,2 to 3 drops of phenolphthalein indicator was added and titrated against 0.1 M Sodium Hydroxide till solution turns to pink colour. Blank assay was exercised same as above but with distilled water instead of soil enzyme.

Microbial Enumeration : Microorganisms viz, fungi 10^5, bacteria 10^6 and actinomycetes 10^6 colonies were isolated and enumerated by following standard serial dilution plate technique. Asthana and Hawker's medium for Fungi 10^5. Nutrient Agar medium for Bacteria 10^6 .

Results and Discussion

The present study was conducted with a primary objective of determining the influence of soil profile on microbial biomass and activities of some enzymes in the soils of paddy, corn, cotton and groundnut cropping systems. Further, the relationship between microbial activities and relevant soil properties in different depths was also examined. The influence of different horizons in four agro-ecosystems showed considerable changes in the properties and enzyme activities and presented in figures 1 to 5.

Certain important physical properties and quantities of accumulated enzymes in soil profiles were measured. Schutz et al (1970), Singer & Munns (1999), Sinsabaugh et al (2009), Page & Guillet (1991). The pH gradually reduced to acidic side as the soil depth is increased. In all the agro-ecosystems 'A,' and 'A_2' horizons are with pH 6 but in 'B,' of site II and site III agro ecosystem -pH was 5.5. In general the pH was around 5.0 in B_2. In all the soils of four agro ecosystems the pH was always 6.0 in A and A2 horizon. Nannipieri et al (2012), Page & Guillet (1991), Rajamannar & Krishnamurthy (1978). The conductivity of the soil in various depths also showed substantial fluctuations. The conductivity values increased from ' A_1 horizon to B_1 horizon. The value in different sites are as follows: Highest value recorded in Cotton at site III – 0.77 , the lowest value recorded in Paddy at site I - 0.25 soils in A1 horizon. Highest value recorded in Cotton at site III – 1.44 , the lowest value recorded in Paddy at site I- 0.36 soils in A2 horizon. Maximum conductivity values were observed in B_1 deg horizon. They are: Cotton site III - 1.44, lowest value recorded in Paddy site I - 0.74. Highest value recorded in B_2 horizon Cotton site III - 0.72 and the lowest value recorded in Paddy site I - 0.32. The conductivity values increased in concentration upto B_1 horizon. Bartoli et al (1996), Brady & Weil (2000), Burns et al (2013). Maximum organic matter percentage was noticed in A_1 A_2 and B_1 horizons. Highest value recorded in Paddy site I – 2.99% and the lowest value recorded in Corn site II – 2.32% in A_1 horizon. Highest value recorded in Groundnut site IV – 3.10% and the lowest value recorded in Corn site II – 2.84% in A_2 horizon. Highest value recorded in Groundnut site IV – 3.10% in B_1 horizon. Highest value recorded in two crops Cotton site3, and Paddy site I - 2.06% and the lowest value recorded in Groundnut site IV – 1.70% .The organic carbon was correlated positively and significantly in all soils of different horizons and different agro ecosystems. A gradual decrease in organic matter was noticed with the increase in the depth of the soil. Maximum organic matter percentage was noticed in A_1 A_2 and B1 horizons.

The fungal and bacteria population varied considerably with soil profile and type of agro ecosystem-. The bacterial densities were high (61 * 10^6) in site at horizon. The maximum fungal populations were recorded in Cotton site 3-35 and lowest population were recorded in site II – 16 colonies in A1 horizon. The maximum fungal populations were recorded in Cotton site III – 54 colonies and lowest population were recorded in Paddy site I – 21 colonies in A2 horizon. The maximum fungal populations were recorded in Cotton site III – 42 colonies and lowest population were recorded in Paddy site I – 32 colonies in B1 horizon. The maximum fungal populations were recorded in Groundnut site IV – 26 colonies and lowest population were recorded in Corn site II – 18 colonies in B2 horizon. The average bacterial and fungal populations were almost similar in B, horizon. Maximum bacterial densities were observed in A, and A, horizons Almost same averages were observed for S, and S, agro-ecosystems in A. A and B, horizons (S,46, S, 45). In A, horizon the average bacterial populations were Corn at site II - 45, Groundnut at site IV – 61 colonies in A1

figure 1:Conductivity µmho/cm $^{-1}$ in Paddy, Corn, Cotton and Groundnut in soils

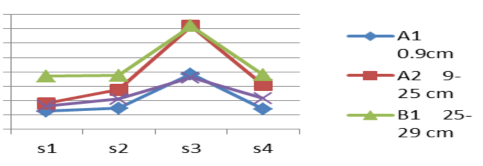
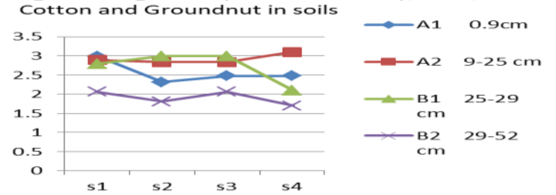
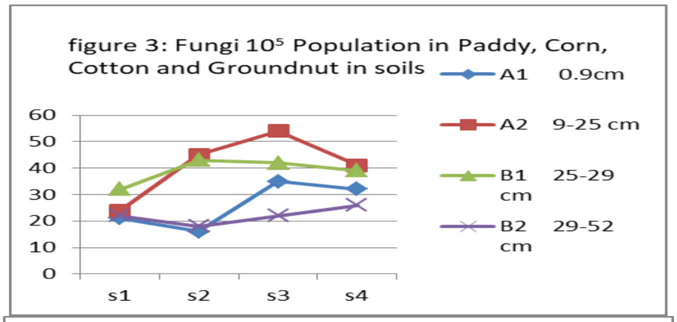
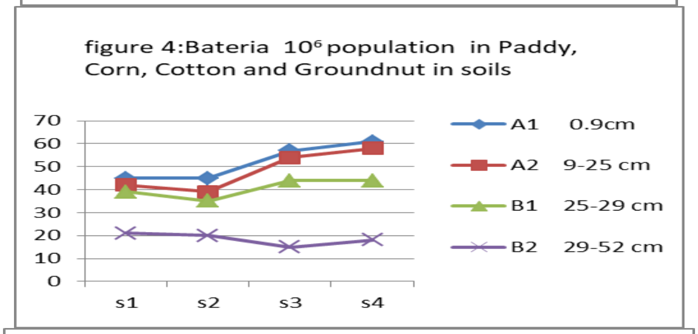
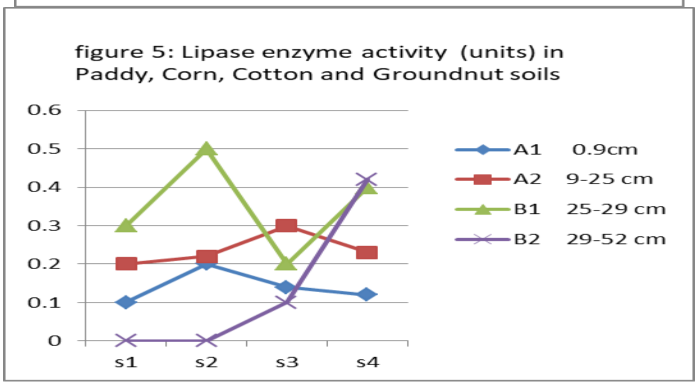


figure 2:Organic Population in Paddy, Corn, Cotton and Groundnut in soils









horizon. The highest recorded value in Ground nut at site IV – 60, lowest recorded value in Corn at site II – 46 colonies in A2 horizon. The same values are recorded in A1 and A2 horizons. The highest values recorded in Paddy at site I – 46 and the lowest value recorded in Corn at site II – 35 colonies in B1 horizon. The highest values recorded in Paddy at site I – 21 and lowest value recorded in at site III – 16 in colonies in B2.

Lipase enzyme was witnessed only upto horizon and afterwards the presence was nil. Highest value recorded in Corn site II – 0.20 units and lowest value recorded in Cotton site III – 0.14 units in A1 horizon. Highest value recorded in Cotton site III – 0.30 units and lowest value recorded in Paddy site I – 0.21 units in A2 horizon. Maximum lipase was noticed in B_1 horizon. Corn site II - 0.5 units, minimum value in Cotton site III - 0.2 units . In B2 horizon lipase enzyme value recorded in Cotton site III - 0.1 unit. No enzyme values were recorded in Paddy site I, Corn site II, Ground nut site IV in B2 horizon.

The above results are in agreement with the finding of Chatterjee and Yadav (1970) who studied soil profile with special reference to chemical characteristics in Deoria district. They observed that electrical conductivity. organic-carbon percentage. pH were decreased with depth Rajamannar and Krishna Murthy (1978) also reported decrease in organic carbon in the profile soil samples with the depth in the forest soils. On the other hand, profile soils of four agro ecosystems in the present study showed low pH, low Electrical Conductivity, low organic carbon and low microbial populations in depth layers of B, which is due to exploitation of soil for various crops and suggests for application of organic manures to improve the soil fertility.

Chitteborough (1992). Sharma et al. (2003) noticed various physico-chemical, geochemical and biochemical changes in soil profile under different ecosystems. A unanimity was observed that the activities decline significantly with increased depth of horizon. The average bacterial and fungal populations were very high in A, and A_2 (surface and subsurface layers) horizons. It is indicated that the availability of high C : N ratio in that area and fungi and bacteria grew better and generally more microbial populations were observed at neutral pH. It was also noticed that the fungal species Aspergillus niger was dominant and acclimatized to edaphic conditions in 'B3' and 'C' horizons (Rai et al., 1970; Page and Guillet, 1991; Hammermeister, 1996). The colour of the surface and subsurface soils of four groecosystems of A1, A2 and B, is different to that of B_2. The physical properties of four ecosystems such as color, combination with clay minerals, buffer action and organic carbon percentage were also in a positive way for more proliferation of microbial colonies. When pH decreased the cation exchange capacity of the soil is generally lowered, as a result pH dependent charged sites under acid conditions and ion exchange also lowered. So, organic carbon and microorganisms effected by this lowered ion exchange and this inturn effected the enzyme activities. Catt (1986), Tate (1987), Wang et al. (1989), Johnson (1990), Bartoli et al. (1996) also observed the decreased trends of enzyme activities in B3 and C horizons and related them to pH dependent ion exchanges.

Jung (1970), Ross and McNelly (1973) and Zhon et al. (1981) noticed substantial variations in biochemical and enzymatic potential of soils taken from different horizons at different times in different ecosystems.

Review of Literature

Soil enzymes play a crucial role in regulating biochemical processes in soil ecosystems. They are primarily produced by microorganisms, plant roots, and soil fauna and are responsible for the decomposition of organic matter and nutrient cycling. Soil enzyme activities are often used as indicators of soil fertility and microbial activity. **Singer and Munns (1999)** described soil as a dynamic biogeochemical system composed of minerals, organic matter, water, air, and living organisms. They emphasized that soil microorganisms play a significant role in maintaining soil productivity through the decomposition of organic materials and the release of nutrients required for plant growth. **Brady and Weil (2000)** reported that soil properties such as pH, organic matter, and electrical conductivity influence microbial populations and enzyme activities in soil profiles. They also noted that organic matter and microbial activity generally decrease with increasing soil depth. **Burns et al. (2013)** reviewed the ecological significance of soil enzymes and reported that enzyme activities vary depending on soil depth, vegetation type, and environmental conditions. Their study highlighted that enzyme activity is generally higher in surface soils due to higher organic matter and microbial populations. Studies by **Sinsabaugh et al. (2009)** emphasized the role of microbial enzymes in nutrient acquisition and organic matter decomposition. They reported that enzyme production is influenced by substrate availability and environmental factors such as soil moisture, temperature, and pH. **Turner and Haygarth (2014)** investigated enzyme activities in agricultural soils and found that soil management practices such as crop rotation and organic amendments significantly affect microbial activity and enzyme production. **Wallace and Terry (2018)** reported that lipolytic microorganisms in soil play an important role in the degradation of lipids and organic wastes. They noted that microbial lipases contribute to soil fertility by participating in the decomposition of complex organic compounds. Recent studies have also highlighted the importance of soil enzymes as indicators of soil quality in agricultural ecosystems. **Liu et al. (2020)** reported that soil enzyme activities are strongly correlated with soil organic carbon, microbial biomass, and agricultural management practices. Overall, previous research indicates that soil enzyme activity is closely linked to microbial populations, soil physicochemical properties, and soil depth. Surface soil layers generally exhibit higher enzyme activity due to higher organic matter content and microbial abundance. However, information on lipase enzyme activity in different soil horizons of specific agro-ecosystems remains limited, which justifies the need for the present study.

Conclusion

The present investigation demonstrated that soil profile characteristics strongly influence microbial populations and lipase enzyme activity in different agro-ecosystems. Surface soil layers contained higher organic matter and microbial biomass, which contributed to enhanced enzymatic activity. Lipase activity was detected mainly up to the B1 horizon and decreased significantly in deeper soil layers. Among the cropping systems studied, the maize ecosystem showed relatively higher lipase activity compared to rice, cotton, and groundnut soils. The reduction in enzyme activity with depth may be attributed to lower organic matter content, reduced microbial population, and changes in soil physicochemical properties. These findings highlight the importance of maintaining soil fertility through the addition of organic manures and sustainable agricultural practices to enhance microbial activity and enzyme-mediated nutrient cycling in soils. The study provides useful insights into the ecological role of soil enzymes in agro-ecosystems and their relationship with soil profile characteristics.

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