DEVELOPMENT AND EVALUATION OF BIOCOMPOSTING TECHNOLOGY FOR VALUE ADDED BIOMANURE PRODUCTION FOR ECO-AGRICULTURE

Thesis submitted in part fulfillment of the requirement for the
Degree of **Master of Environmental Sciences**
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CERTIFICATE

This is to certify that the thesis entitled "**DEVELOPMENT AND EVALUATION OF BIOCOMPOSTING TECHNOLOGY FOR VALUE ADDED BIOMANURE PRODUCTION FOR ECO-AGRICULTURE" s**ubmitted in part fulfillment of the requirements for the degree of **Master of Science in Environmental Sciences** to the Tamil Nadu Agricultural University, Coimbatore is a record of bonafide research work carried out by **S. AKILA** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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ABSTRACT

DEVELOPMENT AND EVALUATION OF BIOCOMPOSTING TECHNOLOGY FOR VALUE ADDED COIR PITH BIOMANURE PRODUCTION FOR ECO-AGRICULTURE

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India has huge biomass of crop residues like sugarcane trash, bagasse, coir pith, farmland waste etc. The overloading of these wastes leads to environmental pollution. Recently composting emerges as the most widely replicable process of handling diverse wastes. Decomposition of organic matter into usable compost depends on the abilities of the microflora to produce and excrete specific degradative enzymes. As microorganisms are important sources of enzymes, the activity of these enzymes correlates with microbial activity. Therefore, an attempt was made to study the microbial load and its enzyme activities during composting of organic wastes.

For composting experiments coir pith was used as substrate. Yeast sludge and *Pleurotus* sp. were used as microbial inoculum cum nutrient source. Since earthworms are found to be the efficient degraders of organic wastes, vermicomposting was also carried out using the earthworms, *Perionyx excavatus.*. Periodical samples were drawn and subjected to quality characterization.

Among the treatments, the treatment involving coir pith with earthworm exhibited marked differences with respect to C/N ratio and increase in macronutrients N, P, K

followed by the treatment involving combined inoculation of coir pith with yeast sludge and *Pleurotus* sp.

The microbial population and their activity at different stages of composting were assessed. The bacterial population was markedly dominant during the composting process. The mesophilic bacteria remained essentially constant whereas the thermophilic population increased during the thermophilic period. The mesophilic fungi increased at the initial stage and decreased during thermophilic and in the later stage of composting. The thermophilic fungi remained stable during the entire period of decomposition. The population of actinomycetes increased during the composting process and obtained maximum on 30^{th} day.

Owing to the nature of the material, enzyme activites largely reflect the diversity of microbial population. The enzyme activities, which are responsible for the recycling of nutrients such as dehydrogenase, urease, phosphates, cellulase and amylase, were found to be increased during the composting process. In general highest enzymes activities were recorded on 30^{th} day of composting. In the treatment involving coir pith with earthworms showed remarkably higher microbial population and the enzyme activities followed by coir pith with combination of yeast sludge and *Pleurotus* sp.

Hence, the findings of the present study clearly indicated the role of microbes and enzyme activities as indicators of composting process that will be highly helpful to monitor the changes and thereby improve the efficiency of the process.

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Chapter - I

INTRODUCTION

The use of pesticide and mineral fertilizers does not necessarily lead to better farming than the use of natural and organic methods in agriculture. There is a need to encourage more productive, environment friendly farming practices. Organic farming techniques are good for farm productivity and possibly better for environment and development. The use of compost have been time-tested production inputs for improving the fertility and productivity of soil because composts are excellent source of humus and plant nutrients (Biswas *et al.,* 1977; Hesse and Mishra, 1982).

In the entire area of waste recycling, composting emerges as the most widely replicable process of handling diverse wastes (Tandon, 1995). The composting process is an essentially aerobic decomposition of complex organic substances by microorganisms. It is believed to involve the action of enzymes accumulating outside the microbial cell as well as intracellularly catalyzed biochemical transformations.

Decomposition of organic matter to a usable compost depends on the abilities of microflora to produce and excrete specific degradative enzymes (Zibiliske,1998).

Most of the organic wastes and crop residues contain population of indigenous microorganisms *viz.,* bacteria, fungi and actinomycetes that can contribute significantly to the composting process. Mesophilic bacteria, fungi and actinomycetes initiate the composting process (at temperature range 20^{o}C to 40^{o}C) which rapidly breakdown the soluble and readily degradable compounds. As the temperature raises thermophiles (temperature above 45^{o}C) takes over and breakdown the proteins, fats and complex carbohydrates like cellulose and hemicellulose. As the supply of these high-energy compounds becomes exhausted, the mesophilic microorganisms once again take over for the final phase of maturation of remaining organic matter (Gaur, 1999).

As microorganisms are important sources of enzymes, the activities of these enzymes correlate with microbial activity. Enzymatic activity has a significant correlation with organic matter. Dalal (1975) found significant correlations between urease activity and organic carbon. The enzyme activities largely reflect the diversity of microbial population. It help organisms in their effects to satisfy their nutritional needs and in their function of degrading and humifying organic materials (Stevenson, 1982).

Dehydrogenase, urease and phosphatase are recognized as very important enzymes involved during composting process. The hydrolytic enzymes that mediate cellulose degradation are known as cellulases. Through the action of cellulase important energy sources for microorganisms are released. Hence, cellulase activity is an indicator of biomass turn over (Tabatabai, 1973). Dehydrogenase has been widely used to measure catabolic activities, which is correlated with microbial activities. The phosphatase enzyme plays an important role in the use of alternative P sources and considered as a general index of microbial activity in compost.

Vermicomposting, employing earthworms for the conversion of organic matter into a more humified material is one of the most efficient processes of waste treatment (Tapiadov, 1981). The earthworm acts as carriers of beneficial microorganisms like nitrogen fixers, phosphorus solubilizers, phosphorus transforming fungi etc.

Presence of earthworm resulted in increased oxygen uptake and further stimulated the activities of enzymes such as cellulase, amylase, urease and phosphatase. Hydrolytic enzymes such as dehydrogenase, amylase and phosphatase were higher in worm casts than in soil (Businelli *et al.,* 1984).

Coir pith, an important by product from coir industry having more moisture retention property (500-600%) and higher potassium content could be exploited for agricultural use. Its annual production is about 1.39 million tones in India and Karnataka alone produces about

140 to 150 thousand tones. Coir pith is a very stable product because of the presence of high percentage of lignin. Hence it takes long time to decompose and posed environmental hazards and disposal problem. Therefore utilization of this waste for production of value added manure would solve the problem of its disposal.

However, the role of free enzymes in composting has been the subject of limited number of report. By considering the importance of the enzymes as a source of microorganisms, an attempt was made with the following objectives.

- Isolation and identification of microbes associated with different stages of composting.
- To assess the changes in microbial load at different stages of composting of coir wastes.
- Assessing the changes in enzyme activities at different phases of composting.
- To identify the waste specific inoculum and optimization of conditions for enhanced decomposition.
- Assessing the changes in microbial load and enzymes during vermicomposting of coir waste.

Chapter-II

REVIEW OF LITERATURE

India has huge bio-mass crop residues like sugarcane trash, bagasse, coir pith waste, cotton waste, farm land waste, industrial wastes etc. The annual production of residues by principal crops is 270 million tones, which can supply 5.6 million tones of $N+P_2O_5+K_2O$. India ranks third in the production of coconut with an annual production of 100.43 million nuts from an area of 1.5 million ha. The coir pith is a waste product in the industry which is released after extracting the coir from husk (Palaniappan and Annadurai, 1999). The coir wastes accumulate in large quantities near the location of coir industries and its disposal is posing serious environmental problems.

Coir industry is a one of the important traditional cottage industries in India, which is over one and half century old. The first factory in India was setup during the preindependence period in Alleppey by a European called James Dhara of Irish origin. It was assessed that in India, 7.5 million tones of coir pith was produced annually (Kamaraj, 1994). The quantity of coir waste available in Tamil Nadu was estimated as 3.26 lakh tones (Santhi and Selvakumari, 2000).

Coir pith with a range of interesting properties finds various applications. It has a lignin (31%) and cellulose (27%) content and carbon-nitrogen ratio of 104:1(Shekar, 1999). It is a very stable product because of the presence of high percentage of lignin. Hence coir pith left to it, takes more time to decompose. Further the coir pith will decompose in the soil only very slowly, as its pentosan lignin ratio is less than 0.5, which is the minimum, required for the slow decomposition of organic matter in the soil (Thampan, 1987).

Palaniappan and Annadurai (1999) opined that the coir pith after composting can be effectively used as manure for both dryland and irrigated crops to increase the crop yield, as an amendment in sodic soils and as an alternative to potash fertilizer. According to Sankaran

et al. (2000) composting of coir pith reduced its bulkiness and converted plant nutrients to the available form.

2.1. Composting

Composting is the biochemical degradation of organic matter by microorganisms. It was believed to involve the action of enzyme accumulating outside the microbial cell as well as intracellularly catalyzed biochemical transformations.

Gaur (1982) defined composting as a controlled microbiological conversion process leading to decomposition of organic materials or wastes to a stable humus form with the evolution of carbon dioxide, production of water and energy.

Composting are to stabilize the putrescible organic matter in raw agricultural/ industrial wastes to reduce offensive odours, to kill weed seeds and pathogenic organisms and finally to produce a uniform slow release organic fertilizer which stimulates soil life, improves soil structure and helps plants to tolerate/ resist pests and diseases (Zibiliske, 1998 and Palaniappan and Annadurai, 1999).

Composting is a very flexible process and can be practiced in many different ways, but a particular practice should be suited to the particular waste management circumstances (Finstein, 1993).

2.2. History of composting

Composting is an ancient technology. There were Roman and biblical references to composting as well as numerous accounts of farmer composting practices in subsequent millennia (Rynk, 1992). George Washington, the united nation's first president, was also the nation's first recognized composter (Arner, 1995). He was acutely aware of the degradative effects of farming on the soil resource, and he built a "dung respository" to make compost from animal manure so he could replenish the soil's organic matter. Sir Albert Howard was

probably the first agricultural scientist to bring a scientific approach to composting almost 75 years ago in India (Howard, 1943).

Composting is considered as one of the oldest biotechnological solid waste disposal methods known to man. The oldest existing reference on composting dates back to the Akkadian Empire of the Mesopotamian valley a thousand years before Moses. In India, the use of dung as manure appears to have been practiced since the Rigvedic age (2500-1500 BC) (Gaur, 1982). Hutchinson and Richards initiated the scientific study of composting in 1921 at Rothamsted (Gaur, 1982).

The anaerobic or 'Bangalore' method of composting was developed by Acharya (1939) who used night soil and refuse for the composting. Waksman and Cordon (1939a and b) carried out basic research on aerobic decomposition of vegetable residues and animal wastes.

2.3. Methods of composting

2.3.1. Indore method

Pioneering work on composting and standardized conditions for degradation of leaves, straw and town refuse, utilizing dung and night soil as starters for promoting microbial activity especially aerobes have been done at indore in India (Howard and Wad, 1931)

2.3.2. Bangalore process

The method for composting of town refuse and night soil in pit was developed by Acharya (1939). The heat fermentation method of manure production, more popularly known as the 'Bangalore Process' solving satisfactorily one of the most difficult problems of sanitary disposal of these offensive wastes and yielding a high quality organic manure.

2.3.3. Synthetic compost

Inorganic nitrogen compounds like ammonium sulphate and urea were utilized equally and effectively for decomposition of carbonaceous materials into compost. The Adco process of preparation of synthetic compost was developed by Hutchinson and Richards (1921).

2.3.4. Leaf compost

Leaf composting can be achieved by heap or ditch method or by windrow method and the finished compost can be used as covering material to supply a heavy inoculum of microorganisms (Gaur and Sadasivam, 1993).

2.4. Physico-chemical and biological changes during composting

2.4.1. Bioconversion of organic materials

During the composting process the conversion of organic matter was carried out by different groups of heterotrophic microorganisms like bacteria, fungi, actinomycetes and protozoa. These microorganisms derive their energy and carbon requirement from the carbonaceous materials. For every ten parts of carbon, one part of nitrogen was assimilated for building up cell protoplasm (Gaur, 1982). The lowering of C/N ratio was brought about the fact that two-thirds of the carbon consumed was given off as CO_2 while the other one-third was combined with nitrogen in the living cells (Golueke, 1991).

2.4.2. Odour and colour

Van der Hoeck and Oosthoeck (1985) reported that during the first stages of bio-oxidaton phase, the unpleasant odour emitted from composting heaps decreased and practically disappeared by the end of composting process. Haider(1992) reported that gradual darkening or melanisation of the material takes place and the final product was found to be dark brown colour during the end of composting.

2.4.3. Changes in temperature during composting

The multiplication of microorganisms in the composting heaps retains the heat of exothermic biochemical reactions leading to an increase in the temperature. The temperature in the middle of the pile goes up to 55 to 70^{o}C after which it was gradually cools to ambient values. The temperature variation will depend upon the type of material being composted as well as probably the size of the heap (Gaur, 1982). The temperature comes down to ambient at the end of composting.

2.4.4. Microbial changes during composting

During the course of composting, both qualitative and quantitative changes occur in the active microflora. When composting begins, the mesophilic flora (at temperature range 20^{o} to 40^{o}C) predominates and was responsible for most of the metabolic activity that occurs. During the initial stages of the decomposition, readily available substrates such as proteins, sugars and starch were rapidly oxidized and the internal temperature rises rapidly to high levels (Zibiliske, 1998). As the temperature increases above 40^{o}C thermophilic bacteria, actinomycetes and fungi replace these organisms. Spore forming bacteria and fungi reappear as the temperature falls towards the final stages (Golueke, 1991). When the temperature falls below 60^{o}C, the actinomycetes attack the long chain polysaccharides i.e., hemicellulose and cellulose. Later the mesophilic strains of microorganisms reinvade.

2.5. Factors affecting composting

2.5.1. Nature of the substrate

Wastes with high content of organic matter are particularly suited, such as from domestic refuse, sewage sludge, leaf litter, grass hedge trimmings, manure from stables, market wastes and agricultural surplus (Adolf, 1990).

2.5.2. **Size of the substrate materials**

Particle size has a major effect on the rate of decomposition. The decomposition of wood and bark saw dusts (Allison, 1965) and of cereal straw (Sadasivam *et al*., 1981) was found to be affected by particle size.

2.5.3. Moisture content

To achieve the optimal decomposition rate, the water content of the compost material should be between 50 and 60% by wet weight. If anaerobic composting was to be followed the maximum moisture level was not important (Gaur *et al*., 1982).

Moisture content varies greatly with different starting materials and with the predominance of the type of microbial population. Maximum requirements exist during the initial decomposition stage when bacteria are dominant. If the moisture content is lower than 35 to 40 percent during this stage, microbial activity was inhibited and the completion of the entire process was accordingly delayed (Gaur, 1999).

2.5.4. Aeration

Aeration is a major consideration in most composting techniques. An adequate supply of gaseous oxygen is required for proper aerobic decomposition. The ultimate amount or total theoretical amount of oxygen required is determined by the amount of carbon to be oxidized (Golueke, 1991).

Mathur *et al.* (1990) applied passive aeration for composting manure, slurry and peat. Perforated pipes can be used at the bottom of compost piles to enhance aeration.

2.5.5. Temperature

Temperature evolution in compost heaps is a reflection of microbial activity. The optimum level should be between 60^{o}C and 71^{o}C(Schulz, 1961) and be maintained between these limits for several days to destroy pathogens and encourage the development of

thermophilic microorganisms, mainly fungi and thermophilic actinomycetes (Finstein and Morris, 1975). Moreover, temperature is a good indicator of the degree of maturity of the compost, which may be considered at its optimum degree of maturity when the temperature remains more or less constant and does not vary with the turning of the material (Stickelberger, 1975).

A maximum temperature of 71^{o}C is the best but should not be allowed to exceed 71^{o}C for long, as decomposition will be slowed due to thermal inactivation and subsequent death of microorganisms. Only a few thermophilic organisms actively carry on decomposition above 70^{o}C (Wiley, 1957;Schulz, 1961)

2.5.6. pH

The pH of compostable material influences the type of organisms involved in the composting process. There was an intrinsic relationship of temperature and pH variation with time during composting. The early mesophilic stages show a decrease in pH (acidic) and with an increase in temperature of the composting mass, there was corresponding increase in pH. The maximum pH rose around 8.0 synchronizes with temperature peak with a subsequent leveling off at alkaline pH (Gray and Biddlestone, 1985).

Pandey (1997) recommended that the pH should be prevented from rising above 8.5 to minimize the gaseous losses of nitrogen in the form of ammonia.

2.5.7. Nutritional factors

The major nutritional factors that affect composting are concentration and the availability of nitrogen, phosphorus, potassium and carbon.

The carbon-nitrogen (C/N) ratio remains the most important balance in composting. It affects the speed of the composting process and volume of finished material. As composting proceeds the microflora use the carbon for energy, and the nitrogen for cell building. The C:

N ratio becomes smaller with time, since the nitrogen remains in the system while the C is released as CO_2.

Generally, the optimum ratio is within the range of 19 to 30 parts. Raising the C/N levels to above 30:1 brings about a corresponding slowing of microbial activity. At C/N ratio lower than 18 excess nitrogen is lost to the atmosphere in the form of ammonia, and the pH may rise to inhibitory levels. An excess of ammonium ions may be toxic to microbes (Golueke, 1991).

Solid wastes usually contain sufficient amount of phosphorus for microbial growth. The carbon to phosphorus (C/P) ratio should be around 100:1 for proper microbial growth and digestion (Hegarty and Curran, 1985).

The maturation of the compost without any of the additional nitrogen sources is not rapid, proving the usefulness of such addition (Jackson *et al.,* 1992). Addition of 0.5 percent nitrogen as urea was found to enhance bioconversion (Son, 1995). A reasonable quality of the final compost was obtained when bovine blood was used as the nitrogen source for composting purposes (Ribeiro, 1994).

2.5.8. Microbial inoculants

Coir pith, a waste from the coir industry is composted by inoculating five bottles of *Pleurotus sajor-caju*, along with the addition of 5 kg of urea for every tone of raw coir pith. The process brought out drastic reduction in the lignin content and resulted in greater proportion of nitrogen and potassium (Nagarajan *et al*., 1985). Increase in relative humidity increased the bacterial population three folds. Actinomycetes population was also enhanced but with lesser magnitude (Mohansingh and Sharma, 1991).

2.6. Microorganisms involved during composting

The break down of substances during composting is referred to certain groups of microorganisms, which were responsible for the different stages of process. Poincelot (1972) discussed the microfloral population during composting of grass clippings and wheat straw, based on the reports of Webley (1947) and Chang and Hudson (1967). The number of microorganisms/g-wet compost reported in the initial mesophilic stage was for bacteria 10^8 and for fungi 10^6. The population of mesophilic actinomycetes was 10^6 initially and the population dropped subsequently, when the thermophilic stage was reached. The count of mesophilic actinomycetes was erratic throughout the composting period.

The controlled optimum temperature near 45^{o}C encourages cellulose-decomposing fungi. Gudrun Bagstam (1978) reported that cellulose decomposition was favoured at a temperature between 40^o and 50^{o}C and the fastest decomposition rate during composting of hard wood bark were attained at a temperature of 40-47^{o}C.

During composting, three distinguished phases were reported namely the mesophilic phase (below 40^{o}C), the thermophilic period (above 40^{o}C) and the cooling period. The bacterial population was markedly dominant during the entire stabilization phase of composting (Gray *et al.*, 1971; Poincelot, 1974). The count of mesophilic bacteria remained essentially constant whereas the thermophilic population increased during the thermophilic period. The thermophilic population growing at 55^{o}C, initially high, suffered a rapid drop from the third to the sixth day. It was increased significantly during the thermophilic stage and decreased again during the cooling down period. The number of mesophilic fungi underwent a marked decrease during the early thermophilic period. No mesophilic recolonization of fungi took place during the subsequent low temperature period of composting process.

In contrast, the population of thermophilic fungi was stable during the entire process. The most temperature responsive microorganisms were the actinomycetes. The mesophilic

counts dropped rapidly during the mesophilic and thermophilic periods to increase again in cooling period. They consequently appear to play a major role during the cooling period of process. During curing, organic materials continue to decompose and are converted to biological stable humic substances. Curing is a critical and often neglected stage of composting (Rynk, 1992).

Compost was considered finished or stable after temperatures within the pile remains greater than 5% for several days. Dinel *et al*. (1996) defined compost maturity as a state when bio-resistant organic compound or humic substances dominated composts.

During the period of greatest heat production, surprisingly few genera and species of microorganism were present in the compost. A few species of *Bacillus* sp. eg: *Bacillus subtilis, B. circulans, B. stearothermophilus,* thermophilic fungi eg: *Aspergillus fumigatus, Mucor pusillus, Chaetomicum thermopile*, the yeast *Torula thermophila* and actinomycetes eg: *Streptomyces* sp., *Thermoactinomyces* sp. etc. were observed during this stage (Storm, 1985).

2.7. Enzyme activities during composting

Enzymes on the basis of mode of action were divided into adaptive and constitutive enzymes. They were also classified as oxidoreductases and hydrolases because of their importance in the recycling of plant nutrients. Among them amylase, cellulase, dehydrogenase, phosphatase and urease were recognized very important as they participate in the mineralisation of nutrients.

At the community or ecosystem level, enzyme production was a function of microbial production, which was regulated by moisture, temperature and nutrient availability (Burns, 1983). Enzyme substrate interactions, such as inhibition, adsorption, stabilization and humification, alter the apparent kinetics and activation energies of the enzymes and determine turnover rates (Sinsabaugh *et al.,* 1991).

The enzyme activities probably largely reflect the diversity of the microbial population. The enzyme activity increases during the mesophilic period and decreases in the late cooling period.Cellulase infact a collection of atleast three distinct activities, has a degradative function of prime import during the composting process (Francis *et al.,* 1978; Poincelot and Day, 1972). A great number of microorganisms mostly fungi, few bacteria and actinomycetes are able to degrade cellulose for their growth.

Cellulolytic bacteria

Many bacteria degrade cellulose by the action of cell bound cellulases. Various cellulose degrading bacteria includes,

Cellulomonas sp

Clostridium thermocellum

Clostridium thermosaccharolyticum

Myrothecium verrucaria

Cellulolytic fungi

The Fungi secrete cellulase into the external environment. They include,

Aspergillus niger

Fusarium lini

Fusarium solani

Gliocladium roseum

Trichoderma reesei

Trichoderma viride (Jaishree paul, 1992)

The extra cellular cellulases from *Trichoderma reesei* were well studied and were known for their potent activity (Rye and Mandels., 1980).

2.7.1. Components of cellulases

Cellulase capable of degrading cellulose is composed of essentially three enzyme species

1) Endo-β-1, 4-Glucanases
2) Exo- β -1, 4-Glucanases and
3) β -Glucosidases

These three enzyme groups work synergistically to hydrolyse crystalline cellulose.

2.7.2. Mechanism of cellulyotic enzymes

Endo-glucanase and cellobiohydrolases worked essentially synergistically in a cyclic process during which cellulose was gradually stripped off from the surface of the cellulosic micro crystal. The product of this synergistic action was cellobiose, which was converted to glucose by the third component of the systems, β-glucosidase. An exoglucanase, which was distinct from cellobiohydrolase, was found in some systems. For effective hydrolysis the components of the cellulase enzyme system must be optimized *in vitro* degradation (Jurasek *et al.,* 1987).

Cellulolytic enzyme systems can be produced by a number of different microorganisms such as aerobic and anaerobic bacteria (Gilkes *et al.,* 1996), white rot fungi (Uzcategui *et al.,* 1991;Thompson *et al*., 1998), soft rot fungi (Kubicek *et al*., 1990). Promising results had been obtained for enzyme producing using spectrum of lignocellulosic sustrates such as wheat straw, bagasse, waste paper sludge, pretreated wood etc. (Pandey *et al*., 1999).

Cellulase of thermophilic actinomycetes had received much attention for the degradation of substrate. The optimum pH for the production of extracellular cellulase ranged from pH 7.0 to pH 7.2. Stutzenberger (1971) found that the optimum pH for extracellular cellulase production by *Thermomonospora curvata* ranged from pH 7.0 to 8.0, optimum temperature for cellulase production ranged from 25-35^{o}C.

The growth and activity of celluloytic fungi were influenced by the composition and degree of complexity of substrate (Targonski and Szajer, 1979; Trivedi and Rao, 1980), which induce or inhibit the biosynthesis of selected components of cellulase complex.

Some of the important factors, which affect relative enzyme susceptibility of cellulose, were crystallinity available surface, admixture with impurities and affinity of cellulose for cellulase (Bisaria and Ghose, 1981).

Enzyme activity was often used as an index of microbial activity in soils as well as their fertility. Dehydrogenase had been widely used to measure catabolic activities, which is correlated with microbial activities (Skujins, 1976).

The urease enzyme was responsible for break down of urea into CO_2 and NH_4. Urease activity significantly correlates with organic carbon and total nitrogen (Frankenberger and Dick, 1982). The climatic and edaphic factors of soil may influence the microbial enzymes (Kiss *et al.*, 1975; Harrison, 1979) and changes the quality and quantity of substrate on which they act.

Reithel (1971) reported that the enzyme urease was present at a high level at the beginning of composting of cattle manure but declined rapidly to a constant lowlevel. This pattern was probably inherent to the nature of starting material, which was rich in excreted urea. This medium was thus favorable for the development of an ureolytic flora which later disappeared or stopped reducing the enzyme when urea gets exhausted.

Paulson and Kurtz (1969) demonstrated that urease activity of soil could be divided into two components; microbial urease, directly associated with microorganisms and adsorbed urease, apparently adsorbed on soil colloids. Skujins (1967) reported that temperature, soil factors such as moisture content, pH, organic matter and population of microorganisms also affect the urease activity in soil. Vasilenko (1968) also reported the

decrease in urease activity with increasing moisture content from 60 to 140 percent of maximum water holding capacity in black spruce humus.

McGarity and Myers (1967) reported that on drying, the urease activity increase initially, possibly, due to release of urease on drying and lysing of microorganisms.

In aerobic environment saprophytic fungi, which can secrete cellulases and other exo-enzymes directly into the environment, constitute a major group of the decomposer community (Burns, 1983).

Ross and Roberts (1973) reported the microbial and enzyme activity during litter decomposition. Initially fungi and bacteria were few, but a rapid increase in their numbers was recorded as decomposition progressed and then decreased towards the end of the process. Cellulase activity was low at the beginning of litter decay but increased as the decomposition progressed. A positive correlation between cellulase activity and fungal and bacterial numbers was found in litter decomposition. Amylase activity also increased with litter decomposition and showed a marked seasonal variation, with values generally highest in June and lowest during the winter months. The changes in amylase activity during litter decomposition are attributed to changes in numbers of microorganisms.

Dhurva Kumar *et al*. (1992), reported the soil enzyme activities in relation to altitude and forest degradation. Dehydrogenase activity showed a significant positive correlation with number of soil microorganisms and amounts of organic carbon. At both low and high altitudes, activity was significantly higher in less degraded stand than the more degraded one. Harrison (1979) obtained seasonal variation in phosphatase activity. The increased phosphatase activity in summer may be attributed to high temperature and bacterial population (Chonkar and Tarafdar, 1984). The high concentration of various enzymes like dehydrogenase, urease and phosphatase is less in disturbed site than more disturbed one and was due to high organic carbon and microbial population.

Phosphatases had been used to describe a broad group of enzymes that hydrolyse organic phosphorus compounds, pyrophos, metaphos and inorganic polyphos, which occurs in soil. The distinction between acid and alkaline phosphatase was based on the marked difference in the pH ranges in which these enzymes were active (Browman and Tabatabai, 1978). The phosphatase plays a role in the use of alternative P sources and considered as a general index of microbial activity in compost (Lampen, 1971; Speir and Ross, 1978; Reid and Wilson, 1971). The level of this enzyme increased during the mesophilic period and remained approximately constant during the later periods of the process.

Garcia *et al.* (2000) reported that enzyme involved in intracellular microbial metabolism, such as dehydrogense and catalase, increased with the organic amendments. The highest values for dehydrogenase and glucosidase activities were found in the cow manure followed by municipal soild waste compost. Dehydrogenase activity basically depends on the metabolic state of the soil biota. A significant increase in dehydrogenase activity with manure is due to the humified organic matter added with compost, which is more resistant to mineralization.

Glavica *et al*. (2002) reported that the enzyme activities during composting of mixture of wood chips and waste microbial biomass from pharmaceutical industry. A beneficial effect of mixing of the compost leads to the increase of amylase, cellulase, esterase and xylanase. Extracellular and membrane attached cellulases were initially present in waste microbial biomass. Presence of membrane bound cellulase seems to be more pronounced than the extracellular ones. The enzyme activities of microorganisms during the process were intensive and diverse.

2.8. Vermicomposting

The role of earthworms in soil environment has been well recognized since ancient times. Earthworms were among the most ancient of terrestrial animal groups with many known fossils (650-750 million years old) of marine polychaete worms in south Australia

(Glaessner *et al.,* 1969). Aristotle, the Greek philosopher referred earthworms as intestines of the earth because of their habit of ingesting and ejecting the soil (Minnich, 1977).Vermicomposting, employing earthworms for the conversion of organic matter into a more humified material was one of the most efficient processes(Tapiadov, 1981). Vermicomposting gives a product that was rich in chelating and phytohormonal elements and had no further need of processing before being used (Bhiday, 1994). The survival of the worms after the completion of vermicomposting process reflects the lack of toxicity in the final product. The product had a high content of microbial agents and stabilized substances(Chandana, 1981).

Vermicomposting with earthworms is an excellent technique for recycling food waste as well as composting yard wastes. Earthworm castings often contain 5 to 11 times more nitrogen, phosphorus and potassium as the surrounding soil. Secretion in the intestinal tracts of earthworms, along with soil passing through the earthworms, makes nutrients more concentrated and available for plant uptake including micronutrients.

The organic waste materials like carbohydrates, cellulose in agricultural, industrial and municipal wastes were very good microbiological nutrients. Earthworm consumes almost all kinds of organic matter. Chemical changes in the degradation of organic matter were carried out through enzyme digestion, enrichment by nitrogen excrements, transport of organic and inorganic materials (Dash and Patra, 1979).

Earthworm population of biomass increase in fields in relation to organic matter present (Barley, 1961). Biology and potential of the earthworm in the management of activated sludges and cattle manures was found to be more (Hartenstein *et al.,* 1979). Thus earthworms can be gainfully employed in a wide variety of biodegradable waste conversion system.

2.8.1. Nutrient status in worm casts

Several works had compared the chemical decomposition of worm casts with soil. It had been claimed that nitrification was enhanced in casts and casts contain more nitrate, amino acids and total nitrogen than soil (Shrinkhande and Pathak, 1951).

Kang and Ojo (1996) and Parle (1963) investigated the stabilizing role of fungi by the measurements of aggregate stability on casts of different ages containing different amounts of fungal hyphae. The stability alters as the casts age rising to a peak at 15 days and then diminishing.

The major role of earthworms in the soil is the decomposition of organic materials, developing soil structure and altering physico-chemical properties (Zhang and Schrader, 1993; Edwards and Bohlen, 1996).

Lee (1985) found that the earthworm cast contains more nitrogen, phosphorus, calcium and carbonate. Nitrogen was excreted nearly as ammonia in urine released in the gut, it was mixed with the soil and can be found in the cast. Mba (1978) reported that the earthworm *Eudrilus eugeiniae* was capable of ingesting and excreting organic materials at a high rate.

Graff (1970) and Sharpley and Syres (1976) found that the exchangeable P measured isotopically was three times greater in casts than in the underlying soil. Worm casts were characterized by a modified soil structure and an increased content of organic matter and nutrients especially N, P and K. (Parthasarathi and Ranganathan, 1999). High P_2O_5 content in casts supported the phosphate availability which was required for root growth, microbial enhancement and in turn may help drive biological nitrogen fixation (James, 1991).

2.8.2. Physico chemical characteristics and microbial population of vermicasts

Earthworm casts form a suitable base for free living beneficial microbes, whose activity was essential for the release of nutrients to plants (Ross and Cairns, 1982). Several studies had shown that vermicasts were enriched in microbial population, micro and

macronutrients and enzyme activities (Mulongoy, 1989; Parthasarathi and Rangananthan, 1999). Increased microbial activity continues outside the gut in the casts (Lavelle *et al*., 1983) and results in an increased mineralization rate of organic N.

Karmegam and Daniel (2000) reported that casts of *Pontoscolex corethrurus* had the pH and EC values slightly different from the values obtained in surrounding soil. The organic carbon, total nitrogen, microbial population in the casts was significantly different from adjacent soil. It was due to the addition of intestinal mucus and also because of selective feeding of soil fractions enriched in organic compounds by the earthworms (Blair *et al*., 1994; Dash, 1999).

Parle (1963) found that the earthworm casts harbored increased number of microorganisms. Ismail (1997) reported that the earthworm promotes microbial population, either by virtue of their own intestinal mechanism or by their casts, serving as best culture media.

2.8.3. Enzyme activities in vermicast

The presence of earthworm resulted in increased oxygen uptake and further stimulated the activities of cellulase, invertase, amylase, urease and phosphatase. Businelli *et al.* (1984) reported that enzymatic activity of worm casts obtained from animal dungs had increased. Hydrolytic enzymes such as dehydrogenase, amylase and phosphatase were higher in worm casts than in soil. Dehydrogenase would be the only enzyme affected by the concentration of heavy metals.

Eisenia foetida, *Dendrobaena veneta* and *Lumdricus rubellus*, the three species of earthworm, which feeds on dung, were investigated. When cowdung was used as a substrate phosphatase activity was found to be high for the effects of earthworm activity to be detected. The faecal phosphate activity showed too peaks, at about pH 3-5 and pH 9-10. The peak

activity at pH 3-5 indicates the phosphatase produced by microbial activity and at pH 9-10 indicate increased activity directly from earthworms.

Satchell and Martin (1984) reported that enhanced phosphate content in earthworm casts was caused by an increased microbial and phosphatase activity in faeces. Phosphatase catalyzes the release of inorganic phosphate from organically bound phosphate. The activity of phosphatase in casts was 3-13 fold higher than in the corresponding surface soils.

Lee (1985) reported increased enzyme activity and microbial population in worm casts as compared with the underlying soil. Urease catalyzes the hydrolysis of urea added to soil. Casts showed 3-5 times more urease activity than the corresponding surface soils.

Dehydrogenase enzyme linked to the respiratory electron transport system (Bolton *et al.,* 1985) and was correlated with respiration in casts.

Total microbial population and enzyme activities (dehydrogenase and phosphatases) in the fresh casts and 30 days old pressmud vermicasts of two compost worms, *Lampito mauritii and Eudrilius euginiae* have been reported by Lavelle and Martin (1992). Enhancement of microbial population and enzyme activities in the fresh casts was due to enhanced mineralisation of nutrients, high substrate concentrations and high moisture level. As the vermicast become aged, there was a reduction in the moisture level leading to reduction in microbial population and enzyme activity.

Ross and Cairns (1982) reported the effects of earthworms and rye grass on enzyme activities of soil. The presence of earthworm resulted in increased oxygen uptake and activities of amylase, urease, cellulase, invertase and phosphatase. Thus earthworms stimulate the biochemical activities and nutrient cycling.

2.9. Compost maturity

Compost maturity is defined as the status of biological stability of compost conferring immediate improvement of soil productivity (Kalaiselvi and Ramasamy, 1996). The expression ‘mature compost’ or ‘stabilized compost’ implied that the product must fit diverse characteristics such as a minimum content of organic matter with stabilized materials. Physico-chemical and biological parameters which are often used as indicators are cation exchange capacity, C/N ratio, humification parameters, degree of polymerization, specific respiration activity, plant growth response test, analysis of water extract, water soluble organic matter, germination index, pH, temperature and oxygen uptake (Zibiliske, 1998).

Harada *et al.* (1993) also emphasized the use of biological and chemical indices for determining compost maturity. Characteristics like temperature, colour, texture, extent of solubility in sodium hydroxide or sodium pyrophosphate solutions, C/N ratio, absence of flies and odour and the sanitary quality were useful for assessing the maturity of composts (Gaur, 1982).

Manna *et al*. (2000) suggested that chemical parameters like water soluble carbohydrates, cation exchange capacity, total organic carbon, biodegradability index and lignin/ cellulose ratio may be used as compost maturity parameters irrespective of organic raw materials.

Chapter - III

MATERIALS AND METHODS

The present investigation on the development and evaluation of biocomposting technology for value added coir pith biomanure production for eco-agriculture was carried out in the department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore. The composting of coir waste was done using *viz., Pleurotus* sp. and yeast sludge and earthworms. The microbial load at different phases of composting and its enzymological changes that occur during composting were assessed. The methods followed for composting and the analysis are briefly described in this chapter.

3.1. Collection and characterization of materials for composting

Coir waste was collected from Central Farm, Tamil Nadu Agricultural University. The inoculum *viz., Pleurotus sajor-caju* was obtained from the mushroom training centre, Tamil Nadu Agricultural University, Coimbatore and yeast sludge from the nearby distillery unit. The samples collected were shade dried, sieved through 2 mm nylon mesh sieve and stored in polythene bags. The samples thus processed were analyzed for assessing physico-chemical parameters. The standard methods used for the analysis of composting materials are shown in Table 3.1.1.

3.1.1. Standard methods followed for the analysis of composting materials

Estimation	Remarks	References
pH	1: 10 organic waste: distilled water using pH meter	Falcon *et al*. (1987)
EC	1: 10 organic waste: distilled water using conductivity bridge.	Falcon *et al*. (1987)
Preparation of di acid extract	H_2SO_4 : $HClO_4$ @5:2	Biswas *et al.* (1977)
Preparation of triacid extract	HNO_3 : H_2SO_4 : $HClO_4$ @ 9:2:1	Piper *et al*. (1966)
Organic carbon	Chromic acid wet digestion method	Walkley and Black (1934)

Total nitrogen	Semi automatic Kjeldhal apparatus	Bremner (1965)
Total phosphorus	Vanado molybdate yellow color method	Jackson (1973)
Total potassium	Flame photometer	Jackson (1973)

3.2. Composting process

Composting and vermicomposting of coir pith were carried out by following the standard methods (Rajannan *et al*., 2002). The treatment details are presented below:

3.1.1. Treatment details

T_1: Coir pith alone (uninoculated control)

T_2: Coir pith + *Pleurotus sajor-caju*

T_3: Coir pith +yeast sludge

T_4: Coir pith + yeast sludge+ *Pleurotus sajor-caju*

T_5: Coir pith + earthworms

As an inoculum *Pleurotus* sp. was added @ 5 bottles/ tone of waste and as a nitrogen source urea @ 5 kg/tone and yeast sludge @ 200 kg/tone of waste were added.

3.2.1. Method of composting coir pith

Composting of coir pith was carried out by following the methods described by Rajannan *et al.,* (2002).

For composting coir pith with yeast sludge alone, 200 kg of yeast sludge was added as inoculum/ tone of coir waste. They were added in layers and water was sprinkled to maintain the moisture. In another treatment coir pith was added with five packets of *Pleutrous* sp./ tone of coir wastes. For combined treatments, 200 kg of yeast sludge, five packets of *Pleurotus* sp. and five kg of urea / tone of coir waste was added for enhancing the composting process. After mixing the materials it was formed as heap. Moisture of the composting materials was

maintained to 60%. To enhance aeration in compost heaps, turning was given at fortnightly intervals. The compost samples were collected at an interval of 15 days and analyzed for physico-chemical, biological and enzymatic changes. The composting of coir pith is shown in Plate.1

3.2.2. Method of composting coir pith with earthworms

The vermicomposting was done by following the standard methods (Kale *et al.*, 1992). The vermicomposting is shown in Plate.2

A suitable shady place was selected and the floor was covered with thick plastic sheet. A finely powdered waste was spread over the sheet and water was sprinkled until it becomes wet. The earthworm (*Perionyx excavatus*) collected from the field was released @ 2000 worms/ m^2 . The samples were collected at an interval of 15 days and analyzed for physico-chemical, biological and enzymatic changes.

3.3. Physico- chemical parameters and microbial analysis of compost

3.3.1. Temperature

Temperature of the composting heaps was monitored using digital thermometer (Davis *et al*., 1992).

3.3.2. Sampling procedure

Samples were taken from compost heaps and vermicomposting bed at fortnightly intervals. They were processed for analysis by following the method described by Faure and Deschamps (1990).

3.3.3. Chemical and microbiological analysis

Samples drawn at 15 days interval were air-dried, powdered, sieved and analysed for pH, EC, N, P, K and C/N ratio by following standard methods of analysis (Table 3.1.1.). The

population dynamics of bacteria, fungi and actinomycetes were assessed in fresh samples as per the standard methods (Table 3.3.3.1.).

3.3.3.1. Standard methods followed for Microbial analysis

Organisms	Medium used	References
Bacteria	Nutrient agar	Pramer and Schmidt, 1966
Fungi	Martin's rose Bengal agar	Pramer and Schmidt, 1966
Actinomycetes	KenKnights agar	Pramer and Schmidt, 1966

3.3.4. Organic carbon

The organic carbon content of samples was estimated by wet digestion method of Walkley and Black (1934).

3.4. Methods of Enzymatic analysis

3.4.1. Assay of Dehydrogenase enzyme

Dehygrogenase activity of the samples were assayed by following the method described by Chendrayan *et al*. (1980). Five gram of sample was transferred to a conical flask and to this 0.2 g of $CaCl_2$ was added. The contents were mixed thoroughly and 1 mL of 3% aqueous solution of 2,3,5-Triphenyl Tetrazolium chloride (TTC) and 1 mL of 1% glucose solution and 2.5 mL of distilled water were added. The flasks were corked and incubated for 24 hours at room temperature. Hot methanol was added to remove Triphenyl Formazan (TPF) extract and filtered. The wine red colour developed was measured at 485 nm using methanol as blank. TPF was used as standard. Dehydrogenase activity was expressed as µg of TPF released /g of sample/day.

3.4.4. Assay of Urease enzyme

Urease activities of the samples were estimated by the method described by Hofman (1965). To a 100 mL volumetric flask, 10g of dry and sieved sample and 1.5 mL of toluene were added and incubated for 15 minutes. Then 10 mL of 10 % urea solution and 20 mL of

citrate buffer (pH 6.7) were added and incubated for 3 hrs. at 37^{o}C in an incubator. Finally the volume was made up to 100 mL with distilled water and filtered. The filtrate was used as enzyme source. For each sample the blank was prepared similarly containing distilled water instead of urea solution. For enzyme assay, 1 mL of filterate, 9 mL of distilled water, 4 mL of phenate solution and 3 mL of sodium hypochlorite solution were added in a 50 mL volumetric flask and allowed to stand for 20 minutes for colour development. The green colour developed was measured at 630nm. Ammonium sulphate was used as a standard solution and the enzyme activity was expressed as μg N/g of sample.

3.4.3. Assay of Phosphatase enzyme

Phosphatase activities of the samples were estimated by the method described by Tabatabai and Bremner (1969). To five gram of sample, 10 mL of distilled water, 0.25 mL toluene and 1 mL of para- nitrophenyl phosphate were added, mixed and incubated at 27^{o}C. After one hr, 5 mL of 0.5 M $CaCl_2$ and 20 mL of 0.5 M NaOH were added and filtered through Whatman No.42 filter paper. The yellow colour developed was measured at 405nm. The p-nitrophenol was used as the standard solution.

3.4.5. Assay of Cellulase enzyme

Cellulase activities of the compost samples were estimated by the method described by Pancholy and Rice (1973). To 5g of dried sample, 0.5 mL of toluene was added and incubated for 15 minutes at room temperature. Then 10 mL of 0.5% carboxymethylcellulose (CMC) was added and incubated for 24 hours at room temperature. After incubation, 1 mL of 10% Trichloro Acetic Acid (TCA) was added and centrifuged at 2000 rpm for 10 minutes. The reducing sugar released was estimated by method described by Sadasivam and Manickam (1992).

To 0.2 ml of the extract, 1mL of alkaline copper tartarate reagent was added and heated in boiling water bath for 10 minutes. After cooling 1mL of arsenomolybdate reagent

was added and colour development was measured using spectrophotometer at 620nm. The result was expressed as µg of reducing sugars/ g of sample.

3.4.2. Assay of Amylase enzyme

Amylase activities of the sample were estimated by the method given by Bernfield (1955). One gram of the sample material was extracted with 10 volumes of ice-cold, 10 mM $CaCl_2$ solution kept overnight at 4^oC. The extract was centrifuged at 54,000 rpm at 4^oC for 20 minutes. The supernatant was used as enzyme source. For enzyme assay, 1 mL of starch solution was pipetted out into the test tube containing 1 mL of enzyme extract and incubated at 27^oC for 15 minutes. The reaction was stopped by the addition of 2 mL of dinitrosalicylic acid (DNS) reagent. The solution was heated in a boiling water bath for 5 minutes and 1 mL of potassium sodium tartarate solution was added when the tubes were warm. The volume was made to 10 mL and the absorbance was measured at 560 nm. The standard solution was prepared using maltose with range of 0-1000 µg. The unit of amylase was expressed as µg of maltose produced during 5 min. incubation with 1% starch.

The substrates and methods followed for enzyme assays are briefed in Table 3.5.

3.5. Standard methods followed for the Enzymatic analysis

S.No	Enzyme	Substrate	Methods	References
1.	Dehydrogenase	2,3,5-Triphenyl tetra zolium chloride	Using spectrophotometer at 485nm	Chendrayan *et al*. (1980)
2.	Urease	10% urea solution	Using spectrophotometer at 630nm	Hofmann (1965)
3.	Phosphatase	P-nitrophenol phosphate	Using spectrophotometer at 420nm	Tabatabai and Bremner (1969)

4.	Cellulase	Carboxymethylcellulose	Reducing sugar estimated using spectrophotometer at 620nm	Pancholy and Rice (1973)
5.	Amylase	1% starch solution	DNS method using spectrophotometer at 560nm	Bernfield (1955)

3.6. Compost maturity assessment test

The decision whether the compost is mature or not is reached at is based on the following qualitative and quantitative tests.

3.6.1. Qualitative tests

3.6.1.1. Starch-iodine test

About 1g of finely powdered compost samples was placed in a 100ml beaker and few drops of ethanol was added to wet the samples. About 20 ml of perchloric acid was added, stirred and filtered through Whatman No.40 filter paper. Few drops of the filtrate were placed on a white tile and 2 drops of iodine reagent (0.8 g of iodine added to 500 mL of distilled water containing 2g KI) was added. Finished compost gives a yellowish colour and very little precipitate; poor or unfinished compost gives a dark blue colour and heavy precipitate (Lossin, 1970).

3.6.1.2. Quantitative tests

The quantitative test of compost maturity involves the determination of humification parameters. In the breaker to 10g of the compost 100mL of 0.5 N NaOH was added and incubated overnight. The extract was centrifuged at 8000 rpm for 10 minutes. The organic carbon content of that supernatent was estimated by following the method of Walkley and Black (1934). The remaining supernatant was acidified with 2N HCl and centrifuged again at 8000 rpm for 10 minutes. The organic carbon content of the residue was also analysed. Using these organic carbon contents of different fractions humification parameters like humic acid

percent (Sequi *et al.,* 1986), polymerization ratio and degree of humification (Ciavatta *et al.,* 1990) were determined.

Humic acid (%) = (Cha/Cex) x 100

Fulvic acid (%) = (Cfa/Cex) x 100

Polymerization ratio = Cfa/Cha

Degree of humification(%) = {(Cha+Cfa)/Cex} x 100

Where, Cha = Organic carbon content in humic acid fraction

Cfa = Organic carbon content in fulvic acid fraction

Cex = Organic carbon contents in alkali extract

3.7. Statistical analysis

The experimental data were statistically analysed as suggested by Panse and Sukhatme (1985). The critical differences were worked out at 5 per cent (0.05) probability level.

Chapter – IV

EXPERIMENTAL RESULTS

Human and animal activities generate large quantities of organic residues. In India, a fraction of these residues is wasted and dumped in the environment thus causing overloading of land and water bodies, which leads to environmental pollution. The bioconversion of wastes to useful products has tremendous potential and it can help to meet the increasing world demand for food and energy. The composting technique has gained renewed attention as an alternative technique for the treatment of organic wastes. The microbial population and its enzyme activities are the major contributors, which are involved in the breaking down of complex molecules into a stable product, humus or compost.

Agricultural residues like coir pith were composted utilizing the yeast sludge and *Pleutrous* sp. Since the microbial population and its enzyme activities plays a major role in the decomposition of organic waste, the changes in microbial population and its enzyme activities were studied at different stages of composting to know their distribution. The results obtained from these experiments are presented below:

4.1. Physico-chemical composition of materials used for composting (Table 4.1)

The yeast sludge was acidic in pH (5.13) with high EC content recorded as 8.98 dSm^{-1} and the organic carbon content was 14.75 per cent. It had also recorded the highest N, P, K content of 2.73 per cent, 0.79 per cent and 5.86 per cent respectively. The C/N ratio of the sludge was 5.40 whereas the coir pith was at neutral pH (6.9) with EC of 0.83 dSm^{-1} and the organic carbon of 40.12 per cent. The N, P, K contents of coir pith was 0.37 per cent, 0.05 per cent and 0.74 per cent respectively. The C/N ratio recorded for coir pith was 108.43.

4.2. Analysis of compost

4.2.1. Changes in physico-chemical properties during composting

Analysis of composting of coir pith showed encouraging results, as there was an increase in the content of major plant nutrients and favorable reduction in C/N ratio.

4.2.1.1. Temperature (Table 4.2)

Significant differences in temperature were observed among the treatments throughout the period of composting. The temperature significantly increased in all the treatments upto 30^{th} day of composting. Then it decreased gradually and reached the ambient temperature. Among the different treatments, the highest temperature was recorded in the treatments T_4 (37.67 ^oC) and T_3 (36.91^oC), which were on par and the lowest value in T_1 (34.43 ^oC).

4.2.1.2. pH (Table 4.3)

In the treatments T_1 to T_4, pH increased till 30^{th} day and then decreased gradually as the composting progressed, reaching neutral range in the final compost. In T_5, there was a progressive increase in pH till 45^{th}, day after which there was a slight decrease. Among the treatments the highest mean value was recorded in T_5 (7.31) and lowest in T_1 (6.68). With respect to days, the highest pH was observed on 30^{th} day (7.19) and lowest at the 15^{th} day of composting (6.68).

4.2.1.3. EC (Table 4.4)

The EC increased significantly and reached maximum during 30^{th} day and then decreased, in all the treatments except T_5. Among the treatments, the maximum value for EC was recorded in T_5 (1.50 dSm^{-1}).Regarding the days, the maximum value was recorded on 30^{th} day (1.47 dSm^{-1}) and minimum value at the beginning of the composting (1.05 dSm^{-1}).

4.2.1.4. Organic carbon (Table 4.5)

Organic carbon content decreased with the progress of composting in all the treatments. Among the treatments, highest organic carbon content was observed in treatments T_2 (36.89 per cent) and T_3 (36.86 per cent), which was on par and minimum in T_5 (33.73 per

cent). With regards to days, the initial samples at the beginning of the composting showed the maximum value (43.05 per cent) while the minimum value (28.9 per cent) was observed in the final compost. Among the treatments and days interactions, the highest value (45.34 per cent) was in T_4 at the beginning of the composting and lowest (26.06 per cent) in T_5 at the end of composting. The per cent reduction in organic carbon content was highest in T_5 (47.58 Per cent) followed by T_4 (36.10 per cent) and lowest in T_1 (25.82 per cent). In T_5, there was 12.43 per cent increase in organic carbon content reduction over control.

4.2.1.5. Total nitrogen (Table 4.6)

The total nitrogen content showed significant difference among the treatments with the highest value in T_5 (1.09 per cent) and lowest value in T_1 (0.44 per cent). The treatments T_4 and T_5 were statistically on par. There was a significant increase in total nitrogen content as the composting progressed with the maximum value on 60^{th} day (1.21 per cent). The treatments and days interaction also showed significant difference with the highest value in the T_5 on 60^{th} day (1.75 per cent) of composting.

4.2.1.6. Carbon/ nitrogen (C/N) ratio (Table 4.7)

The C/N ratio decreased progressively with composting for all the treatments. Among the treatments, the highest value was recorded in T_1 (81.91 per cent) and lowest in T_5 (41.30 per cent). With respect to days, minimum value was recorded on the 60^{th} day (28.82 per cent). The interaction also showed significant difference with the highest value in the T_1 at the beginning of the composting (108.43 per cent) and lowest in T_5 on 60^{th} day of composting (14.89 per cent). The per cent reduction in C/N ratio was highest in T_5 (84 per cent) followed by T_4 (79 per cent) and lowest in T_1 (48 per cent).

4.2.1.7. Total phosphorus (Table 4.8)

The total phosphorus content in different treatments was found to increase with composting period and the rate of increase was less during later stage of composting. Among the treatments, there was a significant difference with the highest value in T_5 (0.44 per cent) and lowest value in T_1 (0.13 per cent). Among the various periods, the increase in phosphorus

content was maximum at maturity phase while the increase was low at early stages of composting. The total phosphorus content also showed significant difference among the treatments and days interaction with highest value recorded in T_5 (0.75 per cent) on 60^{th} day.

4.2.1.8. Total potassium (Table 4.9)

The total potassium content increased as the composting progressed with the maximum value recorded on 60^{th} day (1.37 per cent). Among the treatments, the highest value was observed in T_5 (1.33 per cent), which was significantly higher than all the other treatments, and T_1 had the lowest value at the initial day of composting (0.88 per cent). The interaction also showed significant difference with the highest value in T_5 (1.62 per cent) on 60^{th} day and lowest value in T_1 (0.74%) at the beginning of the composting.

4.2.2. Changes in microbial population during composting

4.2.2.1. Bacteria (Table 4.10 and 4.11)

The population of mesophilic bacteria increased from the initial stage of composting and reached maximum on 30^{th} day. Among the treatments, there was a significant difference with the highest value in T_5 (79 x 10^6 CFU/g) and lowest value in T_1 (38 x 10^6 CFU/g). The interaction effect between the treatments and days also showed significant difference with highest value in T_5 on 30^{th} day (119 x 10^6 CFU/g) and lowest value in T_1 (20 x 10^6 CFU/g) on initial day of composting.

The population of thermophilic bacteria increased and reached maximum on 30^{th} day. There was a significant increase during the thermophilic stage and suffered a rapid drop again during the cooling period. The population trends found to similar for both mesophilic and thermophilic bacteria but distribution in number were found to differ. Among the treatments, there was a significant difference with the highest value in the treatment T_4 (87.8 x 10^6 CFU/g) and lowest value in T_1 (47.4 x 10^6 CFU/g). With respect to days, maximum value was recorded on 30^{th} (113.8 x 10^6 CFU/g) and minimum value at the beginning of the composting

(19 x 10^6 CFU/g). The interaction effects also showed the significant difference. The bacterial culture isolated and identified from the compost is shown in Plate.3

4.2.2.2. Fungi (Table 4.12 and 4.13)

The population of mesophilic fungi increased during the initial period of composting and decreased during the thermophilic period. The population dropped rapidly from the thermophilic period till the end of the composting. Among the treatments, there was a significant difference with the highest value in the treatment T_4 (42.2 x 10^4 CFU/g) and lowest value in T_1 (19.2 x 10^4 CFU/g). With respect to days, maximum value was recorded on 15^{th} day (61.4 x 10^4 CFU/g) and minimum value on 60^{th} day (9.4 x 10^4 CFU/g). There was also a significant difference between the treatments and days.

The population of thermophilic fungi was found to be stable during entire process. Among the treatments there was a significant difference with highest value in treatments T_2 and T_4, which was on par, and lowest value in treatment T_1 (12.5 x 10^4 CFU/g). There was a highly significant difference between the treatments and days interaction. The fungal culture identified from the compost such as *Aspergillus* sp., *Mucor* sp., *Fusarium* sp. are shown in Plate.4 (a,b and c).

4.2.2.3. Actinomycetes (Table 4.14 and 4.15)

The population of mesophilic actinomycetes was found to be unusual throughout the composting period. The mesophilic counts increased and reached maximum during the end of composting. There was a significant increase among the treatments with the highest value in T_5 (48.4 x 10^3 CFU/g) and lowest value in T_1 (35.2 x 10^3 CFU/g). The interaction effects also showed significant difference with the highest value in T_4 (80 x 10^3 CFU/g) on 45^{th} day and lowest value in T_1 (17 x 10^3 CFU/g) at the beginning of the composting.

The population of thermophilic actinomycetes was found to be similar with that of the mesophilic counts. The population increased on the 30^{th} day and then decreased at the end of composting. Among the treatments there was a significant difference with the highest value

in T_4 (54.6 x 10^3 CFU/g) and lowest value in T_1 (38 x 10^2 CFU/g). The interaction effects also showed the significant difference.

4.2.4. Changes in enzyme activities during composting

4.2.4.1. Dehydrogenase (Table 4.16)

The changes in dehydrogenase activity showed significant difference among the treatments with the highest value in T_5 (42.67 µg of TPF/g) and lowest value in T_1 (18.38 µg of TPF/g). With respect to days, the maximum value was recorded on 30th day (31.39 µg of TPF/g) and the minimum value on 60th day (19.67 µg of TPF/g). The interaction effect between treatments and days also showed significant differences with highest value in T_5 (61.4 µg of TPF/g) on the 30th day and lowest value in T_1 (14.24 µg of TPF/g) on the initial day of composting.

4.2.4.2.Urease (Table 4.17)

Among the treatments, the urease activity showed a significant difference with the maximum activity in T_5 (813.59 µg N/g) and minimum activity in T_1 (409.8 µg N/g). The urease activity was seen to increase on 30th day, after which it showed a decreasing trend for all the treatments except T_5. The interaction also showed significant difference with the lowest value in T_1 (80.04 µg N/g) at the beginning of the composting and highest value in T_5 (1241 µg N/g) on 30th day of composting.

4.2.4.3. Phosphatase (Table 4.18)

The changes in phosphatase activity showed significant difference among the treatments with the highest value in the treatment T_5 (28.2 µg of PNPP/g) and lowest value in the treatment T_1 (19.59 µg of PNPP/g). During the process of composting, the phosphatase activity was found to be maximum on 30th day and remain slightly static till end of composting. The treatment and days interaction showed significant difference with highest value in the treatment T_5 on 30th day (44.5 µg of PNPP/g) and lowest value in the treatment T_1 on the initial day (17.05 µg of PNPP/g).

4.2.4.4. Cellulase (Table 4.19)

The changes in cellulase activity showed significant difference among the treatments with the highest value in T_5 (387.6 µg of reducing sugar/g) and lowest value in T_1 (345.1 µg of reducing sugar/g). The treatments T_2 and T_4 were on par. The interaction effect between treatments and days also showed significant differences with the highest value in T_5 on 60^{th} day (540.6 µg of reducing sugar/g) and lowest in T_1 at the beginning of the composting. With respect to days, maximum value was recorded on 60^{th} day (485.7 µg of reducing sugar/g) and minimum value on the initial day (308.7 µg of reducing sugar/g).

4.2.4.5. Amylase (Table 4.20)

The change in amylase activities during composting is presented in Table 4.20. The changes in amylase activity showed significant differences among the treatments with the highest value in T_5 (721.7 µg of glucose/g) and lowest value in T_1 (480.2 µg of glucose/g). With respect to days, the maximum value was recorded on 45^{th} day (787.4 µg of glucose/g) and minimum value was recorded at the end of the composting (421.6 µg of glucose/g). The interaction between treatments and days also showed significant differences with the highest value in T_5 on 60^{th} day (1015 µg of glucose/g) and lowest value in T_1 at the beginning of the composting (470.0 µg of glucose/g).

4.2.5. Compost maturity tests

The samples taken on the 60^{th} day of composting were subjected to different compost maturity tests, both qualitative and quantitative and results are furnished below.

4.2.5.1. Qualitative tests

4.2.5.1.1. Starch iodine test

The starch iodine test resulted in a yellow coloured solution without any precipitate, for all the treatments. This indicated the maturity of the compost samples.

4.2.5.2. Quantitative test (Table 4.21.)

The quantitative tests for compost maturity involved the estimation of humification parameters and results are given below.

4.2.5.2.1. Humic acid content

The humic acid contents showed significant difference among treatments with the maximum value in the treatment T_4 (28.46 %) and the minimum value in the treatment T_5 (12.69%). The treatments T_2, T_3, T_4 were statistically on par with each other with the humic acid contents ranging from 26.06 to 28.46 %.

4.2.5.2.2. Fulvic acid content

The fulvic acid content also showed significant difference among the treatments. The maximum value was estimated in the treatment T_4 (27.10 %) that was on par with the treatment T_3 (26.12 %). The lowest value was recorded in treatment T_5 (10.13%).

4.2.5.2.3. Degree of humification

The degree of humification was highest in the treatment T4 (55.56%) and lowest in the treatment T_5 (22.82%). The treatments T_2 and T_1 were on par with each other ranging from 45.10 to 46.6%.

4.2.5.2.4. Polymerisaton ratio

Polymerisation ratio was maximum in the treatment T_3 (0.96 %) which was on par with T4 (0.95 %) and minimum polymerization ratio was observed in the treatment T_2 (0.79 %).

4.2.5.2.5. C_{ha}/C_{fa} ratio

C_{ha}/C_{fa} ratio was highest in the treatment T_2 (1.27) that was on par with T_5 (1.25) and the lowest C_{ha}/C_{fa} ratio was recorded in the treatment T_3 (1.04).

Chapter - V

DISCUSSION

The agricultural sector, which is the mainstay of Indian economy, is in the forefront in terms of the quantity of waste generation. Organic residues like coir pith and farmland wastes are generated in huge quantities throughout the year. More precisely, composting is a breaking down as well as building up process where enzymes play the major role. A big significance for the process of composting represents the cell wall of microorganisms through which mass transfer is possible. To attack high molecular weight components, which can't pass through the cell wall, microorganisms secrete extra cellular enzymes. They break molecules down into fragments that can be assimilated, while the rest is converted into a stable product, compost. Owing to the nature of starting materials, enzyme activities largely reflect the diversity of the microbial population.

Bacteria are responsible for most of the decomposition and heat generation in compost. They are the most nutritionally diverse group of compost organisms, using a broad range of enzymes to chemically breakdown a variety of organic materials. In composting, actinomycetes with their enzymes play an important role in degrading complex organics such as cellulose, lignin and proteins.

In this investigations on development and evaluation of biocomposting technology for value added coir pith biomanure production for eco-agriculture were carried in order to achieve the objectives set forth in the chapter I. The results obtained are discussed in this chapter.

5.1. Physico-chemical properties of composting materials

The solid wastes generated from the distillery units (yeast slugde) and agricultural residues like coir pith were analysed for their chemical properties.The pH of the yeast sludge was acidic and that of coir pith was sightly neutral. The electrical conductivity were more in

yeast sludge (8.98 dSm^{-1}) and very low in coir pith (0.83dSm^{-1}). The content of organic carbon was maximum in coir pith and minimum in yeast sludge. The content of phosphorus and potassium were also highest in yeast sludge. The C/N ratio of the wastes varied from 5.04 (yeast sludge) to 118.43 (coir pith). The results were in agreement with the findings of Senthil kumar *et al*. (1996) and Sankaran *et al.* (2000).

These results indicated that yeast sludge is rich in nitrogen, resulting in a narrow C/N ratio. The inclusion of yeast sludge in the composting process helps in the proliferation of microorganisms during the initial phase of composting. Coir pith, because of its porous nature, gives vent for decomposing waste materials, facilitating aerobic conditions and thereby hastening the rate of aerobic decomposition (Srikanth, 1997).

5.2. Changes in physical chemical properties during composting

The temperature of the composting heaps fluctuated significantly with time (Fig. 5.1). During aerobic composting, there is a rapid rise in temperature. In this also there was a rapid rise in temperature in the first 30 days of composting and then a gradual decrease was noticed. Among the treatments, the waste receiving combined inoculation of yeast sludge and *Pleurotus* sp. attained the highest temperature of 38.7^{o}C on 30th day and then the temperature got decreased. It was statistically on par with T_3 and different from other treatments. Temperature evolution in compost heaps is a reflection of microbial activity. The increase in temperature during composting might be due to the release of exothermic energy during oxidation of carbon by aerobic decomposers. The rise in temperature of compost heaps were also reported by many researchers. The temperature during the composting process is considered to be a reflection of the metabolic activity of the microbial populations involved (Jimenez and Garcia, 1989). Finstein and Morris (1975) reported that the bacterial metabolism is responsible for increase in temperature. Gaur (1982) reported that the extent of temperature rise depend on the type of material being composted. Several authors concur that the thermophilic phase has to be maintained for atleast 10 days to destroy thermosensitive pathogens and to permit the hatching of parasite eggs whose larvae are destroyed by the

thermal exposure (Gray *et al.*, 1971; Poincelot, 1974 and De Bertoldi *et al.,* 1983). According to Tiquia *et al.* (1997), temperature is a good indicator of the degree of maturity of the compost.

The compost pH is a good indicator of the development of composting (Fig. 5.2). In the treatments T_1 to T_4, the pH showed an increasing trend till 30^{th} day and then decreased to neutral levels at the end of composting. The increase in pH of the compost mixtures during the initial stages could be attributed to the metabolic degradation of organic acids or intensive proteolysis of ammonia compounds (Gaur, 1987). The decrease in pH during the final stages may be due to the synthesis of organic acids and phenolic compounds (Mahimairaja *et al.,* 1994). In treatment T_5 there was an increasing trend till 45^{th} day and then decreased to slightly alkaline to neutral level at the end of the composting. Lee (1985) reported that higher pH in casts could be a consequence of ammonia excretion into the worm intestine or the action of calciferous glands in the worm pharynx when substrate is being ingested. Microbial activities play an important role in controlling pH. During mineralistion, degradation of easily hydrolysed polysaccharides, synthesis of organic acids by microorganisms to breakdown the substrate leads to decrease in pH (Jimenez and Garcia, 1991). These may be the valid reason for decrease in pH at the end of composting.

Regarding the electrical conductivity, in all the treatments, a gradual decrease in electrical conductivity was observed during composting. The concentration of soluble salts (EC) during composting increased till 30^{th} day for all the treatments and then decreased except in T_5 (Fig. 5.3). In case of T_5, increase in EC in the later stage of composting may be due to the volume reduction, which might have increase, the concentration of nutrients and insoluble salts. This view was supported by the results of Helkaih *et al.* (1995). Lee (1985) reported that increase in EC might be due to high soluble salts in the excretion of urine when mixed with vermicast. The increase in EC is due to mineralistion process promoted by activity of earthworms along with microorganisms. The decrease in soluble salt contents of the composting wastes might be due to the utilization of these salts by the microorganisms for the synthesis of their biomass (Talashilkar, 1986 and Garcia *et al.*, 1992).

The organic carbon content showed a decreasing trend with the advancement of composting (Fig. 5.4). The organic carbon content decreased significantly from the initial day to the end of composting. The treatments T_4 and T_5 had significantly lower organic carbon content at the end of the composting. At the end of the period, it ranged from 29.76 to 26.06 per cent. Minimum organic carbon content of 26.06 per cent was recorded in T_5. In the case of T4, the addition of yeast sludge as nitrogen source might have increased biological activity thereby bringing about faster decomposition of organic matter. According to Mathur *et al.* (1980) organic carbon content of organic waste decreased with the time of composting. Gaur and Sadasivam (1993) reported that the reduction in organic carbon might be due to utilization by microorganisms as an energy source to build up protoplasm. Earthworms and microorganisms are active in carbon rich substrate. Lee (1985) reported that the carbon in casts gets reduced due to the use of carbon for tissue build up. According to Daniel and Karmegam (1999) the reduction in organic carbon during vermicomposting is due to the respiratory activity of the worms and microorganisms present in the substrates.

The total nitrogen content was found to increase till the end of composting period (Fig. 5.5). Initially, the nitrogen content of the treatments ranged from 0.37 per cent to 0.46 per cent. At maturity stage, higher nitrogen content of 1.75 per cent was recorded in T_5 and it was statistically on par with T_4. The increase in nitrogen content in T_4 may be attributed to the addition of yeast sludge and *Pleurotus* sp. According to Mahimairaja *et al.* (1994) the weight loss due to release of CO_2 and the mineralistion of nitrogen during decomposition of organic matter might have resulted in an increased nitrogen content in the finished compost. Bernal *et al*. (1998) indicated that the nitrogen usually increase during composting process when volatile solid loss is greater than the loss of ammonia. The increased amount of total nitrogen in vermicast is due to the excretory products and mucus from the earthworms as well as through increased rates of mineralization of organic nitrogen by microorganisms in the casts (Lavelle and Martin, 1992; Edwards and Bohlen, 1996). Lee (1983) reported that nitrogenous products of earthworm metabolism also results nitrogen to the substrate in the form of urine, mucoproteins and dead worm tissue, which contains about 12 per cent

nitrogen. Earthworms also house nitrogen-fixing bacteria in their gut in a true symbiotic relation (Karsten and Drake, 1995). It may be reason for higher nitrogen content in vermicast. According to Laverack (1963) nitrogen was excreted nearly as ammonia in the urine release in the gut, it is mixed and can be found in the cast.

The C/N ratio was much wider at the beginning and narrowed down at the end of the composting (Fig. 5.6). The progressive decrease in organic carbon and increase in total nitrogen content consequently resulted in a significant decrease in C/N ratio during composting. Treatment T_5 registered, the maximum decrease in C/N ratio of about 84 per cent followed by T_4 (79 per cent). Reduction in C/N ratio during composting is due to the conversion of carbonaceous materials into cell biomass, CO_2, water and humus (Kalaiselvi and Ramasamy, 1996). It is this reason that might have led to decrease in C/N ratio. The faster reduction in C/N ratio in the few weeks of composting might be due to the addition of mineral nutrient sources and also due to prevalence of thermophilic conditions. The reduction in C/N ratio was minimum in treatment T_1 (48 per cent). In the case of treatment T_1, the addition of mineral nutrient sources was nil. Thus the C/N ratio was more when compared to other treatments. Bernal *et al.* (1998) reported that C/N ratio is considered to be one of the simple indices to evaluate any organic compost for its fitness for application.

There was a significant increase in the total phosphorus and potassium content during composting in all the treatments (Fig. 5.7 and 5.8). It was followed by the treatment that received yeast sludge and *Pleurotus* sp. The enhancement in the concentration of these nutrients was due to mineralisation of native carbon of wastes accompanied by a reduction in the total volume of the wastes under ideal conditions. This is in agreement with Metting (1993) who reported that during decomposition, microorganisms assimilate complex organic substances and release inorganic nutrients. The treatment T_5 had highest nutrient content when compared to other treatments. In the case of vermicomposting the enhancement of macronutrients had been due to mineralisation, microbial and enzyme activity in the vermicast (Baskar *et al.*, 1993; Neeta Sharma, 1994; Parthasarathi and Ranganathan, 1999).

Satchell and Martin, 1984 reported that the increase in phosphorus is due to the gut secreted phosphatase enzyme, which increases the lability of organically bound P.

5.3. Changes in microbial load during composting

Bacteria, fungi and actinomycetes play a major role in composting process. Bacteria are responsible for most of the decomposition and heat generation in compost.The population of mesophilic bacteria increased from the beginning of the composting till thermophilic phase and remained static till the end of this phase and decreased during the later stage (Fig. 5.9). The microbial population was considerably higher in the treatments T_3 and T_4 with the maximum bacterial population of $81x10^6$ CFU g^{-1} and $87x10^6$ CFU g^{-1} respectively on 30^{th} day , which may be due to the incorporation of yeast sludge as nutrient source.The treatment T_5 recorded the highest bacterial population of $119x10^6$ CFU g^{-1} when compared to other treatments. Davis *et al.* (1992) reported that the number of bacterial colony forming units (CFU) was higher than fungal colony forming units in mesophilic and thermophilic stages. Finstein and Morris (1975) showed the importance of bacteria in the composting process, particularly in the initial stages and have concluded that bacterial metabolism is responsible for the dramatic temperature increase during composting.

When the temperature increases bacteria dominates during composting (Fig. 5.10). The thermophilic population growing at high temperature will be initially high and then decreases rapidly. The diversity species is fairly high during thermophilic stage. The treatment T_4 recorded the maximum thermophilic bacterial population of 150 $x10^6$ CFU g^{-1} on 30^{th} day and decreased to population of 51 $x10^6$ CFU g^{-1} at the end of the composting. This is in confirmation with Gray *et al.* (1971) who reported that the count of mesophilic bacteria increased and remained essentially constant whereas the thermophilic population increased during the thermophilic period and drop rapidly. In the case of vermicast, the increase of microorganisms is due to the activity of earthworms and their castings, which encouraged the growth of microorganisms (Heijnen and Marinissen, 1995; Daniel and Karmegam, 1999). According to Dash (1999) the ammonia content and partially digested

organic materials in the earthworm casts provide beneficial nutritive medium for the growth of microbes and the mucus in the casts ejected by earthworms induce the activity of microorganisms persistently. Ismail (1997) reported that the earthworm promotes microbial population, either by virtue of their own intestinal mechanism or by their casts, serving as best culture media. The prominent bacterial species isolated from composting were *Bacillus* and *Pseudomonas.*

The fungi are responsible for the decomposition of many complex polymers and enabling bacteria to continue the decomposition process. The fungal population was maximum during initial day of composting and the lowest population was recorded at the end of composting (Fig. 5.11). The treatments T_2 and T_4 recorded maximum fungal population of 78 $x10^4$ CFU g^{-1} and 86 $x10^4$ CFU g^{-1} respectively on 15^{th} day and then decreased rapidly. The thermophilc fungi were found to be stable till 45^{th} day and then decreased at the end of the composting (Fig. 5.12). In the treatment T_4 maximum thermophilic population of 29 $x10^4$ CFU g^{-1} on 15^{th} day was recorded and then found to be stable till the end of the composting. Gray *et al*. (1971) and Poincelot (1974) reported that the number of mesophilic fungi underwent a marked decrease during the early thermophilic period and no recolonization of fungi took place during the subsequent low temperature period of composting process. In contrast, the population of thermophilic fungi was stable during the entire process. Most of the fungi isolated and identified include *Aspergillus flavus, A. terrius, A. niger* and *Trichoderma* sp.

In composting actinomycetes play an important role in degrading complex organics such as cellulose, lignin and proteins. Paterson and Bridge (1994) reported that the most temperature responsive microorganisms were the actinomycetes and they helps in degrading the complex organics like cellulose. The population of mesophilic actinomycetes was low during the initial stage of composting and increased during the later stage of composting (Fig. 5.13). The treatments T_4 and T_5 were recorded the maximum mesophilic counts of 85 $x10^4$ CFU g^{-1} and 88$x10^4$ CFU g^{-1} respectively on 45^{th} day which were statistically on par and it

significantly different from other treatments. The population of thermophilic actinomycetes reached maximum in treatment T_4 (90 x10^4 CFU g^{-1}) on 30th day and decreased at the end of the composting. Chang and Hudson (1967) reported that mesophilic actinomycetes were erratic throughout the composting period. Gray *et al.* (1971) reported that the mesophilic counts dropped rapidly during the mesophilc and thermophilc periods to increase again in the cooling period. The population of thermophilic actinomycetes also showed the similar trend as that of mesophilic population but during the cooling stage the population of thermophilic actinomycetes declined (Fig. 5.14).

5.4. Changes in enzyme activities during composting

Dehydrogenase is the intracellular enzymes that are involved in microbial oxidoreductase metabolism. The activity of these enzymes basically depends on the metabolic state of the microbes. It has been widely used to measure catabolic activities, which is correlated with microbial activities. Thus it is a good indicator of microbial activity. In the case of present study maximum dehydrogenase activity was recorded in the treatment T_5 (61.70 µg of TPF/g 24 hrs) followed by T_4 (25.44 µg of TPF/g 24 hrs) on 30th day (Fig. 5.15). Activity increased during the mesophilic period of composting and reached maximum during the thermophilic period and subsequently declined to a constant value. In the case of treatment T_4, a significant increase in dehydrogenase activity may be due the humified organic matter added with compost, which is more resistant to microbial mineralization. For the treatment T_5, dehydrogenase activity was correlated with respiration in casts. The highest dehydrogenase activity in T_5 may be due to rich microbial diversity in the gut of the earthworm and also earthworm casts form a suitable base for free living benefical microbes. Pedrazzini and Mc Kee (1984) reported that increased dehydrogenase activity was largely due to available nutrients and higher amounts of organic carbon. Bolton *et al,* (1985) reported that dehydrogenase is believed to be an intracellular enzyme linked to the respiratory electron transport and it was higher in casts than in the soils. Garcia *et al.,* (2000) reported that enzyme involved in intracellular microbial metabolism such as dehydrogenase increased with the organic amendments. Enhancement of enzyme activities in the fresh casts are due to

enhanced mineralization of nutrients, high substrate concentration and high moisture level (Martin, 1978).

The enzyme urease was responsible for breakdown of urea into CO_2 and NH_4. During the composting process urease activity was found to be increased and reached maximum during thermophilic period but declined to a constant low level at the end of the composting (Fig. 5.16). Maximum urease activity was recorded in treatment T_5 (813.6 µg N/g) followed by T_4 (617.8 µg N/g). In case of treatment T_4, maximum activity was recorded on 30^{th} day (813.9 µg N/g) and then decreased. Pallab De *et al.* (1990) reported a significant positive correlation between the organic carbon and urease activity. Since yeast sludge contain high amount of nitrogen source and coir pith with high organic carbon the urease activity was found to be increased. High urease activity may also be attributed to a faster rate of turnover of the organic matter. Frankenberger and Dick (1983) reported that urease activity was significantly correlated with organic carbon and total nitrogen. Reithel (1971) reported that the increase in urease activity depends on the nature of the starting material, which is rich in excreted urea. Thus it is favourable for the development of an ureolytic flora, which later reduces, or stops producing the enzyme when urea becomes exhausted. Skujins (1967) reported that the temperature, organic matter and population of microorganisms also affect the urease activity. In the case of treatment T_5 the urease activity was found to increase till the end of the composting. This may be due to excretion of ammonium in the urine released in the gut and can be found in the cast. Mulongoy and Bedoret (1989) reported that casts showed 3-5 times more urease activity than the corresponding soils. They found no correlation between urease and biomass carbon indicating that the enzyme was likely not of microbial origin or that ureolytic microbes didn't vary as a function of total microbial biomass.

Phosphatase has been used to describe a broad group of enzymes that catalyze the release of inorganic phosphate from organically bound phosphate. Pallab De *et al,* (1990) reported that phosphatase play the most important role in transforming the organic

phosphorus into the available form of phosphorus. In all the treatments the level of this enzyme increased during the mesophilic period and remained approximately constant during the later periods of the process except T_5, which declined during the cooling period. The maximum phosphatase activity was seen in T_5 (44.50 μg of PNPP/g) on 30th day followed by T_4 (28.45 μg of PNPP/g) (Fig. 5.17). In case of T_4 it might be due to rich nutrient source supplied by yeast sludge and *Pleurotus* sp. The increase in phosphatase activity may be attributed to high temperature and bacterial population (Chonkar and Tarafdar, 1984). Satchell and Martin (1984) reported that enhanced phosphate content in earthworm casts is caused by increased microbial and phosphatase activity in faeces. The activity of phosphatase in casts was 3-13 fold higher than in the soils. It reached maximum upto 30th day and then starts declining. Martin (1978) reported that when vermicast become aged, there is a reduction in moisture level leading to reduction in microbial population and enzyme activity. Speir and Ross (1978) reported that the phosphatase is considered as a general index of microbial activity in compost.

Cellulases play an important role during the composting process because of its degradative function. A great number of microorganisms mostly fungi, bacteria and actinomycetes are able to degrade cellulose for their growth. The enzyme activity increased during the mesophilic period and again in the late composting period (Fig. 5.19). The increase in cellulase activity may be due to break out of complex organic materials by fungal population and simple glucose molecules available on the later stage. Ross and Roberts (1973) reported that cellulase activity was low at the beginning of litter decay but increased as the decomposition progressed. The cellulase activity was found to be maximum in T_5 (540.6 μg of reducing sugar/g) followed by T_4 (524.6 μg of reducing sugar/g) during the later stage. Francis *et al.* (1978) reported that the cellulase enzyme activity increased during the mesophilic period, declined during thermophilic period and increased again in the later period. Ross and Cairns (1982) reported that the presence of earthworms resulted in increased oxygen uptake and further stimulated the activites of cellulase and amylase. Stutzenberger (1971) reported that cellulase of thermophilc actinomycetes had received much attention for

degradation of celluose. According to Speir and Ross (1981) cellulase activity was correlated positively and significantly with the moisture content of the litter, suggesting the favorable role of moisture in the synthesis of cellulase.

Amylase assumes significance from the point of break down of easily decomposable organic material such as starch. The enhanced enzyme activity means the enhanced phase of nutrient component from chelated complex molecules of compost (Balasubramanian *et al.*, 1974). In all the treatments the amylase activity was found to be increased during the decomposition and declined at the end of composting except T_5 (Fig. 5.20). Ross and Roberts (1973) reported that the amylase activity increased with litter decomposition and it is positively correlated with fungal and bacterial numbers and also reported that changes in amylase activity during decomposition are attributed to changes in the number of microorganisms. In the treatment T_5 the amylase activity was found to be maximum (1015 µg of maltose/g) on 60^{th} day. Lavelle and Martin (1992) reported that enhancement of enzyme activity in the casts was due to increased oxygen uptake, enhanced mineralisation of nutrients, high substrate concentration and high moisture level.

5.5. Compost maturity tests

The compost sample collected at the end of the 60^{th} day gave good results for the qualitative tests for compost maturity *viz.*, starch iodine test showing complete decomposition of the wastes. Humic substances present in composting are good indicator of compost maturity. The humic acid content at the end of the composting ranged from 12.69 to 28.46 per cent, which indicated high humification and maturity of the compost. This is in agreement with the observations made by Bernal *et al.*(1998). The ratio between carbon content of humic acid and fulvic acid ranged between between 0.8 to 1.27 which can be considered as mature according to Jimenez and Garcia (1992). Higher value of humic acid, degree of humification , Cha/Cfa and lower value of fulvic acid and polymerization ratio are indices of compost maturity (Kalaiselvi and Ramasamy, 1996). Businelli *et al,* (1984) reported that casts contained more fulvic or humic acid than the corresponding soil due to gut transit

contributing to humification processes. The ratio of humic and fulvic acid in T_5 is 1.25. This was in confirmation with Stevenson (1982) who reported that humic acid: fulvic acid ratio in cast averaged to 1.29 indicating that humification was more advanced in casts.

Chapter – VI

SUMMARY

In the present investigation, attempts have been made to study the changes in microbial and enzyme activities at different stages of composting of coir pith. Composting was carried out using agricultural residue, coir pith as a substrate. Yeast sludge and *Pleurotus sajor-caju* were used as inoculum to enhance microbial activity and as a nutrient supplement for composting. Composting of coir pith was done in three different combination (i.e) coir pith with yeast sludge, coir pith with *Pleurotus* sp. and coir pith with yeast sludge and *Pleurotus* sp. Uninoculated coir pith was used as a control. Vermicomposting was also done to study its microbial load and enzyme activities during composting.

The quality of compost has also been evaluated with respect to the content of nutrients and degree of maturity. The findings of the investigation are summarized under:

- All the treatments with coir pith developed remarkably higher temperature and it increased upto 30^{th} day, declined gradually thereafter stabilized during maturity phase.
- The pH of the compost increased slightly upto 30^{th} day, thereafter decreased marginally. At the final stage of composting all the treatments showed slightly neutral pH, which is a positive characterisitic for the use of compost as a growing medium. In the vermicomposting pH increased upto 45^{th} and at the end of compost it was stabilized.
- Among the composting treatments, yeast sludge inoculated treatment recorded the maximum electrical conductivity. The electrical conductivity of compost decreased and stabilized at maturity phase. In the vermicomposting EC slightly increased at the end of the composting.
- Organic carbon content decreased during composting process and the rate of decomposition was found to be higher in treatments involving combination of yeast sludge and *Pleurotus* sp. with coir pith (T_4). In the vermicomposting (T_5) the organic carbon content was highest when compared to composting with yeast sludge and *Pleurotus* sp.

- Macronutrients such as nitrogen, phosphorus and potassium increased during composting process and Treatments T_4 and T_5 registered the highest nutrient contents at the end of the composting.
- The C/N ratio decreased progressively and during the maturity stage, it was satisfactory in all the treatments as they were well within the limit of 30.
- In general in all the treatments, the mesophilic bacterial population increased as the composting progressed, reached maximum on the 30^{th} and then decreased and got stabilized during the maturity phase. The microbial population was found to be more in the vermicasts (T_5) followed by T_4.
- The thermophilic bacterial population at the initial stage was less and increased on 30^{th} day. It was found to be stabilized till 45^{th} day and declined rapidly with advancement of composting process.
- The population of mesophilic fungi increased upto 15^{th} day and T_2 and T_4 recorded the maximum population. The population at thermophilic stage got reduced and it was maintained till the maturity stage. The thermophilic fungi were found to be stable during the composting process.
- The actinomycetes during mesophilic stage recorded minimum population and at thermophilic stage it was slightly increased. The maximum population was registered at the later stage. Maximun thermophilic actinomycetes was observed at 30^{th} day and then decreased during the maturity phase. Actinomycetes population was found to be higher in the treatments T_3, T_4 and T_5.
- The microorganisms isolated from the composting samples were identified as *Bacillus* sp., *Pseudomonas* sp. *Aspergillus* sp., Mucor sp., and Fusarium sp.
- The activities of enzymes such as dehydrogenase, urease, phosphatase, cellulase and amylases which plays an important role in degrading the wastes and enhancing the nutrients was found to be more pronounced during composting.
- Dehydrogenase enzymes, which are involved in hydrolytic activity, was found to be increased upto 30^{th} day and maintained till the thermophilic stage. Its activity decreased

during the maturity stage. The dehydrogenase activity was found to be more in Treatment T_5 followed by T_4 and T_3.

- The urease enzymes increased from 15th day and reached maximum on 30th day and maintained stability during the maturity phase. Among the treatments the urease activity were higher in T_2 and T_4. In the treatment T_5 the urease activity increased till the maturity stage.
- The phosphatase enzyme, which is involved in transforming the organic phosphorus into the available form, increased in all the treatments. Its activity reached maximum on 30th day and maintained its stability till the end of the composting. The highest phosphatase activity was recorded in treatments T_4 and T_5.
- The cellulase enzyme activity increased as the composting proceeds. At the mesophilic stage the activity slightly increased and then decreased during the thermophilic stage. Among the treatments the maximum activity was recorded in treatments T_2, T_4 and T_5.
- The activities of amylase during composting process increased from the mesophilic stage to thermophilic stage. Its activity decreased at the maturity stage except T_5. Among the treatments maximum activity was registered in treatments T_3 and T_5.
- The qualitative and quantitative tests of compost maturity gave favourable results for all the treatments, indicating their maturity. The treatment T_4 gave the highest values for humic acid and degree of humification.

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Research Findings

DEVELOPMENT AND EVALUATION OF BIOCOMPOSTING TECHNOLOGY FOR VALUE ADDED COIR PITH BIOMANURE PRODUCTION FOR ECO-AGRICULTURE

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India has huge biomass of crop residues like sugarcane trash, bagasse, coir pith, farmland waste etc. The overloading of these wastes leads to environmental pollution. Recently composting emerges as the most widely replicable process of handling diverse wastes. Decomposition of organic matter into usable compost depends on the abilities of the microflora to produce and excrete specific degradative enzymes. As microorganisms are important sources of enzymes, the activity of these enzymes correlates with microbial activity. Therefore, an attempt was made to study the microbial load and its enzyme activities during composting of organic wastes.

For composting experiments coir pith was used as substrate. Yeast sludge and *Pleurotus* sp. were used as microbial inoculum cum nutrient source. Since earthworms are found to be the efficient degraders of organic wastes, vermicomposting was also carried out using the earthworms, *Perionyx excavatus.*. Periodical samples were drawn and subjected to quality characterization.

Among the treatments, the treatment involving coir pith with earthworm exhibited marked differences with respect to C/N ratio and increase in macronutrients N, P, K followed by the treatment involving combined inoculation of coir pith with yeast sludge and *Pleurotus* sp.

The microbial population and their activity at different stages of composting were assessed. The bacterial population was markedly dominant during the composting process. The mesophilic bacteria remained essentially constant whereas the thermophilic population increased during the thermophilic period. The mesophilic fungi increased at the initial stage and decreased during thermophilic and in the later stage of composting. The thermophilic fungi remained stable during the entire period of decomposition. The population of actinomycetes increased during the composting process and obtained maximum on 30^{th} day.

Owing to the nature of the material, enzyme activites largely reflect the diversity of microbial population. The enzyme activities, which are responsible for the recycling of nutrients such as dehydrogenase, urease, phosphates, cellulase and amylase, were found to be increased during the composting process. In general highest enzymes activities were recorded on 30^{th} day of composting. In the treatment involving coir pith with earthworms showed remarkably higher microbial population and the enzyme activities followed by coir pith with combination of yeast sludge and *Pleurotus* sp.

Hence, the findings of the present study clearly indicated the role of microbes and enzyme activities as indicators of composting process that will be highly helpful to monitor the changes and thereby improve the efficiency of the process.

Table 4.1. Physico- chemical properties of composting materials

Parameters	Materials	
	Yeast sludge	**Coir pith**
pH	5.13	6.9
EC (dSm^{-1})	8.98	0.83
Organic carbon (%)	14.75	40.12
Total Nitrogen (%)	2.73	0.37
Total Phosphorus (%)	0.79	0.05
Total Potassium (%)	5.86	0.74
C/N ratio	5.40	108.43

Table 4.2. Changes in temperature during composting of coir pith

Treatments	Temperature (^{o} C) Sampling period (Days)													
	Initial	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**55**	**60**	**Mean**
T_1	29.9	33.0	34.5	36.0	37.9	38.5	39.9	38.4	35.9	33.0	31.0	29.9	29.7	**34.43**
T_2	30.0	34.9	36.0	37.8	38.7	39.0	40.8	40.0	38.0	36.2	34.7	32.5	30.0	**36.05**
T_3	31.0	35.5	36.6	38.6	39.0	40.1	42.4	41.3	39.1	37.5	35.0	33.0	30.7	**36.91**
T_4	32.9	35.9	37.1	38.9	39.8	41.2	43.6	42.9	39.0	37.9	35.4	33.6	31.5	**37.67**
Mean	**30.95**	**34.83**	**36.05**	**37.83**	**38.85**	**39.70**	**41.68**	**40.65**	**38.00**	**36.15**	**34.03**	**32.25**	**30.48**	**36.26**

	SE(d)	**CD (0.05)**
T	0.55	1.07
D	0.30	0.60
TXD	1.10	2.17

T_1- Coir pith alone (uninoculated control) ; **T_2-** Coir pith with *Pleurotus* sp.; **T_3-** Coir pith with Yeast sludge; **T_4-** Coir pith with Yeast sludge and *Pleurotus* sp.

Table 4.3. Changes in pH during composting of coir pith

Treatments	pH Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	6.90	6.65	7.00	6.54	6.30	**6.68**
T_2	6.85	6.80	7.06	7.00	6.47	**6.84**
T_3	6.79	6.51	7.13	7.04	6.90	**6.87**
T_4	7.00	6.56	7.20	7.12	7.00	**6.98**
T_5	7.01	6.90	7.56	7.68	7.41	**7.31**
Mean	**6.91**	**6.68**	**7.19**	**7.08**	**6.82**	**6.94**

	SE(d)	CD (0.05)
T	0.05	0.10
D	0.05	0.10
TXD	0.12	0.23

Table 4.4. Changes in Electrical Conductivity (dSm^{-1}) during composting of coir pith

Treatments	EC(dSm^{-1}) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	0.83	1.00	1.30	0.98	0.90	**1.00**
T_2	1.01	1.10	1.35	1.18	1.11	**1.15**
T_3	1.15	1.23	1.45	1.31	1.23	**1.27**
T_4	1.29	1.34	1.55	1.43	1.40	**1.40**
T_5	0.98	1.35	1.68	1.81	1.70	**1.50**
Mean	**1.05**	**1.20**	**1.47**	**1.34**	**1.27**	**1.26**

	SE(d)	CD (0.05)
T	0.02	0.04
D	0.02	0.04
TXD	0.12	0.08

T_1- Coir pith alone (uninoculated control) ; **T_2-** Coir pith with *Pleurotus* sp.;
T_3- Coir pith with Yeast sludge; **T_4-** Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5-** Coir pith with earthworms.

Table 4.5. Changes in organic carbon content (Per cent) during composting of coir pith

Treatments	Organic carbon (per cent) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	40.12	38.43	35.98	31.65	29.76	**35.19**
T_2	43.13	40.35	36.94	33.88	30.14	**36.89**
T_3	44.90	40.62	38.28	30.92	29.56	**36.86**
T_4	45.34	39.84	35.35	30.13	28.97	**35.93**
T_5	41.75	37.56	35.15	28.13	26.06	**33.73**
Mean	**43.05**	**39.36**	**36.34**	**30.94**	**28.90**	**35.72**

	SE(d)	CD (0.05)
T	0.26	0.52
D	0.26	0.52
TXD	0.57	1.15

Table 4.6. Changes in total nitrogen content (Per cent) during composting of coir pith

Treatments	Total nitrogen (per cent) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	0.37	0.40	0.42	0.50	0.53	**0.44**
T_2	0.42	0.62	0.71	0.75	0.96	**0.69**
T_3	0.45	0.78	1.02	1.25	1.30	**0.96**
T_4	0.50	0.80	1.10	1.35	1.53	**1.06**
T_5	0.46	0.79	1.05	1.42	1.75	**1.09**
Mean	**0.44**	**0.68**	**0.86**	**1.05**	**1.21**	**0.85**

	SE(d)	CD (0.05)
T	0.01	0.02
D	0.01	0.02
TXD	0.02	0.04

T_1- Coir pith alone (uninoculated control) ; **T_2-** Coir pith with *Pleurotus* sp.; **T_3-** Coir pith with Yeast sludge; **T_4-** Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5-** Coir pith with earthworms.

Table 4.7. Changes in Carbon/nitrogen ratio during composting of coir pith

Treatments	C/N ratio Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	108.43	96.01	85.67	63.30	56.15	**81.91**
T_2	102.69	65.08	52.03	45.17	31.04	**59.27**
T_3	99.78	52.08	37.53	24.74	22.74	**47.37**
T_4	90.68	49.80	32.14	22.32	18.93	**42.77**
T_5	90.76	47.54	33.48	19.81	14.89	**41.30**
Mean	**98.47**	**62.10**	**48.17**	**35.07**	**28.82**	**50.89**

	SE(d)	CD (0.05)
T	0.39	0.78
D	0.39	0.78
TXD	0.87	1.75

Table 4.8. Changes in total phosphorus content (Per cent) during composting of coir pith

Treatments	Total phosphorus (per cent) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	0.05	0.09	0.12	0.17	0.21	**0.13**
T_2	0.04	0.17	0.19	0.21	0.38	**0.20**
T_3	0.07	0.12	0.25	0.34	0.51	**0.26**
T_4	0.05	0.27	0.38	0.51	0.71	**0.38**
T_5	0.06	0.30	0.48	0.62	0.75	**0.44**
Mean	**0.05**	**0.19**	**0.28**	**0.37**	**0.66**	**0.28**

	SE(d)	CD (0.05)
T	0.01	0.01
D	0.01	0.01
TXD	0.01	0.02

T_1- Coir pith alone (uninoculated control) ; **T_2**- Coir pith with *Pleurotus* sp.; **T_3**- Coir pith with Yeast sludge; **T_4**- Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5**- Coir pith with earthworms.

Table 4.9. Changes in total potassium content (Per cent) during composting of coir pith

Treatments	Total potassium (per cent) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	0.74	0.78	0.88	0.90	1.10	**0.88**
T_2	0.83	0.98	1.05	1.12	1.34	**1.06**
T_3	0.80	0.85	0.92	0.98	1.20	**0.95**
T_4	0.83	0.91	1.10	1.27	1.42	**1.11**
T_5	0.80	1.06	1.56	1.59	1.62	**1.33**
Mean	**0.80**	**0.92**	**1.10**	**1.17**	**1.34**	**1.07**

	SE(d)	CD (0.05)
T	0.01	0.02
D	0.01	0.02
TXD	0.02	0.04

Table 4.10. Population dynamics of mesophilic bacteria during composting of coir pith

Treatments	Mesophilic bacterial population (10^6 CFU g^{-1}) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	20	33	61	49	27	**38.0**
T_2	26	49	74	58	33	**48.0**
T_3	30	56	81	62	36	**53.8**
T_4	35	60	87	75	44	**60.2**
T_5	33	75	119	99	69	**79.0**
Mean	**63.4**	**80.6**	**103.6**	**73.6**	**46.4**	**55.8**

	SE(d)	CD (0.05)
T	0.67	1.35
D	0.67	1.35
TXD	1.50	3.01

T_1- Coir pith alone (uninoculated control) ; T_2- Coir pith with *Pleurotus* sp.; T_3- Coir pith with Yeast sludge; T_4- Coir pith with Yeast sludge and *Pleurotus* sp.; T_5- Coir pith with earthworms.

Table 4.11. Population dynamics of thermophilic bacteria (45°C) during composting of coir pith

Treatments	Thermophilic bacterial population (10^6 CFU g^{-1}) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	11	43	78	76	29	**47.4**
T_2	22	76	109	99	35	**68.2**
T_3	18	60	118	105	43	**68.8**
T_4	25	75	150	138	51	**87.8**
Mean	**19.00**	**63.50**	**113.8**	**104.5**	**39.5**	**68.1**

	SE(d)	CD (0.05)
T	0.64	1.29
D	0.57	1.15
TXD	1.28	2.58

Table 4.12. Population dynamics of mesophilic fungi during composting of Coir pith

Treatments	Mesophilic fungi population (10^4 CFU g^{-1}) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	28	35	17	10	6	**18.2**
T_2	35	78	35	26	10	**41.0**
T_3	38	53	21	17	8	**25.8**
T_4	36	86	43	29	17	**46.4**
T_5	43	55	24	10	6	**29.4**
Mean	**36.0**	**61.4**	**28.0**	**18.4**	**9.4**	**32.2**

	SE(d)	CD (0.05)
T	0.28	0.57
D	0.28	0.57
TXD	0.64	1.28

T_1- Coir pith alone (uninoculated control) ; **T_2**- Coir pith with *Pleurotus* sp.; **T_3**- Coir pith with Yeast sludge; **T_4**- Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5**- Coir pith with earthworms.

Table 4.13. Population dynamics of thermophilic fungi (45°C) during composting of coir pith

Treatments	Thermophilic fungal population (10^4 CFU g^{-1}) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	12	16	14	10	10	**12.40**
T_2	27	20	25	17	13	**20.40**
T_3	20	18	18	16	9	**16.20**
T_4	25	29	20	18	11	**20.60**
Mean	**21.00**	**20.75**	**19.25**	**15.25**	**10.75**	**17.40**

	SE(d)	CD (0.05)
T	0.15	0.30
D	0.13	0.27
TXD	0.30	0.60

Table 4.14. Population dynamics of mesophilic actinomycetes during composting of coir pith

Treatments	Mesophilic actinomycetes population (10^3 CFU g^{-1}) Sampling period (Days)					Mean
	Initial	15th Day	30th Day	45th Day	60th Day	
T_1	17	24	38	69	56	**40.8**
T_2	20	31	39	80	63	**46.6**
T_3	32	40	40	74	65	**50.2**
T_4	28	52	55	85	70	**58.0**
T_5	23	48	50	88	72	**56.2**
Mean	**24**	**39**	**44.4**	**79.2**	**65.2**	**50.4**

	SE(d)	CD (0.05)
T	0.70	1.40
D	0.70	1.40
TXD	1.14	2.98

T_1- Coir pith alone (uninoculated control) ; **T_2**- Coir pith with *Pleurotus* sp.; **T_3**- Coir pith with Yeast sludge; **T_4**- Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5**- Coir pith with earthworms.

Table 4.15. Population dynamics of thermophilic actinomycetes (45°C) during composting of coir pith

Treatments	Thermophilic actinomycetes population (10^3CFU g^{-1}) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	17	24	55	49	45	**38.0**
T_2	24	33	73	59	55	**48.8**
T_3	21	29	85	63	53	**50.2**
T_4	26	41	90	60	58	**55.0**
Mean	**22.00**	**31.57**	**75.8**	**57.80**	**52.8**	**48.0**

	SE(d)	CD (0.05)
T	0.58	1.16
D	0.51	1.04
TXD	1.15	2.32

Table 4.16. Changes in dehydrogenase activity during composting of coir pith

Treatments	Dehydrogenase activity (µg of TPF/g 24 hrs) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	14.24	16.05	22.37	22.33	16.90	**18.38**
T_2	15.09	17.21	23.22	23.10	17.30	**19.18**
T_3	15.76	19.05	24.53	24.50	18.11	**20.39**
T_4	17.03	19.62	25.44	24.74	18.60	**21.09**
T_5	26.98	54.64	61.40	42.93	27.42	**42.67**
Mean	**17.82**	**25.31**	**31.39**	**27.52**	**19.67**	**24.34**

	SE(d)	CD (0.05)
T	0.20	0.41
D	0.20	0.41
TXD	0.45	0.91

T_1- Coir pith alone (uninoculated control) ; **T_2**- Coir pith with *Pleurotus* sp.; **T_3**- Coir pith with Yeast sludge; **T_4**- Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5**- Coir pith with earthworms.

Table 4.17. Changes in urease activity during composting of coir pith

Treatments	Urease activity (µg N/g) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	80.04	283.8	595.8	559.9	529.6	**409.8**
T_2	85.26	496.4	918.9	772.5	608.1	**576.2**
T_3	90.15	317.0	813.9	717.3	593.1	**506.3**
T_4	93.34	529.6	993.4	797.4	675.9	**617.9**
T_5	82.74	830.5	1241	885.7	1028	**813.6**
Mean	**86.31**	**491.5**	**912.6**	**746.6**	**721.4**	**584.8**

	SE(d)	CD (0.05)
T	5.65	11.34
D	5.65	11.34
TXD	12.63	25.36

Table 4.18. Changes in phosphatase activity during composting of coir pith

Treatments	Phosphatase activity (µg of PNPP/g) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	17.05	19.44	22.63	20.02	18.81	**19.59**
T_2	19.80	20.71	26.51	20.93	20.35	**21.66**
T_3	18.92	19.77	27.64	22.94	21.64	**22.18**
T_4	21.01	24.42	28.45	27.72	24.40	**25.20**
T_5	26.65	29.48	44.50	21.40	22.00	**28.81**
Mean	**20.69**	**22.76**	**29.95**	**22.60**	**21.44**	**23.49**

	SE(d)	CD (0.05)
T	0.18	0.35
D	0.18	0.35
TXD	0.39	0.79

T_1- Coir pith alone (uninoculated control) ; **T_2-** Coir pith with *Pleurotus* sp.; **T_3-** Coir pith with Yeast sludge; **T_4-** Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5-** Coir pith with earthworms.

Table 4.19. Changes in Cellulase activity during composting of coir pith

Treatments	Cellulase activity (µg of reducing sugar/g) Sampling period (Days)					Mean
	Initial	15	30	45	60	
T_1	297.5	327.0	300.6	383.5	416.7	**345.1**
T_2	305.8	324.7	308.9	408.4	508.7	**371.3**
T_3	315.6	323.2	311.9	383.5	438.6	**354.6**
T_4	317.6	330.0	314.9	391.8	524.6	**375.8**
T_5	307.1	328.5	315.1	446.5	540.6	**387.6**
Mean	**308.7**	**326.7**	**310.3**	**402.7**	**485.7**	**366.9**

	SE(d)	CD (0.05)
T	2.74	5.51
D	2.74	5.51
TXD	6.13	12.32

Table 4.20. Changes in amylase activity during composting of coir pith

Treatments	Amylase activity (µg of maltose/g) Sampling period (Days)					Mean
	Initial	15th Day	30th Day	45th Day	60th Day	
T_1	470.0	570.9	569.5	589.3	201.2	**480.2**
T_2	539.7	586.1	748.5	748.5	338.5	**592.3**
T_3	521.3	748.5	794.9	965.1	237.9	**653.5**
T_4	485.5	526.9	539.7	983.6	315.3	**570.2**
T_5	515.9	524.2	903.3	650.3	1015	**721.7**
Mean	**506.5**	**591.9**	**710.6**	**787.4**	**421.6**	**603.6**

	SE(d)	CD (0.05)
T	6.86	13.79
D	6.86	13.79
TXD	15.35	30.83

T_1- Coir pith alone (uninoculated control) ; **T_2**- Coir pith with *Pleurotus* sp.; **T_3**- Coir pith with Yeast sludge; **T_4**- Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5**- Coir pith with earthworms.

Table 4.21. Humification parameters of composted coir pith

Treatments	Humic acid (%)	Fulvic acid (%)	Degree of humification(%)	Polymerisation ratio (%)	C_{ha}/C_{fa}
T_1	24.13	20.97	45.10	0.87	1.15
T_2	26.06	20.54	46.60	0.79	1.27
T_3	27.12	26.12	53.24	0.96	1.04
T_4	28.46	27.10	55.56	0.95	1.05
T_5	12.69	10.13	22.82	0.80	1.25
Mean	**23.69**	**20.97**	**44.66**	**0.87**	**1.15**
SE(d)	0.78	0.68	1.45	0.03	0.04
CD(0.05)	1.73	1.52	3.24	0.07	0.08

T_1- Coir pith alone (uninoculated control) ; **T_2**- Coir pith with *Pleurotus* sp.; **T_3**- Coir pith with Yeast sludge; **T_4**- Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5**- Coir pith with earthworms.

Fig. 5.1. Changes in temperature during composting of coir pith

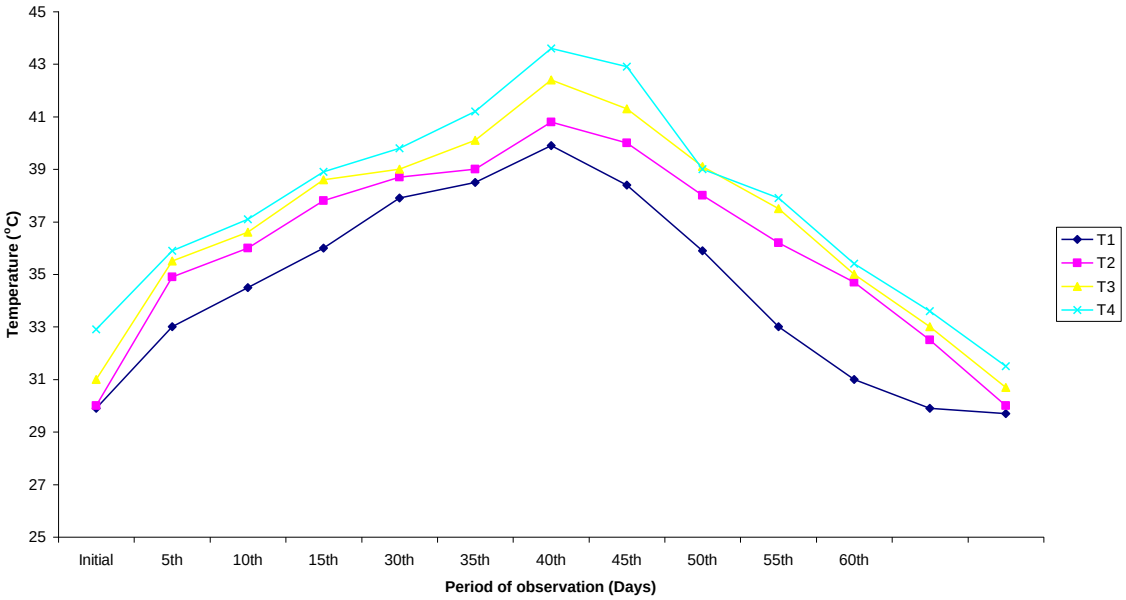
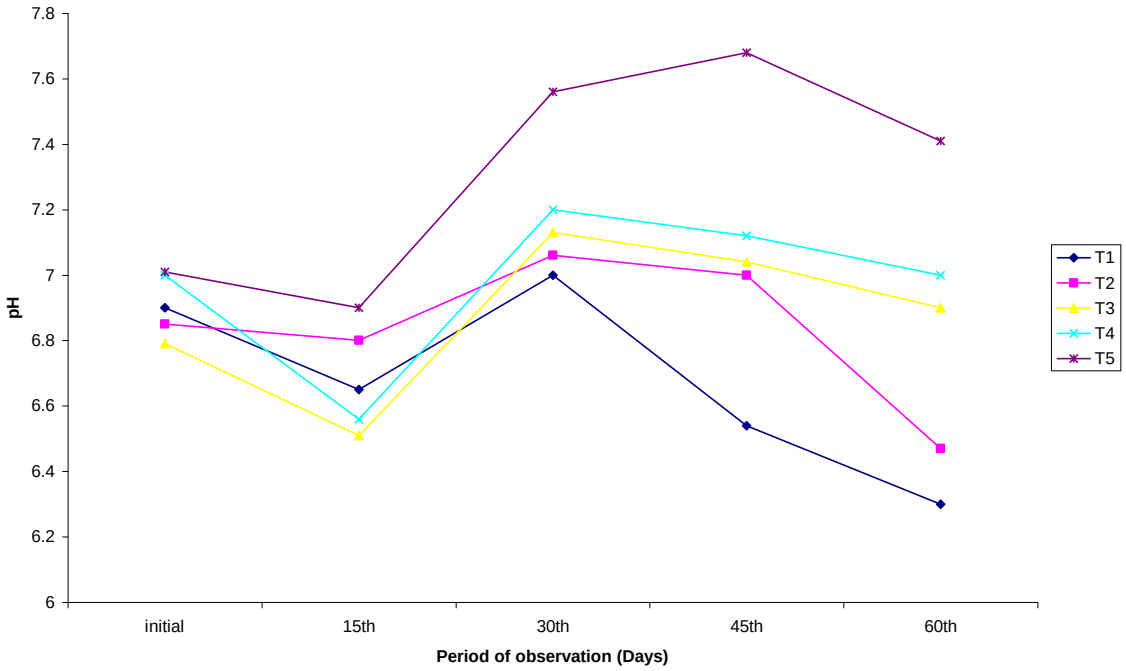


Fig 5.2. Changes in pH during composting of coir pith



T_1- Coir pith alone (uninoculated control) ; **T_2**- Coir pith with *Pleurotus* sp.; **T_3**- Coir pith with Yeast sludge; **T_4**- Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5**- Coir pith with earthworms.

Fig. 5.3. Changes in electrical conductivity during composting of coir pith

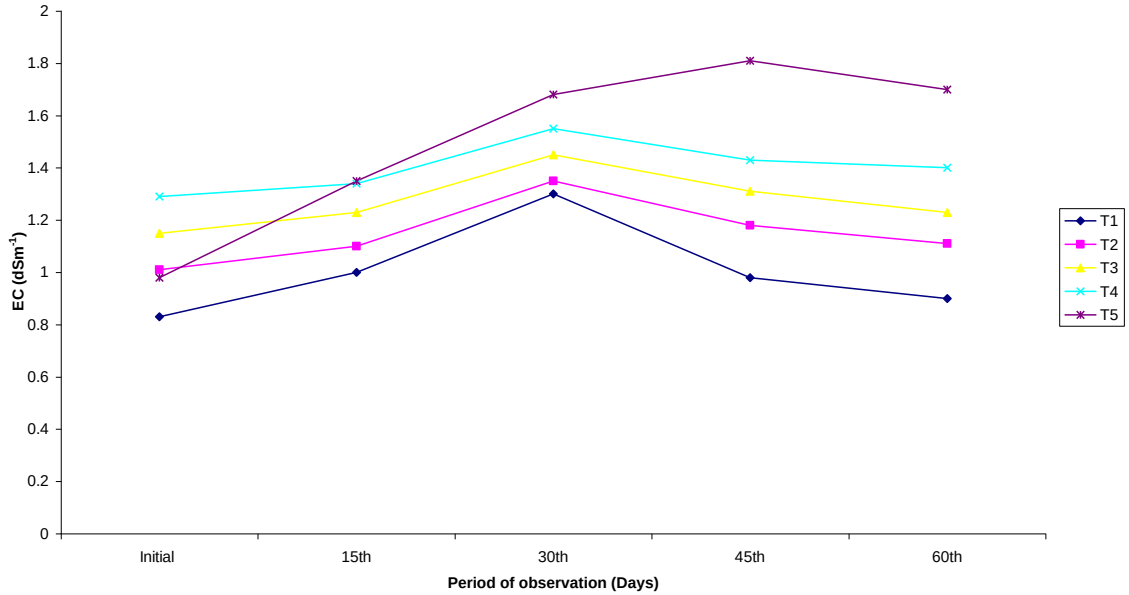
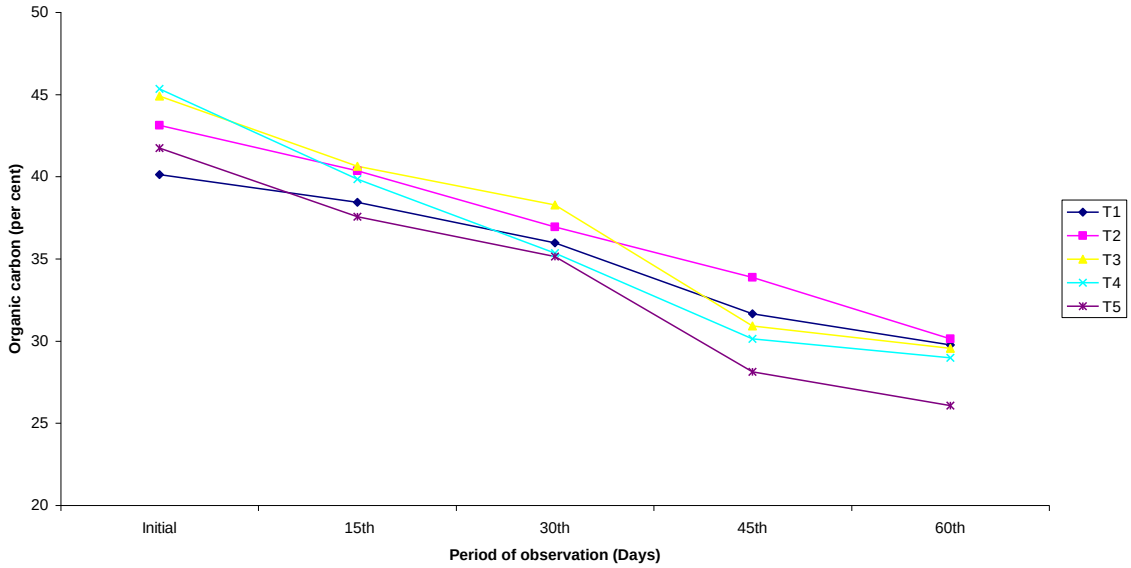


Fig.5.4. Changes in organic carbon content (per cent) during composting of coir pith



T_1- Coir pith alone (uninoculated control) ; **T_2-** Coir pith with *Pleurotus* sp.; **T_3-** Coir pith with Yeast sludge; **T_4-** Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5-** Coir pith with earthworms.

Fig.5.5. Changes in total nitrogen content (per cent) during composting of coir pith

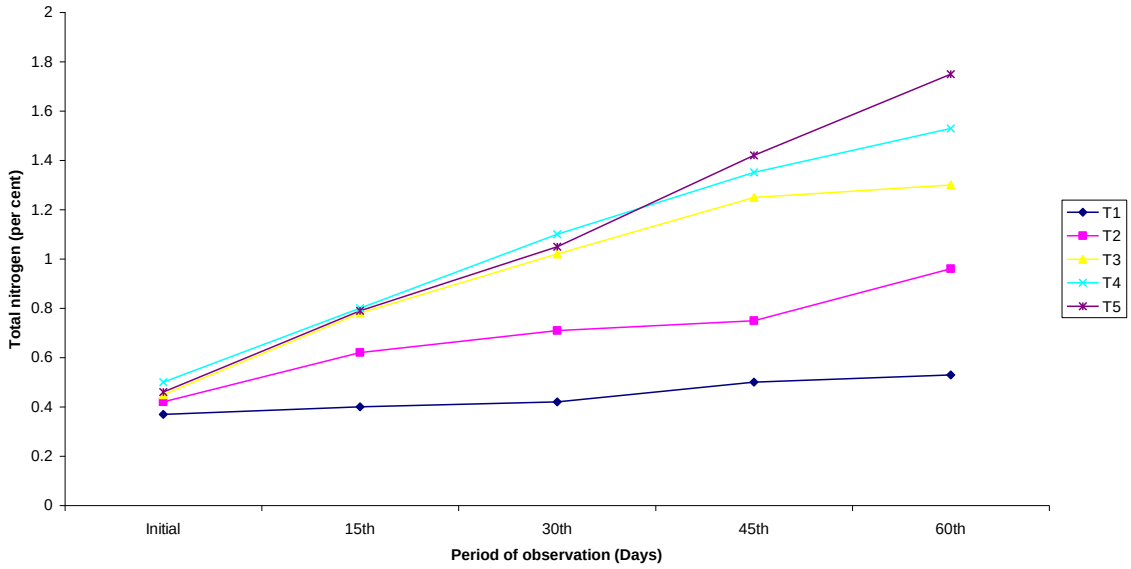
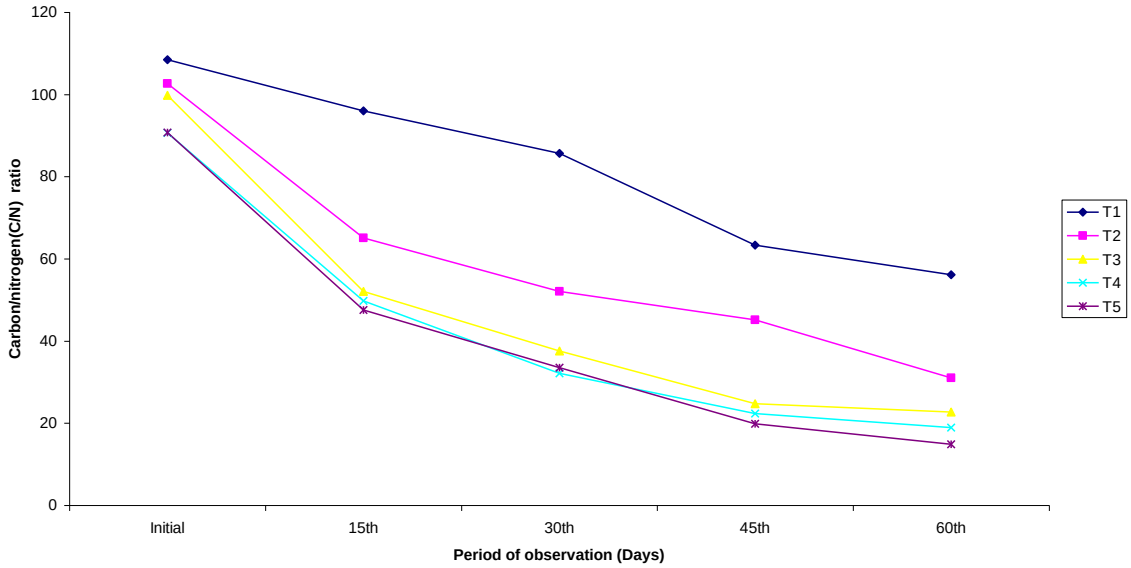


Fig.5.6. Changes in carbon/nitrogen(C/N) ratio during composting of coir pith



T_1- Coir pith alone (uninoculated control) ; T_2- Coir pith with *Pleurotus* sp.; T_3- Coir pith with Yeast sludge; T_4- Coir pith with Yeast sludge and *Pleurotus* sp.; T_5- Coir pith with earthworms.

Fig.5.7. Changes in total phosphorus (per cent) during composting of coir pith

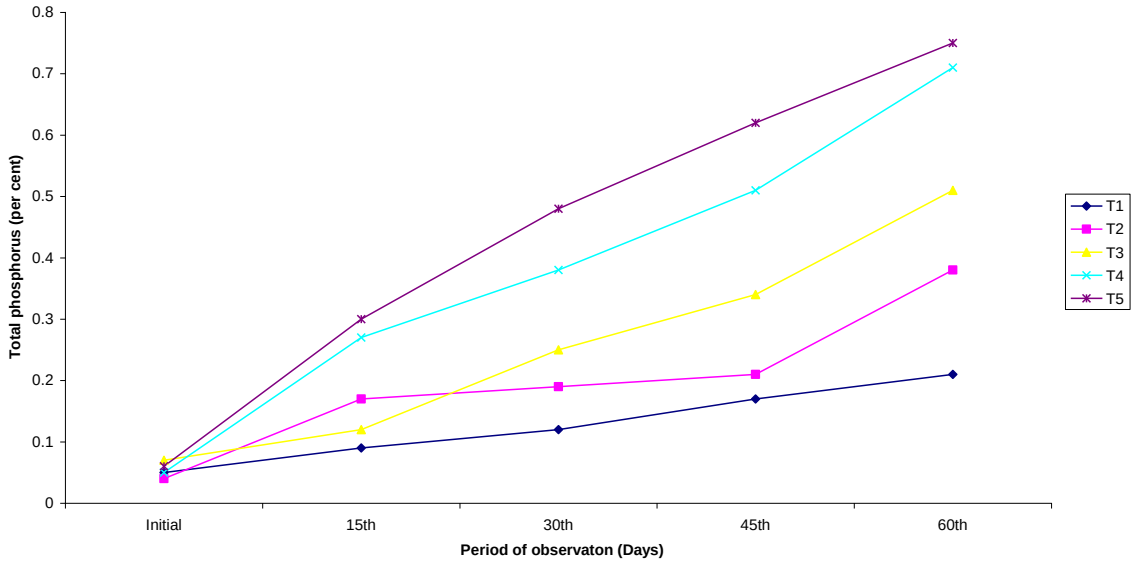
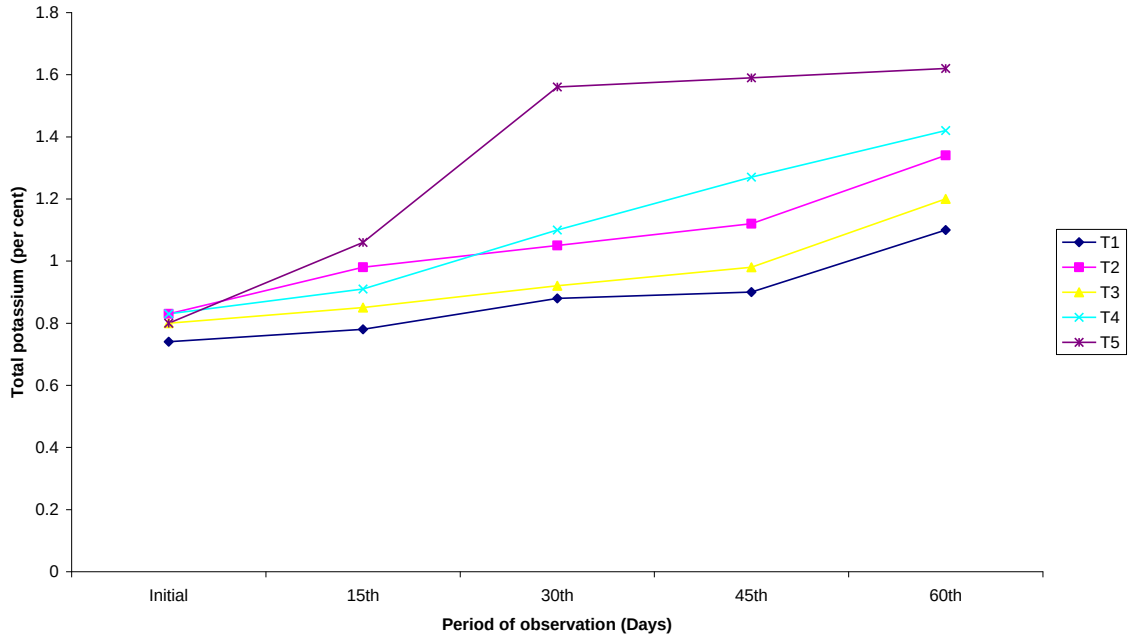


Fig.5.8. Changes in total potassium (per cent) during composting of coir pith



T_1- Coir pith alone (uninoculated control) ; **T_2-** Coir pith with *Pleurotus* sp.; **T_3-** Coir pith with Yeast sludge; **T_4-** Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5-** Coir pith with earthworms.

Fig. 5.9. Population dynamics of mesophilic bacteria during composting of coir pith

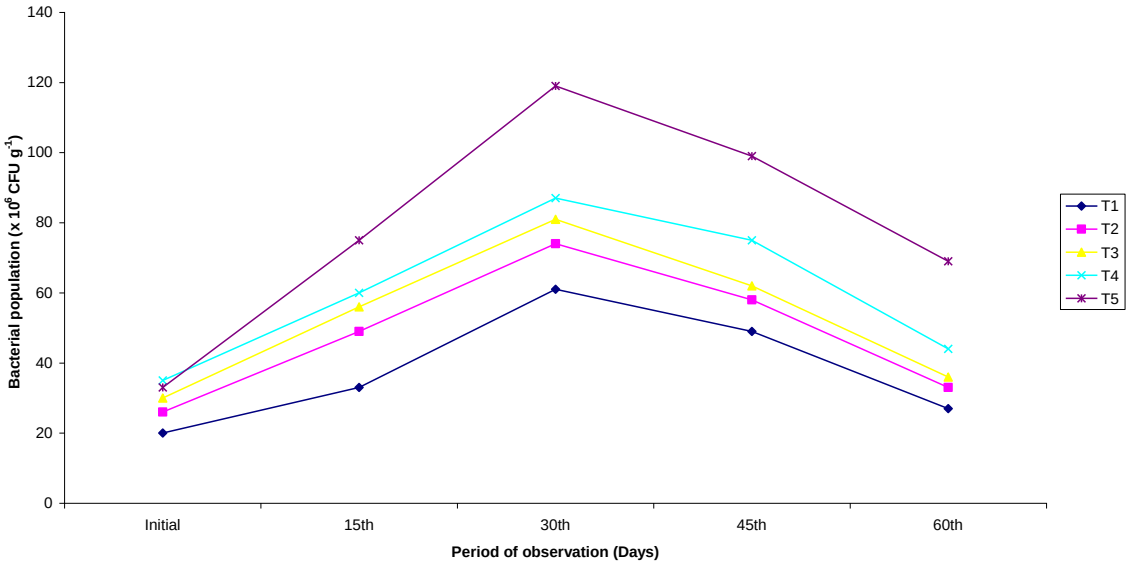
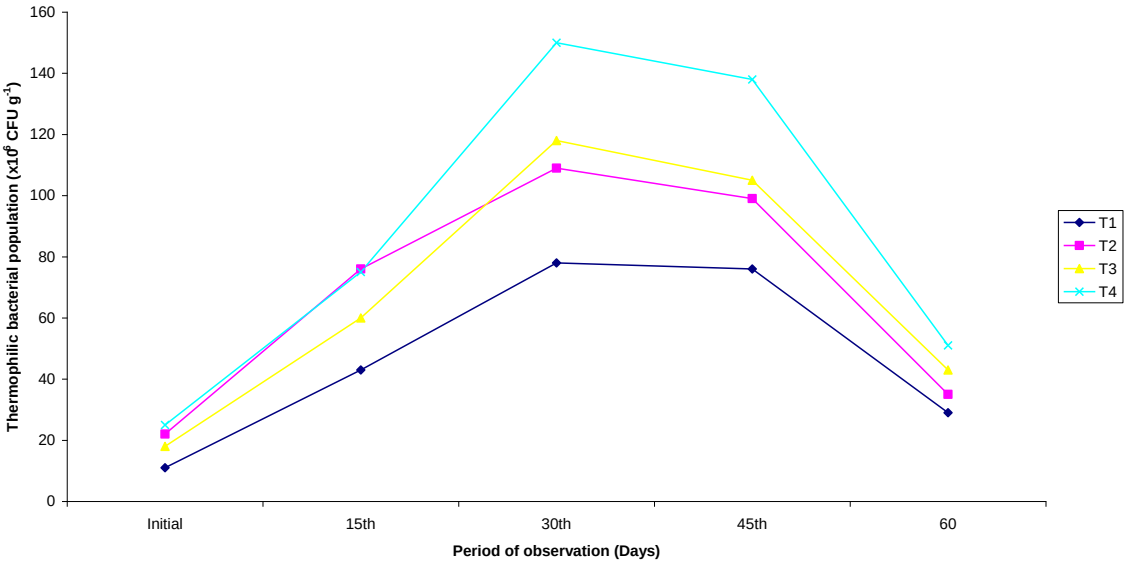


Fig. 5.10. Population dynamics of thermophilic bacteria (45°C) during composting of coir pith



T_1- Coir pith alone (uninoculated control) ; **T_2-** Coir pith with *Pleurotus* sp.; **T_3-** Coir pith with Yeast sludge; **T_4-** Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5-** Coir pith with earthworms.

Fig. 5.11. Population dynamics of mesophilic fungi during composting of coir pith

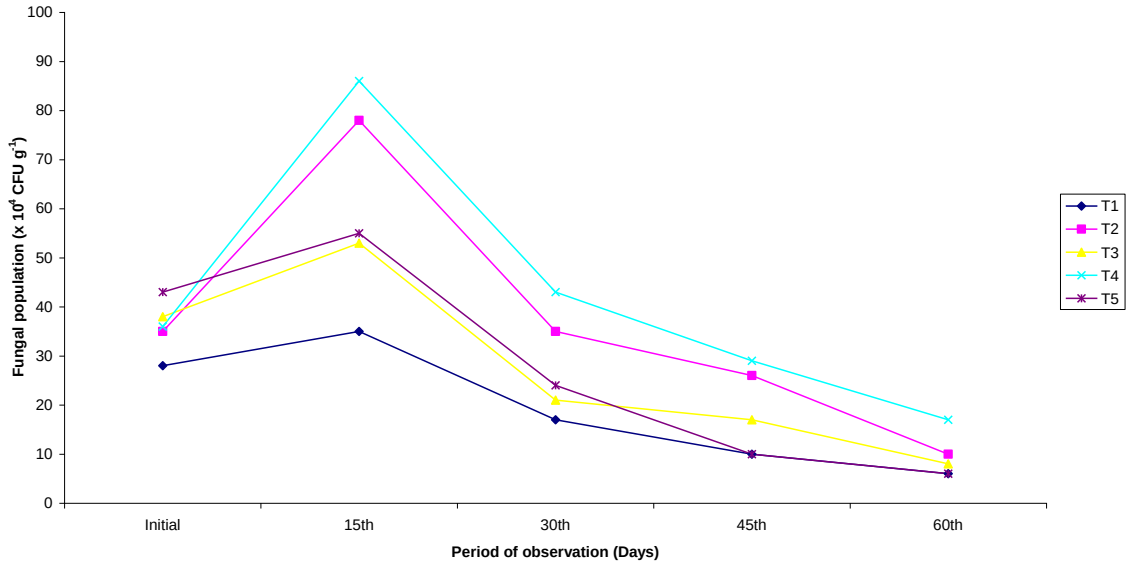
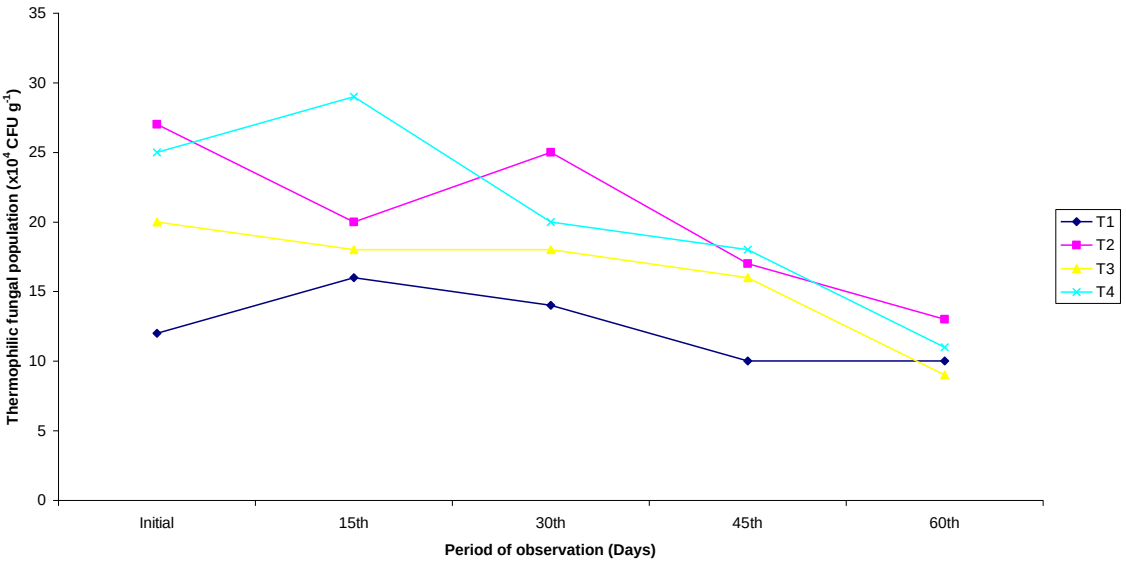


Fig.5.12. Population dynamics of thermophilic fungi (45°C) during composting of coir pith



T_1- Coir pith alone (uninoculated control) ; **T_2-** Coir pith with *Pleurotus* sp.; **T_3-** Coir pith with Yeast sludge; **T_4-** Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5-** Coir pith with earthworms.

Fig.5.13. Population dynamics of mesophilic actinomycetes during composting of coir pith

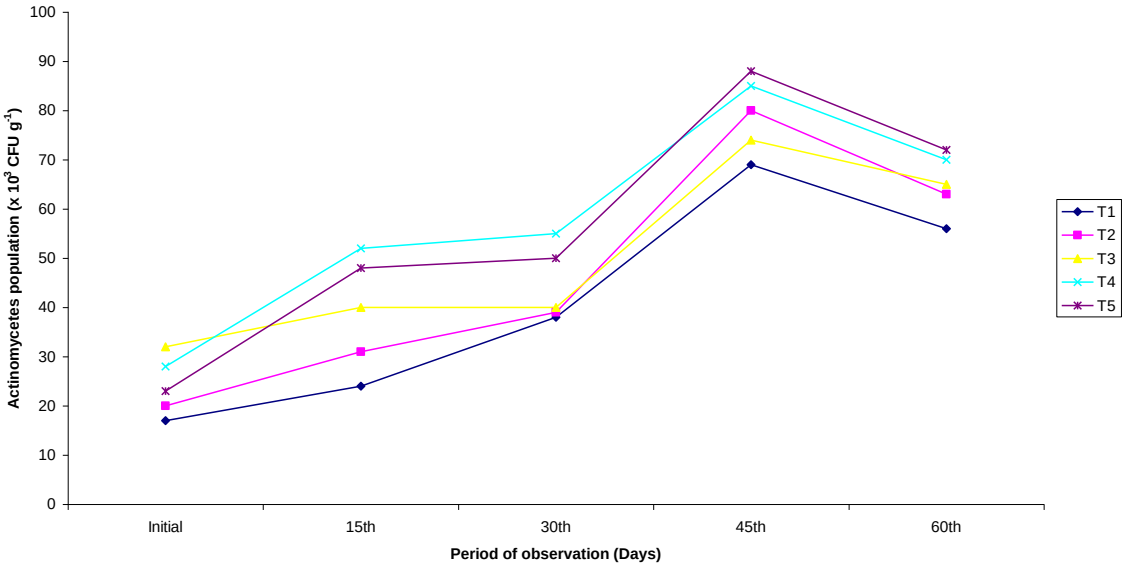
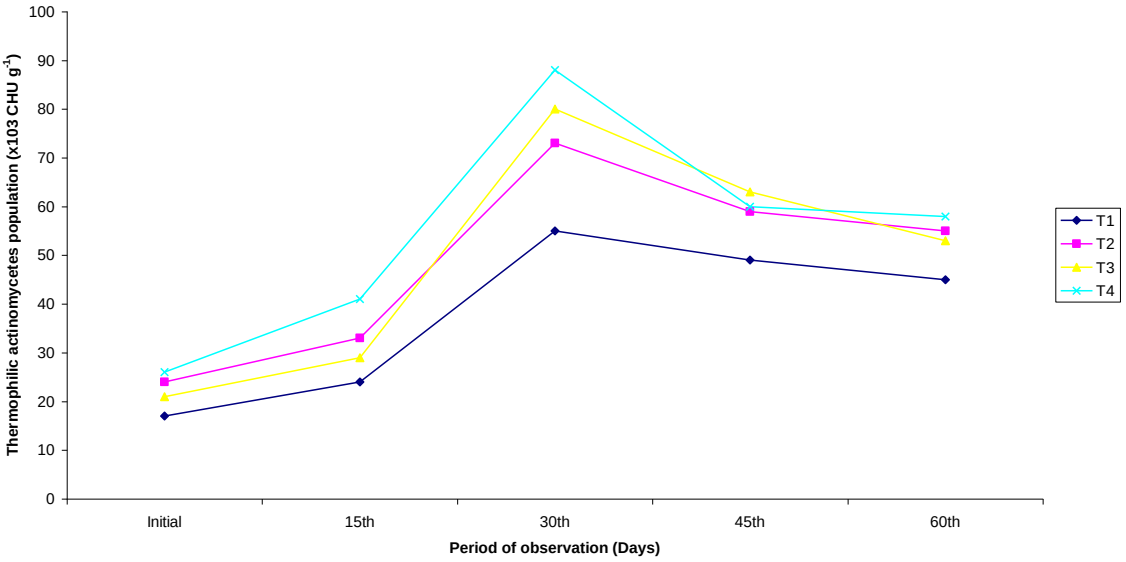


Fig.5.14. Population dynamics of thermophilic actinomycetes (45°C)during of coir pith



T_1- Coir pith alone (uninoculated control) ; **T_2-** Coir pith with *Pleurotus* sp.; **T_3-** Coir pith with Yeast sludge; **T_4-** Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5-** Coir pith with earthworms.

Fig.5.15. Changes in dehydrogenase activity during composting of coir pith

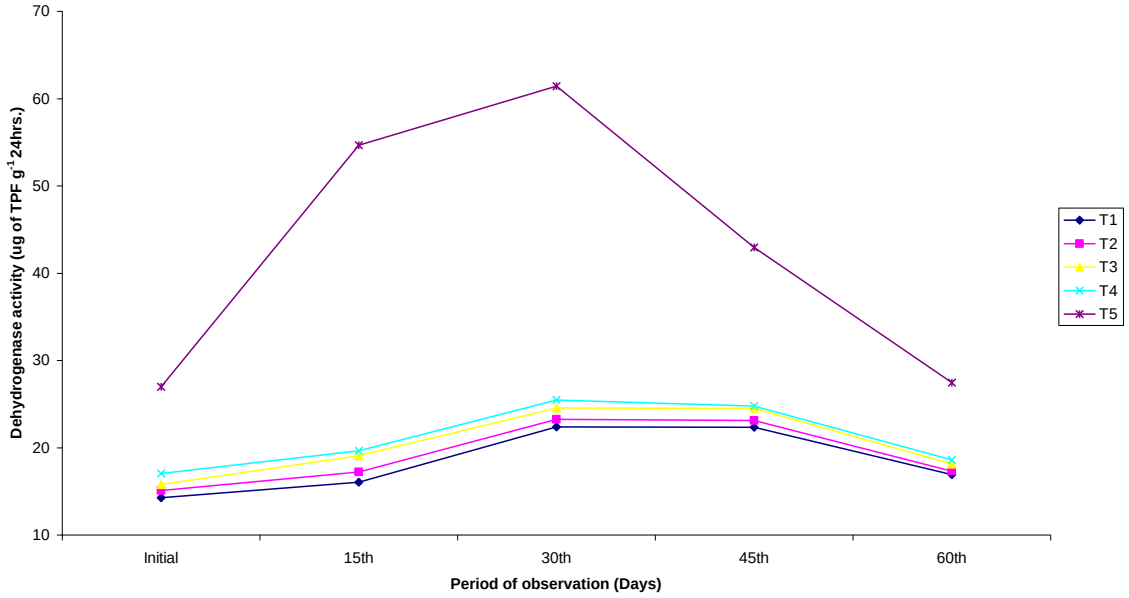
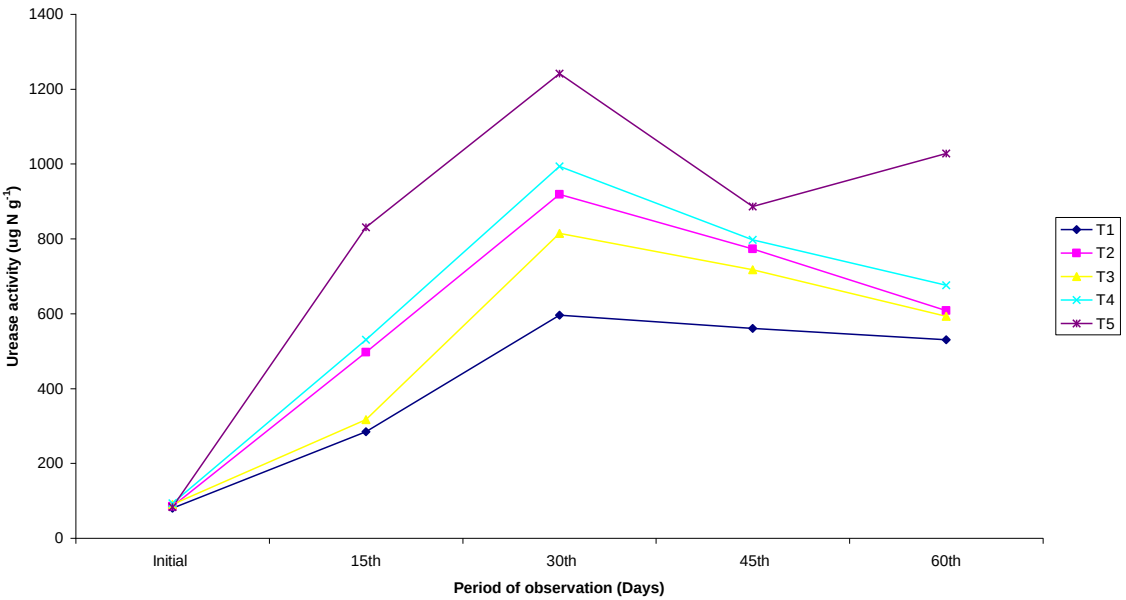


Fig. 5.16. Changes in urease activity during composting of coir pith



T_1- Coir pith alone (uninoculated control) ; **T_2-** Coir pith with *Pleurotus* sp.; **T_3-** Coir pith with Yeast sludge; **T_4-** Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5-** Coir pith with earthworms.

Fig. 5.17. Changes in phosphatase activity during composting of coir pith

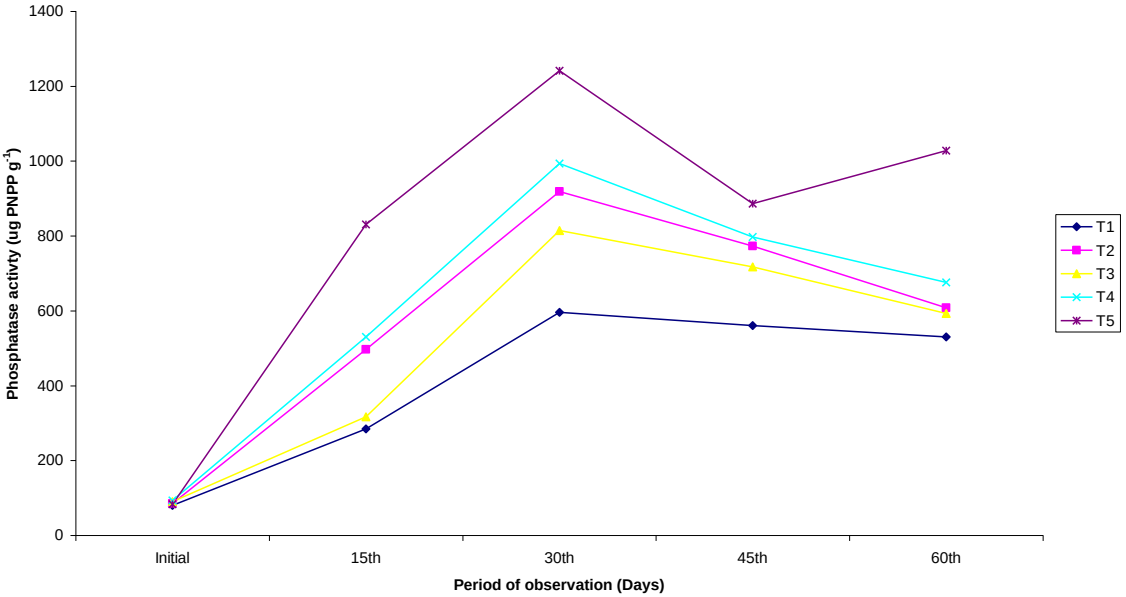
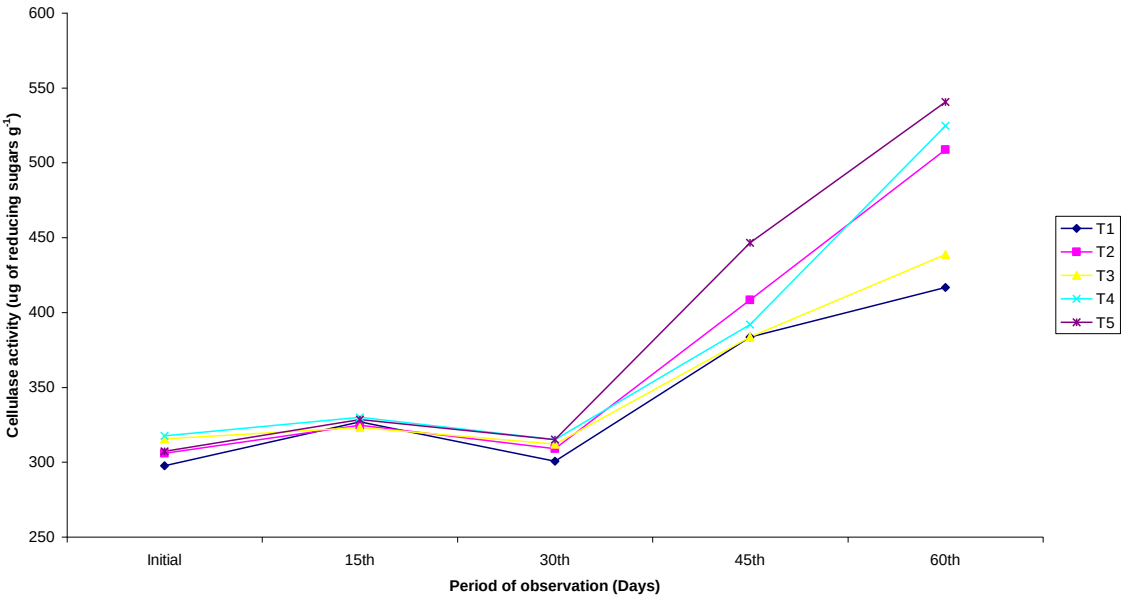
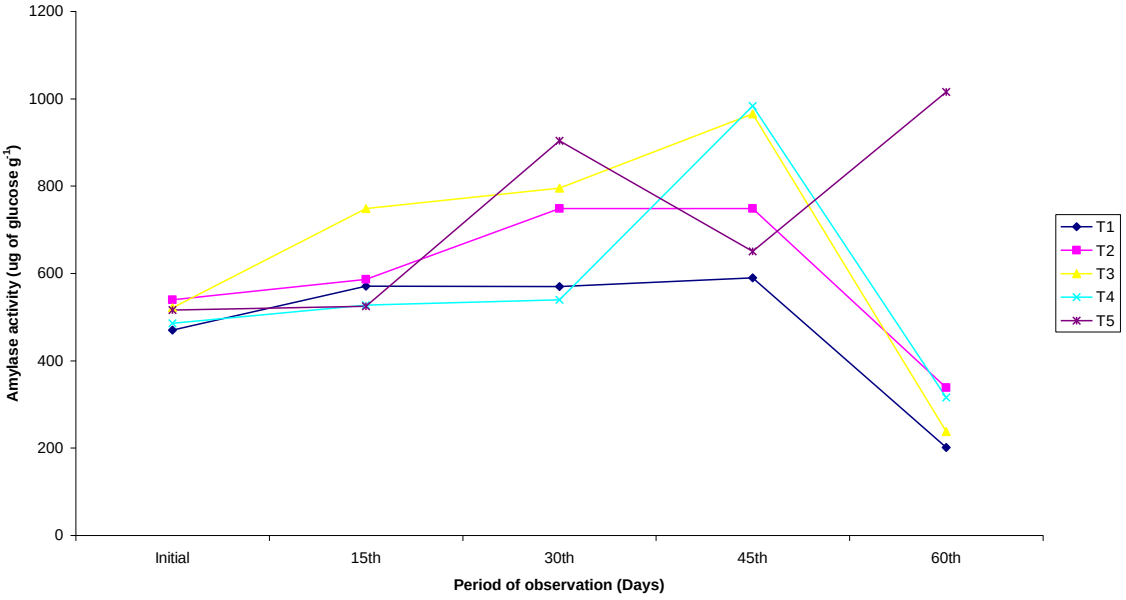


Fig.5.18. Changes in cellulase activity during composting of coir pith

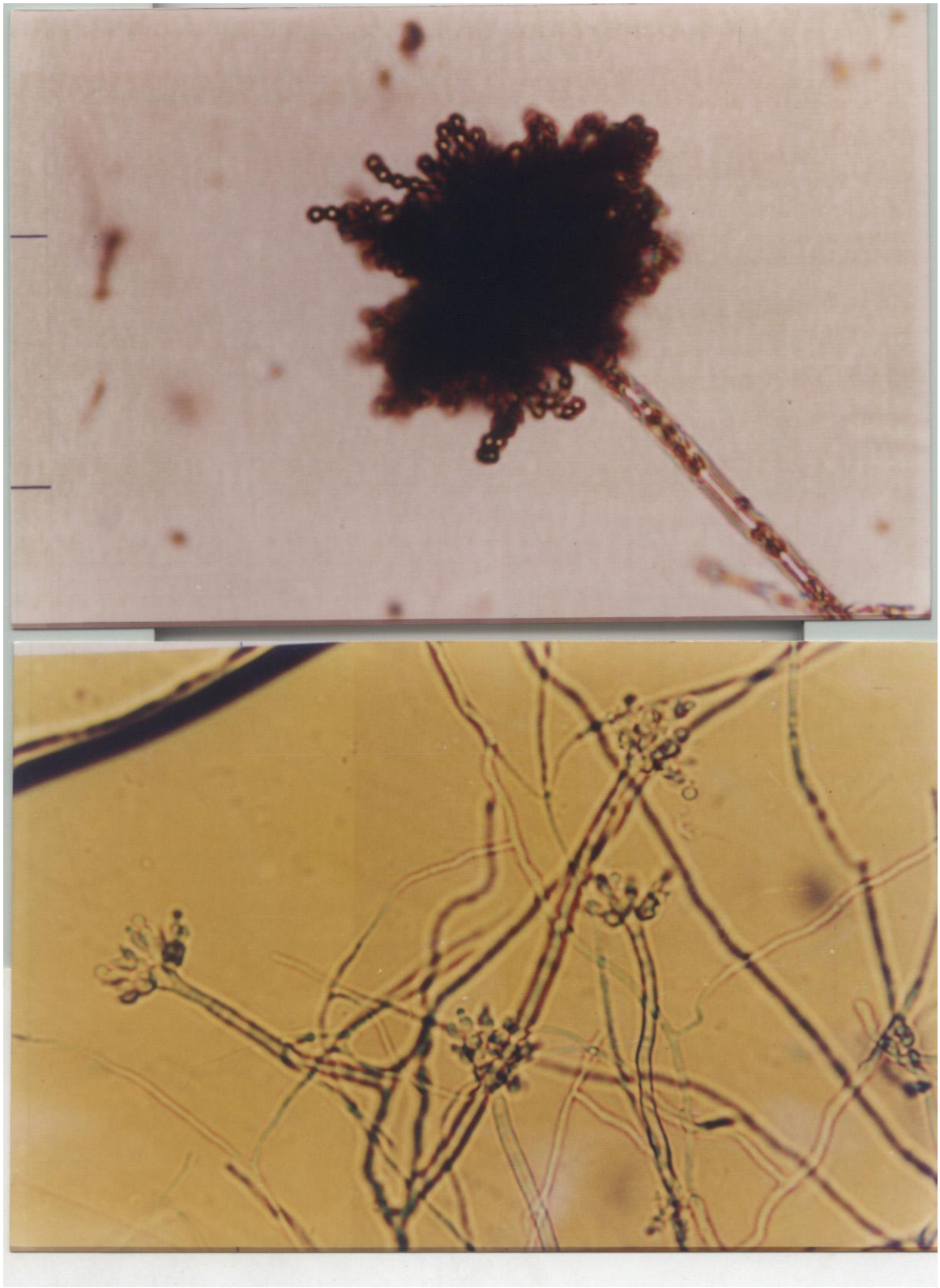


T_1- Coir pith alone (uninoculated control) ; **T_2-** Coir pith with *Pleurotus* sp.; **T_3-** Coir pith with Yeast sludge; **T_4-** Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5-** Coir pith with earthworms.

Fig. 5.19. Changes in amylase activity during composting of coir pith



T_1- Coir pith alone (uninoculated control) ; **T_2**- Coir pith with *Pleurotus* sp.; **T_3**- Coir pith with Yeast sludge; **T_4**- Coir pith with Yeast sludge and *Pleurotus* sp.; **T_5**- Coir pith with earthworms.




STUDY ON ENZYMOLOGICAL CHANGES DURING
COMPOSTING OF ORGANIC WASTES
T1
T2
T3
T4