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# Journal of Advanced Studies in Agricultural, Biological and Environmental Sciences

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Proceedings of Two day National Seminar on

## "Microbial Technologies for Sustainable Environment"

(MTSE-2017)

24<sup>th</sup> & 25<sup>th</sup>, January, 2017



Sponsored by  
**UGC-SERO & APCOST**



Organised by

Department of Microbiology

**GOVERNMENT COLLEGE FOR WOMEN (A), GUNTUR**

Accredited with NAAC 'A' Grade

College with Potential for Excellence by UGC

Guntur-522001, A.P



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ज्ञान - विज्ञानं विमुक्तये

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## MESSAGE

Evolution of Life is amazing from Unicellular Organism to Multi Cellular Organisms. The origin of life is a scientific problem which is not yet solved. Though it is generally agreed that all life today evolved by common descent from a single primitive life form. We do not know how this early form came about, but scientists think it was a natural process which took place perhaps 4 billion years ago.

Discovery of microorganisms in 1674 by Antonie van Leeuwenhoek, using a microscope of his own design triggered exploration of Life and into the magic world of different species of Life and living organisms as Microbiology. Microbes are everywhere and often in very large numbers. One can think of microbes beyond the world in which we all live (i.e., from earth to space). Microbes are an integral part of biogeochemical cycles which are important for our sustenance. A large number of these microbes are beneficial to plants, animals and humans in one form or the other but some are harmful as well.

“Microbes generate more than half of the oxygen we breathe”. It is not an exaggeration to state that life originated with microbes and all life is derived from microbes. Life without higher organisms is possible, but life without microbes is not. Microbiota of an ecosystem has the capability to keep its environment clean, provided it's not overloaded with the pollutants.

Microbial world, a treasure in itself, if exploited judiciously, can contribute to the sustainable development. There are three super challenges of the 21<sup>st</sup> century: (1) climate change, (2) food security, (3) dependence on imported petroleum, and I think microorganisms are efficient enough in meeting out these challenges.

**Climate Change:** The ongoing global climate changes caused by increase in green house gases represent one of the biggest scientific and political challenges of the 21<sup>st</sup> century. There is quite a possibility that changes in climate and land use can be compensated by the homeostasis of the microbial communities.

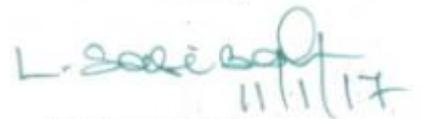
**Food Security:** Population rise is a major concern of our country. As per estimates Indian population will touch to 1.3 billion by 2020 and will require more that 300 million tonnes of grains. Thus there is need of many green revolutions like one we had in late sixties. The constant researches in the area of agricultural microbiology have proved that microbial fertilizers/biopesticides/bioherbicides can contribute significantly in improving the crop productivity. It can be rightly said that microbes—a

goldmine for biofertilizers, but unfortunately, the biofertilizers are not widely utilized by farmers, which could be because of lack of awareness among masses.

Dependence on imported Petroleum: Other greatest challenge in the 21st century is to meet the growing energy demand world over and reduce the dependency on imported petroleum. The future risks of global warming, depletion of oil reserves and environmental concerns promote the production and usage of alternative fuels; bioalcohols. The ethanol is economically produced from sugarcane and corn. To make ethanol bioprocessing from lignocellulosics economically microbial intervention is the key.

Government College for Women (Autonomous) at Guntur attempts to rediscover the magical potential of these microbes integrating with the most current technological initiatives in order to restore the eco balance and potentiate the environmental sustainability. I congratulate every member of faculty in the Department of Micro Biology, for organising this innovative brainstorming by way of a National Seminar supported by the UGC, and wish them all a grand success.

Yours Sincerely



(Dr. L. Sasibala)

## MESSAGE

It gives me great pleasure to note that the Department of Microbiology of Government College for Women (Autonomous), Guntur is organizing a UGC Sponsored National Seminar on Microbial Technologies for Sustainable Environment (MTSE-2017) on 24<sup>th</sup> & 25<sup>th</sup> January 2017 in Collaboration with Andhra Pradesh State Council of Science & Technology (APCOST).

The environmental friendly processes have been gaining international attention in recent years as an advanced technology in many production processes and industries. The seminar will focus on various applications of microbial technologies in the fields of agriculture, bio-energy, waste management, effluent treatment, biomining, bioplastics, carbon sequestration etc. leading to sustainable environment.

Microbes have been exploited for their specific biochemical and physiological properties from the earliest times for baking, brewing, and food preservation and more recently for producing antibiotics, solvents, amino acids, feed supplements and chemical feedstuffs. In addition, microorganisms are factories for industrial enzymes production, which are used to enhance detergents, to clean up toxic wastes, to replace chemicals in paper, pulp and leather processing, and for oil extraction. Furthermore, microorganisms and their enzyme systems are responsible for the degradation of organic matter and xenobiotics. Currently, wastewater treatment uses microbes to decompose organic matter in sewage. Microbes can also be used to create biofuels like biogas or bioethanol and in metal extraction.

The seminar on Microbial technologies leading to sustainable environment has identified a number of current areas which will throw light during current sessions under various themes. On behalf of MTSE-2017 and the Advisory Committee I, cordially invite all to this seminar and assure a lot of insights from eminent speakers and delegates.

Dr. K. Sucharita

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**ENZYME CATALYSIS IN GREEN CHEMISTRY : A SUSTAINABLE TECHNOLOGY****RAFIYA SULTANA, M. KAMALA KARUNA**

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**Abstract**

Enzyme catalysis is a rapidly emerging technology in green chemistry which is a environmental benign green synthesis, economically feasible, environmental friendly. Enzymes play an important role in green chemistry. Use of enzymes turns a chemical synthesis into green synthesis by accelerating the rate of the reaction, increasing the specificity of a reaction, reducing/avoiding solvents, decreasing the number of steps, increasing the yield and most importantly by substituting the hazardous chemicals. Enzyme catalysis work by lowering the activation energy of a chemical reaction. Enzymes are reusable and biodegradable that make them green catalysts. Enzyme catalysis mainly increases our quality of life and natural environment, focusing mainly on the sustainable development.

**Keywords :** Enzymes, Biocatalysts, Green chemistry, Non hazardous, stereo specific

**Introduction**

Ever since the World War II, industrialization accelerated the use of chemicals and reagents in laboratories and industries depleting not only the environment but also human health and safety. This resulted in increased global warming, depletion of the ozone protective layer that protects against harmful UV radiation, contamination of both land and water due to the release of toxic chemicals by industry, and the reduction of nonrenewable energy resources like petroleum [1]. However, there is a growing awareness about the risks that are associated with chemicals and the need of substitution from any chemical that are recognized as being hazardous. In this regard, that there is a emerging need for more environmentally acceptable processes in the world of chemistry. This resulted in greener chemistry or what is known as 'Green Chemistry' [2] or 'Sustainable Technology'. Green chemistry is efficiently utilizes preferably renewable raw materials, eliminates waste and avoids the use of toxic or hazardous substances and minimal or no use of solvents.

Green chemistry focuses the environmental impact of both chemical products and the processes by which they are produced. Green Chemistry is a primary pollution prevention rather than waste remediation. Therefore green chemistry is the ultimate goal for sustainable development and meeting the needs of the present and future generations. The technology that has become a central part of green chemistry is Biocatalysis/Enzyme catalysis as enzymes are biodegradable especially in pharmaceutical industries. Especially enzyme catalysis is useful when a drug is difficult to synthesize or require multiple steps or large amount of solvents . Using enzymes that are proteins accelerates chemical reactions and has immediate advantage in turning a chemical process/synthesis a green synthesis. Enzymes are natural catalysts, present in every living organism that carry out a wide variety of chemical reactions. In addition, because they are typically produced by fermentation from sugars, enzymes are truly renewable catalysts.

Enzymes catalyzed reactions have emerged as preferred tools in green chemistry and increasingly used in industrial processes as they catalyze reactions with high catalytic efficiency and specificity without polluting environment by avoiding by-products or waste products. Enzymes have found their application in many environment friendly industrial and chemical processes today because they have the ability to avoid the use of metals and organic solvents [3, 4]. Since most of the enzyme catalyzed reactions are in aqueous phase, they have wide applications in several aspects of our life, such as detergents in pharmaceutical and food processing industries, for clinical and diagnostics research and also for organic synthesis in non-aqueous phase. Besides their role as catalysts some enzymes act as because of their binding affinity and specificity [5].



### Few Examples of Enzymes Catalysis

**Chiral Intermediates for Atazanavir, HIV inhibitor:** Atazanavir [6, 7] is an acyclic aza-peptidomimetic, a effective HIV protease inhibitor approved by the Food and Drug Administration (FDA) for treatment of AIDS. It is prepared from (1S,2R)-[3-chloro-2-hydroxy-1-(phenylmethyl) propyl]carbamic acid, 1,1-dimethylethyl ester 22, a key chiral intermediate required for the total synthesis of the HIV protease inhibitor atazanavir that was carried out using *Rhodococcus*, *Brevibacterium*, and *Hansenula* strains [8]. C-13 Paclitaxel Side-Chain Synthon of Anticancer Drug paclitaxel: a variety of cancers were treated with paclitaxel which was approved by FDA for treatment of ovarian cancer and metastatic breast cancer [9]. C-13 Paclitaxel Side-Chain Synthon of paclitaxel was synthesized using two strains of *Hansenula* [10].

**Anti-Alzheimer's Drugs:** Ethyl-(S)-5-hydroxyhexanoate and (S)-5-hydroxyhexanenitrile are important chiral intermediates in the synthesis of anti-Alzheimer's drugs. Both the chiral compounds were prepared by using *Pichia methanolica* SC 16116 [11].

### Mechanism/Stereochemistry of enzyme catalyzed reaction

Enzyme catalyzed reactions are stereospecific and produces selectively only one stereoisomer because the binding site of an enzyme is chiral in nature. The chiral binding site also known as active site of the enzyme restricts the reagents to bind to only one side of the functional group of the reactant. As a result, selectively produces only one stereoisomer is formed or selectively catalyses the reaction of only one stereo isomer.

The enzyme will bind only the stereoisomer whose functional groups/substituents are in the correct positions with respect to the substituents in the chiral binding site. Other stereoisomers do not have substituents in the proper positions, so they cannot bind efficiently to the active site of the enzyme. An enzyme's stereospecificity can be compared to that of right handed glove which fits only the right hand.

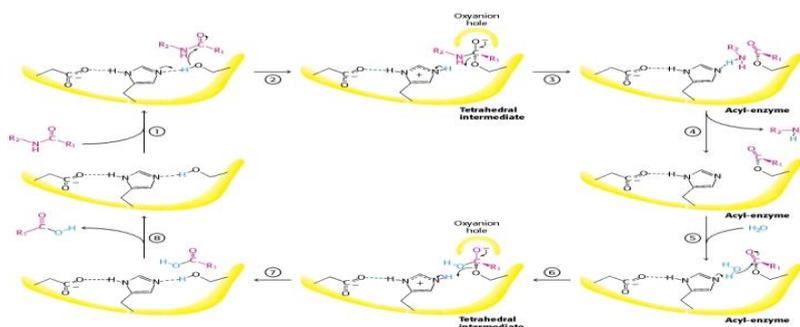
Active sites are generally clefts or grooves present on the surface of an enzyme, usually made up of amino acids of the properly folded protein. Substrates bind to the active site by non-covalent interactions such as including hydrogen bonds, ionic bonds, van der waal's interaction and hydrophobic interactions. Once a substrate is bound within the active site of an enzyme, multiple mechanisms can accelerate its conversion to the product of the reaction. The enzyme provides a template upon which the reactants are brought together and properly oriented to favor the formation of the transition state in which they interact [12].

The configurations of both the enzyme and substrate are modified by substrate binding a process called induced fit but not lock and key model as proposed earlier. The stress created by such distortion of the substrate can promote its conversion to the transition state by weakening critical bonds. Furthermore, the transition state is stabilized by its tight binding to the enzyme, thereby lowering the required energy of activation that is the reason for the increased rate of the reaction. In addition to bringing substrates together, many enzymes can affect the catalytic process, where specific amino acid side chains in the active site region may react with the substrate and forming reaction intermediates. Generally acidic and basic amino acids such as aspartic acid, glutamic acid and histidine etc., are often involved in these catalytic mechanisms.

For example Chymotrypsin is a member of a family of serine proteases that digest proteins by catalyzing the hydrolysis of specifically peptide bonds present between hydrophobic amino acids such as tryptophan, phenylalanine and trypsin. digests The structure of all serine proteases, is similar and use the same mechanism of catalysis. The active sites of these enzymes contain three crucial amino acids serine, histidine, and aspartate that assist the hydrolysis of the peptide bond where serine acts as a nucleophile (Figure 1) [13]. Chymotrypsin preferentially cleaves specifically to bulky hydrophobic amino acids is due to the formation of S1 pockets near the active site which is lined with relatively hydrophobic residues such as Ser, Trp and Gly. Chymotrypsin catalyzes the reaction rate by a factor of  $10^9$ . The reaction has two steps, an acylation stage and a deacylation stage as shown in the following Figure 1. In the earlier stage, the peptide bond is cleaved and an



ester is formed between substrate and enzyme. In the latter stage, this ester is hydrolyzed and the enzyme is regenerated.



**Figure 1:** Enzyme catalytic mechanism of serine proteases indicating Acylation, Tetrahedral Intermediate (oxyanion hole) and Deacylation (Yellow colored line: protein backbone)

**Source:** [https://en.wikibooks.org/wiki/Structural\\_Biochemistry/Enzyme\\_Catalytic\\_Mechanism/Proteases/Chymotrypsin](https://en.wikibooks.org/wiki/Structural_Biochemistry/Enzyme_Catalytic_Mechanism/Proteases/Chymotrypsin)

### Conclusion

Enzyme catalysis are stereo specific reactions that is there are no by-products which attracts the pharmaceutical industries in drug synthesis. The use of enzymes in green synthetic reactions produces selectively the desired product under mild conditions which reduces the resources such as energy and water therefore benefiting the industry and the environment. There is need of educating new generation about the green catalysts (enzymes), green synthesis and green chemistry for the sustainable development of both society and the environment.

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## PROMISING ROLE OF GENETICALLY MODIFIED ORGANISMS IN MITIGATING HEAVY METAL TOXICITY

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### ABSTRACT

Heavy metal contamination is one of the most significant environmental issues, since metals are highly toxic to all the forms of life, as they decline the metabolic activity and diversity, and they affect the structure of living organisms both qualitative and quantitatively. For treating heavy metal contaminated tailings and soils, bioremediation is the most cost-effective method, and promising method, although various heavy metals are beyond the bioaccumulation capabilities of microorganisms. Perhaps, because of the toxicity of these compounds, microorganisms have not evolved appropriate pathways to bioaccumulate them; populations of microorganisms responsible for this bioaccumulation are not large or active enough to remove these compounds completely, or complex mixtures of pollutants resist removal by existing pathways. The tools of genetic engineering looks most promising in helping out to most extent.

**Key words:** Bioremediation, bioaccumulation, heavy metals

### Introduction

Bioremediation involves the use of plants or microorganisms, viable or not, natural or genetically engineered to treat environments contaminated with organic molecules that are difficult to break down (xenobiotics) and to mitigate toxic heavy metals, by transforming them into substances with little or no toxicity, hence forming innocuous products (Dobson & Burgess, 2007; Li & Li, 2011). Basically there are two different strategies.

### List of heavy metals that are posing environmental toxicity:

Heavy metals are considered to be chemical elements with an atomic mass greater than 22 and a density greater than 5g/mL. This definition includes 69 elements, of which 16 are synthetic. Some of these elements are extremely toxic to human beings, even at very low concentrations (Roane & Pepper, 2000; Wang & Chen, 2006).

The main heavy metals associated with environmental contamination, and which offer potential danger to the ecosystem, are copper (Cu), zinc (Zn), silver (Ag), lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), strontium (Sr), cesium (Cs), cobalt (Co), nickel (Ni), thallium (Tl), tin (Sn) and vanadium (V) (Wang & Chen, 2006).

Generally metal ions are classified as: 1) Essential and important for metabolism (Na, K, Mg, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Mo and W); 2) Toxic heavy metals (Hg, Cr, Pb, Cd, As, Sr, Ag, Si, Al, Tl), which have no biological function (in ecotoxicology terms, hexavalent forms of Hg, Cr, Pb and Cd ions are the most dangerous); 3) Radionuclides (U, Rn, Th, Ra, Am, Tc), which are radioactive isotopes and, although toxic to cells, they are nonetheless important in nuclear medicine procedures; 4) Semi-metals or metalloids (B, Si, Ge, As, Sb, Te, Po, At, Se), which exert distinct biological effects on metals.

### Mechanism of action of heavy metal toxicity

Most heavy metals are cations and this determines their sorption to negatively charged functional groups that are present in: cell surfaces, which are generally anionic at a pH of between 4 and 8; surfaces with residual hydroxides (OH<sup>-</sup>) or thiol (SH<sup>-</sup>) and anionic salts, such as PO<sup>4-</sup> and SO<sup>4-</sup>, humic acid, and clay minerals (Roane & Pepper, 2000).



The great electrostatic attraction of Heavy metal ions and high binding affinities compete for sites that essential metal ions normally bind to various cellular structures, causing destabilization of the structures and biomolecules (cell-wall enzymes, DNA and RNA), which results in replication defects, mutations, hereditary genetic disorders and cancer. This occurs, for example, with arsenate, which competes with phosphate, and cadmium, which competes with zinc. By employing microarray technology, Kawata et. al. (2007), found that six heavy metals (arsenic, cadmium, nickel, antimony, mercury and chromium) induce gene expression patterns that are very similar to the pattern induced by DMNQ (2,3-dimethoxy-1, 4-naphthoquinone), the reactive oxygen species (ROS) chemical generating agent, which causes "oxidative stress", leading to deleterious effects (membrane damage or other cellular lipid structures, modification of proteins, fragmentation and cross-links, changes in DNA that can induce mutations or be repaired by repair mechanisms).

Therefore, the ions of heavy metals cause oxidative damage, both directly, by producing ROS, and indirectly, by inactivating the cellular antioxidant system, thus leading to cell damage (Mannazzu et. al., 2000; Liu et. al., 2005).

#### Bioremediation takes hand over conventional technologies

Heavy metals loaded in Environments are treated by means of conventional technologies based on physicochemical principles, which are considered inefficient and uneconomic. It is more complex and requires several steps: 1) precipitation with hydroxides, carbonates or sulfides; 2) redox chemistry; 3) sorption (adsorption with activated carbon/ion exchange); 4) use of membranes (ultrafiltration, electro dialysis and reverse osmosis-RO); 5) electrolytic recovery; 6) evaporation; 7) liquid-liquid extraction; 8) electrodeposition. This procedure produces large quantities of mud in the final wastewater with concentrations of metals in the order of mg/L, which is difficult to dispose off. (Goyal et. al., 2003; Tabak et. al., 2005; Hameed, 2006; Machado et. al., 2008; Wang & Chen, 2009).

1. Biostimulation, is the process where autochthonous microorganisms are grown at the contaminated site, in order to introduce pH-correction substances, nutrients, surfactants and oxygen. where as 2. Bioaugmentation or bioaddition, is the addition of genetically modified organisms (GMO) to indigenous to complement with certain ecophysiological characteristics compatible with the habitat conditions that are conducive to the promotion of bioremediation (Vidali, 2001; Silva et. al., 2004; Gaylard et. al., 2005; Li & Li, 2011).

Here are few examples of microorganisms studied for bioremediation of heavy metal.

**Bacteria** : *Arthrobacter*, *Bacillus sp*, *Citrobacter*, *Cupriavidus metallidurans*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Streptomyces sp*, *Zoogloea ramigera*

**Archea**: *Filo Crenarchaeota*, *Phanerochaete chrysosporium*

**Fungi**: *Aspergillus tereus*, *Penicillium chrysogenum*

**Yeast**: *Candida utilis*, *Hansenula anomala*, *Rhodotorula mucilaginosa*, *Rhodotorula rubra* GVa5, *Saccharomyces cerevisiae*

Table 1. Genetically engineered bacteria for remediation of heavy metals.

Heavy Metal	Initial Conc. (ppm)	Removal Efficiency (%)	Genetically Engineered Bacteria	Expressed Gene
As	0.05	100	<i>E. coli</i> strain	Metalloregulatory protein ArsR
Cd <sup>2+</sup>	--	--	<i>E. coli</i> strain	SpPCS
Cr <sup>6+</sup>	1.4–1000	100	<i>Methylococcus capsulatus</i>	CrR



Cr	--	--	<i>P. putida</i> strain	Chromate reductase (ChrR)
Cd <sup>2+</sup> , Hg	--	--	<i>Ralstonia eutropha</i> CH34, <i>Deinococcus radiodurans</i>	merA
Hg	--	--	<i>E. coli</i> strain	Organomercurial lyase
Hg	7.4	96	<i>E. coli</i> JM109	Hg <sup>2+</sup> transporter
Hg	--	--	<i>Pseudomonas</i> K-62	Organomercurial lyase
Hg	--	--	<i>Achromobacter</i> sp AO22	mer
Ni	145	80	<i>P. fluorescens</i> 4F39	Phytochelatin synthase (PCS)

(Dixit R et al., 2015)

Higher organisms respond and tolerate to some extent the presence of metals, with the production of cysteine-rich peptides, such as glutathione (GSH) (Singhal et al., 1997), phytochelatins (PCs) and metallothioneins (MTs) (Mehra & Winge, 1991), which can bind and sequester metal ions in biologically inactive forms (Hamer, 1986; Bae et al., 2000).

Table 2. List of selected plants reported for phytoremediation of heavy metals.

S.No	Heavy Metal	Plant Species
1	Cd, Cu, Pb, Zn	<i>Salix</i> spp. ( <i>Salix viminalis</i> , <i>Salix fragilis</i> )
2	Cd	Castor ( <i>Ricinus communis</i> )
3	Cd, Pb, Zn	Corn ( <i>Zea mays</i> )
4	Cd, Cu, Pb, Zn	<i>Populus</i> spp. ( <i>Populus deltoides</i> , <i>Populus nigra</i> , <i>Populus trichocarpa</i> )
5	Cd, Cu, Ni, Pb	<i>Jatropha</i> ( <i>Jatropha curcas</i> L.)
6	Hg	<i>Populus deltoides</i>
7	Se	<i>Brassica juncea</i> , <i>Astragalus bisulcatus</i>
8	Zn	<i>Populus canescens</i>

(Dixit R et al., 2015)

### Concern over survivability and stability of GMOs

Although the utilization of GMOs in the field has been limited due to possible risks involved in the horizontal transfer of genetic material, the results that have been obtained are nevertheless important in assessing the benefits and obstacles associated with their applications in bioremediation. Such knowledge is necessary in view of the future possibility of releasing GEMs into contained environments for bioremediation. To be of practical use in the field, a bacterial GMO must be able to survive and grow in such environments. Important parameters in this regard are growth rate, inoculum size, environmental conditions, including spatial distribution, and the presence of competing microorganisms.

The spatial distribution of a GMO introduced into the environment is important because it helps define its interactions with the members of the indigenous bacterial community and other components of the ecosystem. In general, a bacterium that has been recently isolated from a natural environment is more likely to



survive when released back into that same environment. A crucial consideration regarding the introduction of engineered bacteria into field sites is their effect on the structure and function of natural ecosystems.

#### Future Prospects

Rapid industrialization and technology development have adverse side effects like soil contamination and degrading soil health. Due to the complexity involved in the conventional methods for remediation of soil, the use of microbes has arisen as a time-saver for bioremediation. The reluctance among the public to accept GEM for bioremediation also needs to be considered in future studies, and they must prove non-toxic to the environment.

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## THE SCOPE OF BACTERIAL AMYLASES FOR BIODEGRADATION

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### ABSTRACT

Most of the microorganisms present in the nature have decomposing activity. The microbial enzymes are more stable and obtained easily. Amylases are among the most important enzymes and have a great significance in present day industry. Starch degrading bacteria find great applications in industries such as food, fermentation, textile and paper. Thus isolating and manipulating pure culture from various soil and waste materials has manifold importance for various biotechnology industries. In the present investigation bacterial strains producing amylase were isolated from soil sample and inoculated into Casein Peptone Starch medium for fermentative production of amylase. The optimum temperature for production was 35-40°C. The pH range was found to be 7. The fermented broth upon filtration yield amylase produced, which is then tested for its ability to degrade variety of substances. A comparative analysis of biodegradation ability of bacterial amylases was done and degradation capacity of bacterial strains was found to be effective.

**Key words:** Amylases, Biodegradation, fermentation, soil sample.

### INTRODUCTION

Enzymes are biological catalysts which are an indispensable component of biological reactions. They have been in use since ancient times and they have been used in saccharification of starch, production of beverages like beer, treatment of digestive disorders and production of cheese from milk. Among the many enzymes that are widely used Amylases have been in increasing demand due to its crucial role of starch hydrolysis and the applications of this hydrolytic action. Amylases are enzymes that catalyze the hydrolysis of internal  $\alpha$ -1,4-glycosidic linkages in starch in low molecular weight products, such as glucose, maltose and maltotriose units. The substrate that amylase acts upon is starch. Starch is a polysaccharide composed of two types of polymers – amylose and amylopectin. Amylose constitutes 20-25% of the starch molecule. It is a linear chain consisting of repetitive glucose units linked by  $\alpha$ -1, 4-glycosidic linkage. Amylopectin constitutes 75-80% of starch and is characterized by branched chains of glucose units. The linear successive glucose units are linked by  $\alpha$ -1, 4-glycosidic linkage while branching occurs every 15-45 glucose units where  $\alpha$ -1, 6 glycosidic bonds are present. The hydrolysate composition obtained after hydrolysis of starch is highly dependent on the effect of temperature; the conditions of hydrolysis and the origin of enzyme. Amylases are obtained from various origins like plant, animal, bacterial and fungal. The microbial source of amylase is preferred to other sources because of its plasticity and vast availability. Because of their wider applications and thermo stability bacterial amylases are preferred.

### Bacterial Amylases

The production of microbial amylases from bacteria is dependent on the type of strain, composition of medium, method of cultivation, cell growth, nutrient requirement, incubation period, and Ph, temperature and thermo stability. Infact such characteristics were found in the genus Bacillus is exploited commercially due to their rapid growth rate leading to short fermentation cycle, capacity to secrete amylase extracellular and easier harvesting.

Amylases produced by different Bacillus strain such as Bacillus licheniformis, Bacillus stearothermophilus, Bacillus amyloliquefaciens find potential application in a number of industrial processes like food, fermentation, textiles and paper industry. Thermo stability is the desired characteristic of most of the



industrial enzymes. *Bacillus subtilis* is known to produce thermostable amylase and have been widely used for commercial production of enzyme for various applications. These amylases have been produced by submerged fermentations as well as solid state fermentations. Some *Bacillus* strains produce enzyme in the exponential phase whereas some others in the mid stationary phase.

### **Biodegradability by Amylase**

Biodegradation is the biologically catalyzed reduction in complexity of chemical compounds. It is the process by which organic substances are broken down into smaller compounds by living microbial organisms. Some microorganisms have the astonishing, naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), radionuclides and metals. *Bacillus* is known to secrete amylases extracellularly which of commercial importance and is a boon for industrial applications as these amylases were found to be effectively acting upon starch. With the advances in technology and the increase in the global population, plastic materials have found wide applications in every aspect of life and industries. However, most conventional plastics such as polyethylene, polypropylene, polystyrene, poly(vinyl chloride) and poly(ethylene terephthalate), are non biodegradable, and their increasing accumulation in the environment has been a threat to the planet. To overcome all these problems, some steps have been undertaken. The first strategy involved production of plastics with high degree of degradability. Biodegradable plastics are seen by many as a promising solution to this problem because they are environmentally-friendly. They can be derived from renewable feedstock's, thereby reducing greenhouse gas emissions. For instance, polyhydroxyalkanoates (PHA) and lactic acid (raw materials for PLA) can be produced by fermentative biotechnological processes using agricultural products and microorganisms. Biodegradable plastics offer a lot of advantages such as increased soil fertility, low accumulation of bulky plastic materials in the environment (which invariably will minimize injuries to wild animals), and reduction in the cost of waste management. Furthermore, biodegradable plastics can be recycled to useful metabolites (monomers and oligomers) by microorganisms and enzymes.

In our study we focused on isolation of amylase producers, production of amylase and testing the ability of amylases to degrade variety of polymers

### **MATERIALS AND METHODS**

#### **Isolation of Amylase Producing Microorganisms:**

Soil samples were collected from different environment sources of our college-botanical garden area and ground soil. Serial dilution was made and was plated on Casein Peptone Starch medium by spread plate technique. 0.1ml of the sample was plated each and kept for incubation for 48-72 hours at 37°C.

#### **Screening for Amylase Activity (Starch Iodine Test)**

Isolated colonies were picked up from each plate containing pure culture and streaked in straight lines in starch agar plates with starch as the only carbon source. After incubation at 37°C for 24-48 hrs., individual plates were flooded with Iodine Solution (Iodine-1gm, Potassium Iodide- 2 gm, Distilled water-300ml) to produce a deep blue colored starch-iodine complex. In the zone of degradation no blue colour forms, which is the basis of the detection and screening of an amylolytic strain. The colonies which were showing zone of clearance in starch agar plates were selected and further morphological examination was done by Gram's Staining.

#### **Morphological Characteristics (Microscopic Examination)**

Amylolytic colony was processed for staining by Gram staining method and observed microscopically. The colonies were identified as *Bacillus* which are Gram positive, medium sized, occurring singly or in pairs with round ends and sporulating. Spores were oval, central bulging.



## Fermentative Production of Amylase

### Inoculum Building

Amyolytic colonies showing clear zones around them were picked up with an inoculation loop and inoculated into 100ml of Casein Peptone Starch(CPS) medium aseptically and incubated at room temperature for 72 hours. The inoculum grows in the form of a pellicle on the surface of the medium and also makes the medium turbid.

### Enzyme Production Medium

#### Casein Peptone Starch (CPS) Medium

Peptone-0.5 gm, Glycerol-1ml, Casein- 0.5 gm, K<sub>2</sub>HPO<sub>4</sub>- 0.2 gm, MgSO<sub>4</sub>- 0.2 gm

FeCl<sub>3</sub>- 0.01%, Starch – 1gm, Agar-20 gm, Distilled water- 1000 ml, pH - 7.2

10% inoculum was transferred from inoculum medium into fermentation medium and kept at room temperature for 5 days. After 5 days the culture medium was filtered and centrifuged at 5000 rpm for 15 minutes to remove the cell mass. The supernatant was used as sample containing enzyme for further estimation qualitatively.

### Assay for Amylase Activity

#### Amylase Activity

One unit of enzyme activity is defined as the amount of enzyme required for the production of micromole of product from substrate under standard conditions. It is measured in units/ml

Amylase enzyme activity is the amount of amylase which produces 1ml of sugar i.e., glucose/min under standard assay conditions. Amount of glucose formed gives activity of amylase on the substrate starch. Hence the estimation of glucose can be correlated with enzyme activity.

#### Estimation of Glucose in the Broth by DNS Method

DNS reacts with reducing sugar like glucose and maltose at 100°C to form orange-red coloured complex which has absorption maxima at 540nm. A standard graph using different aliquots of standard glucose solution (1mg/ml) was prepared and the concentration of glucose in the sample was determined. Using glucose concentration amylase enzyme activity was found to be **5000 units/lit/min**

### Biodegradation by Bacterial Amylases

Plastic composites containing high levels of gelatinized corn starch (20-40% dry weight basis) in combination with petrochemical-based, hydrocarbon polymers such as polyethylene (PE) and poly(ethylene-co-acrylic acid) (EAA) are being developed in an effort to facilitate the breakdown, or metabolism, of the composites by living organisms. We have developed simple laboratory assay for biological degradation of starch-containing plastics.

**Plastics:** The plastic films used in this study were contained (dry weight basis) 40% starch, 45% poly(ethylene-co-acrylic acid) (EAA), 15% urea (starch/EAA plastic) or 40% starch, 25% EAA, 25% low density polyethylene (PE), 10% urea (starch/PE/EAA plastic).

Plastic films were cut into strips 1.2-1.3 cm wide and about 20 cm long. Each strip was numbered, and its average thickness determined with a micrometer for subsequent tensile strength, measurements. Strips equivalent to 0.75 g (4-6 strips) were added to each flask after being sterilized by soaking for 30 minutes in a solution of 3% H<sub>2</sub>O<sub>2</sub>, followed by several rinses in sterile distilled water. Flasks were inoculated with 2 mL of a freshly produced amylase extract, and were incubated at 28°C with gentle agitation.

**Analytical Methods:** Plastic strips were recovered from the flasks after the desired period of incubation, rinsed carefully in distilled water, and air-dried at constant temperature and humidity (25°C, 50% humidity) for 5 days (final moisture content = 5%). The air-dry strips were weighed.



## RESULTS AND DISCUSSION

Starch-plastic composites contain a mixture of two different types of materials

(i) Hydrophobic, petrochemical-derived polymers (PE, EAA) known to be highly resistant to degradation by living organisms, and

(ii) A hydrophilic, natural polymer (starch) that is easily broken down by a wide array of organisms.

After several weeks of incubation the films tested were found to be showing considerable level of degradation. Though not fully degraded, our work gave us insight into a more interesting area where we all need to focus on finding better alternates to chemical polymers or replacing them by the use of starch based plastics like potato starch derived food eatables, spoons, forks, starch based biofilms containing PHB(which is more readily degradable),corn starch based materials etc.

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## BIOREMEDIATION AND BIODEGRADATION FOR POLLUTION FREE ENVIRONMENT

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### ABSTRACT

*Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. Bioremediation is the process of using organisms to neutralize or remove contamination from waste. It is very important to understand that this form of waste remediation uses no toxic chemicals, although it may use an organism that can be harmful under certain circumstances. A gross, but simple explanation of bioremediation is the use of maggots in wound care control. Wounds that have contamination can have maggots introduced to them. The maggots then eat the contamination, allowing the wound to heal correctly. That is a form of medical bioremediation but there are many other types that are used to control different waste contamination.*

### INTRODUCTION

In fact Biodegradation is the biologically catalyzed modification of an organic chemical's structure. However, this modification can be through different metabolic pathways and does not necessarily mean a reduction in toxicity. Mineralization, one type of biodegradation, is defined as the conversion of an organic substance to its inorganic constituents, rendering the original compound harmless. Transformation is defined as any metabolically-induced change in the chemical composition of a compound.

Keywords: Bioremediation, Biodegradation, pollution, Environment, Microbes

At sites filled with waste organic material, bacteria, fungi, protists, and other microorganisms keep on breaking down organic matter to decompose the waste. If such environment is filled with oil spill, some organisms would die while some would survive. Bioremediation works by providing these organisms with different materials like fertilizer, oxygen and other conditions to survive. This would help to break the organic pollutant at a faster rate. In other words, bioremediation can help to clean up oil spills.

To clean up oil spills, bacteria are introduced to the area of the spill where they break down the hydrocarbons of the oil into carbon dioxide; this is an **example of bioremediation**. Toxic metals, such as mercury (II), can be converted into nontoxic forms, such as mercury, by bacteria.

**Bioremediation** is very **safe** because it uses the same microbes that already naturally occur in soil or water - this process simply adds more of these organisms to those already present. No dangerous chemicals are used in the process, and harmful contaminants are completely destroyed.

According to the EPA, *Bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances."*

### WHY BIOREMEDIATION IS IMPORTANT?

Bioremediation is important for two reasons.

**1. It uses no chemicals** – One of the issues with using man-made chemicals in the treatment and removal of contamination is that the chemicals eventually make it into the water supply. There were many chemicals used at the beginning of the Waste management era that we now know were very harmful to plant, animal and human life once they reached the water supply.

**2. It can allow waste to be recycled** – Another major reason that bioremediation is preferred is that once the waste is treated and the contamination neutralized or removed, the waste itself can then be recycled. When chemical remediation types are used, the waste is still contaminated just with a less toxic substance and in



general, cannot then enter into the recycle process. Bioremediation allows for more waste to be recycled while chemical methods still create waste that cannot be used and has to be stored somewhere

What are the 2 classes of Bioremediation used?

There are two classes of bioremediation used. Don't confuse the class type with the actual types of bioremediation available, the classes describe the general application of the organisms. The two classes are:

- **In-situ** – In situ refers to when contaminated waste is treated right at its point of origin. For example, there may be soil that is contaminated. Rather than remove the soil from its point of origin, it is treated right where it is. The benefit to in situ treatment is that it prevents the spread of contamination during the displacement and transport of the contaminated material.
- **Ex-situ** – Ex situ refers to treatment that occurs after the contaminated waste has been removed to a treatment area. To use soil as the example again, the soil may be removed and transported to an area where the bioremediation may be applied. The main advantage to this is it helps to contain and control the bioremediation products, as well as making the area that was contaminated available for use.

**TYPES OF BIOREMEDIATION:** THERE ARE FAR MORE THAN 9 TYPES OF BIOREMEDIATION, BUT THE FOLLOWING ARE THE MOST COMMON WAYS IN WHICH IT IS USED.

1. **Phytoremediation** – use of plants to remove contaminants. The plants are able to draw the contaminants into their structures and hold on to them, effectively removing them from soil or water.
2. **Bioventing** – blowing air through soil to increase oxygen rates in the waste. This is an effective way to neutralize certain oxygen sensitive metals or chemicals.
3. **Bioleaching** – removing metals from soil using living organisms. Certain types of organisms are drawn to heavy metals and other contaminants and absorb them. One new approach was discovered when fish bones were found to attract and hold heavy metals such as lead and cadmium.
4. **Landfarming** – turning contaminated soil for aeration and sifting to remove contaminants, or deliberately depleting a soil of nitrogen to remove nitrogen based organisms.
5. **Bioreactor** – the use of specially designed containers to hold the waste while bioremediation occurs
6. **Composting** – containing waste so a natural decay and remediation process occurs.
7. **Bioaugmentation** – adding microbes and organisms to strengthen the same in waste to allow them to take over and decontaminate the area
8. **Rhizofiltration** – the use of plants to remove metals in water.
9. **Biostimulation** – the use of microbes designed to remove contamination applied in a medium to the waste.

Factors Responsible For Microbial Bioremediation to be Effective

The major advantage of the bioremediation methods is that it allows for contamination to be treated, neutralized or removed and then produces a waste product itself that is more easily disposed of. In some cases, there is no need for disposal at all. In the case of the plants used in phytoremediation and rhizofiltration, the plant is able to do something called bioaccumulation. This means it holds onto the contaminant. As the plant is still growing, there is no need to remove and destroy it. In many ways it is similar to having a rechargeable battery. In the case of contaminated waste, it is the plant that keeps growing to allow for more storage of waste. This is a uniquely cost effective solution for contaminated waste.

**Microbial Population:** Suitable kinds of organisms that can biodegrade all of the contaminants

**Oxygen:** Enough to support aerobic biodegradation (about 2% oxygen in the gas phase or 0.4 mg/liter in the soil water)

**Water:** Soil moisture should be from 50–70% of the water holding capacity of the soil

**Nutrients:** Nitrogen, phosphorus, sulfur, and other nutrients to support good microbial growth

**Temperature:** Appropriate temperatures for microbial growth (0–40°C)



**pH:** Best range is from 6.5 to 7.5

There are some types of contamination that are very difficult to use bioremediation for. The two biggest concerns are:

1. Cadmium
2. Lead

Both of these are classified as heavy metals and are difficult to remove using microorganisms. As mentioned earlier, a recent discovery about the absorption rate of fish bone has proving successful. In fact, bone seems to hold the clue for removing heavy metal contamination. Char is used to remove small amounts of zinc, lead and cadmium; and it is thought that the calcium in the fish bone is what makes it effective.

#### **Factors Affecting Rates of Biodegradation**

Biodegradation may be influenced by pH, temperature, moisture, carbon sources, soil texture, aerobic versus anaerobic conditions, the number of substituents, and the concentration of the pollutant. It is impossible, however, to make a generalization about the best universal conditions for biodegradation. What's toxic to some microbes is a nutrient to others, what might be a damaging pH to some is beneficial to others, and so on. A greater amount of substituents will cause slower degradation in aerobic environments, but faster degradation in anaerobic ones. Chlorine makes a molecule less degradable due to steric hindrance preventing access to necessary enzymes, therefore molecules with higher chlorination are slower to degrade in aerobic conditions. High concentration of a pollutant generally results in faster rates of degradation. If the concentration drops below a threshold concentration, the enzymes may not detect it and will cease to degrade it.

Soil with small pores, especially clays, may cause biodegradation to take years due to the decrease in bioavailability. Chlorine makes a molecule less degradable due to steric hindrance preventing necessary enzymes from accessing the compound, therefore molecules with higher chlorination are slower to degrade.

The rate at which a compound is transformed, as well as the curves that describe its transformation, is referred to as kinetics, and is affected by all factors listed above. First order kinetics (exponential decay) often describes biodegradation when the initial substrate concentration is low, while zero-order kinetics (linear biodegradation) is often observed when the substrate concentration is very high. In some cases if the concentration of the chemical falls below a critical threshold concentration, the microbes can no longer transform it and the chemical persists.

#### **Advantages of Bioremediation**

1. Bioremediation is a publicly accepted treatment of polluted soil because it is based upon natural processes. Microbes that metabolize contaminants increase in population when the contaminant is present. The inverse is true, degradation of the contaminant causes population declines of those microbes. Usually the products from treatment are harmless; such as carbon dioxide, water, and cellular biomass.
2. Bioremediation is theoretically meant to completely degrade a wide range of pollutants into harmless products on site. This removes the risks involved with transportation for treatment and elimination of contaminated substances.
3. Bioremediation is meant to completely eliminate specific pollutants without the risks of transferring contaminants from one environmental medium to another (land, air, water).
4. Bioremediation can be a cheaper alternative to other technologies used for pollution mitigation.

#### **Disadvantages of Bioremediation**

1. Only biodegradable compounds are capable of undergoing bioremediation. Not every compound is capable of fully degrading quickly.



2. The products of biodegradation may potentially be even more persistent or toxic than the original contaminant.
3. Biological functions are usually extremely specific and require the presence of microbes that are capable of metabolizing the contaminants. In order for the correct microbes to be present, the appropriate environmental conditions, levels of nutrients, and contaminants need to be met.
4. Scaling up the size of studies from small initial studies to commercial-scale field operations is difficult.
5. The real environment contains contaminants that are mixed, unevenly distributed, and in different phases (solid, liquid, gas). More research needs to be completed to create technologies that can adapt.
6. Compared to other treatment technologies, bioremediation often takes more time.
7. Problems with ensuring adequate contact between the microbes and the contaminant. preferential pathway and soil structure can leave uncertainty in remediation dispersal.

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## RHIZODEGRADATION OF HYDROCARBON FROM OILY SLUDGE

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Rhizoremediation involves the use of plant growth promoting rhizobacteria (PGPR) to remove organic pollutants from terrestrial environment. The main objective of this study was to evaluate the efficiency of rhizoremediation through inoculation of hydrocarbon degrading bacterial strains with and without nutrients in the rhizosphere sporadically become contaminated with oily sludge. Bacterial strains viz. *Bacillus cereus*, *Bacillus altitudinis*, *comamonas* and *Stenotrophomonas maltophilia* were isolated from various oily waste pits of oil fields.

Autoclaved soil was treated with oily sludge at 7:3 ratio and Alfalfa plant was inoculated with individual plant growth promoting rhizobacteria (PGPR) with and without diammonium phosphate and ammonium nitrate. The different saturates and total hydrocarbon was extracted by Soxhelt extraction and was analyzed by GC-FID at 0, 5 and 10 d of incubation.

The inoculation 30% sludge with *B. altitudinis* accelerated the rate of degradation of n-alkanes and some methyl branched than un-inoculated soils. Oily sludge inoculated with *B. altitudinis* indicated that more than 80% of hydrocarbons were degraded at day 5 of incubation. No further degradation was observed till the end of the incubation period. *B. altitudinis* is capable of degrading straight chain hydrocarbons rapidly than any other strains.

Alfalfa is a N-symbiotic plant and increased nutrient content under the rhizosphere promote degradation of n-alkanes. All other bacterial strains also showed significant interaction with alfalfa for the biodegradation of oily sludge but the rate of degradation remained slow than with *B. altitudinis*. Inoculation with *B. altitudinis* accelerated the rehabilitation process and within 5 days the rehabilitation occurs.

Keywords: Rhizoremediation; PGPR; Bioremediation; n-Alkanes; Hydrocarbon; Oily sludge

## ENVIRONMENTAL POLLUTION – REMEDIAL TECHNIQUES

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The global environment is under great stress due to urbanization and industrialization as well as population pressure on the limited natural resources. The environmental problems are diverse and sometimes specific with reference to time and space. The nature and the magnitude of the problems are ever changing, bringing new challenges, and creating a constant need for evolving newer and more appropriate technologies. The major environmental threats include organic aqueous waste (pesticides), organic liquids (solvents from dry-cleaning), oils (lubricating, automotive, hydraulic, and fuel oils), and organic sludge/solids (painting operations, tars from dyestuffs intermediates). Mostly soil contaminations are the result of accidental spills and leaks. There are several conventional techniques (chemical treatment process) available to treat some of these chemicals, but due to their cost, end products which in turn are again toxic, these techniques fail to completely eradicate these chemicals. One of the green technologies to treat these hazardous chemicals is bioremediation. Bioremediation is an increasingly popular alternative to conventional chemical methods for



treating waste compounds and media with the possibility to degrade contaminants, since it uses natural microbial activity mediated by different consortia of microbial strains. The ideal solution for pollution abatement is Bioremediation, the most effective innovative technology to come along that uses biological systems for treatment of contaminants. Although, this novel and recent technology is a multidisciplinary approach, its central thrust depends on microbiology. This technology includes biostimulation (stimulating viable native microbial population), bioaugmentation (artificial introduction of viable population), bioaccumulation (live cells), biosorption (dead microbial biomass), phytoremediation (plants) and rhizoremediation (plant and microbe interaction). Rhizoremediation, which is the most evolved process of bioremediation, involves the removal of specific contaminants from waste product of contaminated sites by mutual interaction of plant roots and suitable microbial flora. Phytoremediation is an emerging technology that uses plants for the treatment/ mineralization of pollutants. Pollutants can be taken up inside plant tissues (phytoextraction), adsorbed to the roots (rhizofiltration), transformed by plantenzymes (phytotransformation), volatilize via plants into the atmosphere (phytovolatilization), degraded by microbes in the root zone (rhizoremediation) or incorporated to soil material (phytostabilization).

Key words: Bioremediation, Phytoremediation, Microbiology, Rhizoremediation

## AQUACULTURE PROCESSING WASTES AS POTENTIAL SOURCE FOR VALUE ADDED PRODUCTS: A SPECIAL FOCUS ON SHRIMP WASTE

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Aquaculture is in focus to the growing demand for food and is world fastest growing food production sector with an annual growth rate of 10% since 1984. Asia's contribution to world aquaculture production is 91% and India is 4<sup>th</sup> largest producer of fish and second largest in inland fish production. In last two decades India had shown phenomenal increase in shrimp cultivation from 179 tons in 1996 to 42870 tons in 2005. Globally the annual shrimp production by culture has increased from 8 million tons in 1985 to 154 million tons by the end of 2011. India is one of the major shrimp producing countries in Asia, contributing 9.06 million metric tons and occupying second position next to China. The State of Andhra Pradesh with approximately 700 km coastal belt, major rivers like Krishna Godavari, Penna, and brackish water lakes like Pullkat dominated the sector with over 73 percent of the total production in India (2007–2008) with more than 60 percent of the total water area dedicated to prawn farming (38 819 hectares), followed by West Bengal (4744) with annual yield of 1.5 tons / hectare. About 35 to 45 % by weight of shrimp raw material is discarded as waste when processed into headless shell-on products. Peeling process which involves the removal of the shell from the tail of prawn, increases the total waste production upto 40 - 45%. On a global basis, the shrimp processing in industry produces over 700000 million tons of waste shell. Shell and head wastes of crustaceans contain chitin, proteins and minerals. The constituents of shell waste make them worthy of further processing and utilization. Shell waste is mainly subjected to chemical processing for chitin/chitosan production by demineralization and deproteinizing the waste chitin can be obtained. Bioconversion processes are also taken up for the production of enzymes and bioactive materials by the utilization of waste. Chitosan and its derivatives are examples of value added materials. They are produced from chitin, which is natural carbohydrate polymer found in the skeleton of crustaceans. Nellore district in Andhra Pradesh being the hub of shrimp cultivation known as



shrimp capital of India with 2050 shrimp farms and 7105 Ha of land under culture there is huge availability of these waste which may be deleterious to environment can utilized for conversion in to value added products. The main aim of this work is to use aquaculture waste materials (prawn shells) which are hazard and toxic for environment to prepare chitosan extract from and the use of this chitosan in successfully carried out various applications in laboratory.

Key words : Chitosan, Crustaceans, Chitin, Aquaculture, Shrimp waste

## BIOLOGICAL DEGRADATION OF PLASTICS

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Plastic is a broad name given to different polymers with high molecular weight, which can be degraded by various processes. However, considering their abundance in the environment and their specificity in attacking plastics, biodegradation of plastics by microorganisms and enzymes seems to be the most effective process. Microorganisms such as bacteria and fungi are involved in the degradation of both natural and synthetic plastics .The biodegradation of plastics proceeds actively under different soil conditions according to their properties, because the microorganisms responsible for the degradation differ from each other and they have their own optimal growth conditions in the soil. Polymers especially plastics are potential substrates for heterotrophic microorganisms. This requires understanding of the interactions between materials and microorganisms and the biochemical changes involved. Widespread studies on the biodegradation of plastics have been carried out in order to overcome the environmental problems associated with synthetic plastic waste. This paper reviews microbial and enzymatic biodegradation of plastics and some factors that affect their biodegradability.

Key words: Microorganisms, environmental problems, biodegradation of plastics.

## NATURE FRIENDLY BIODEGRADATION-BIOREMEDIATION

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Environment contain a large number of microorganisms ranging from 10<sup>5</sup>-10<sup>7</sup> per ml in aquatic environment whereas 10<sup>7</sup>-10<sup>9</sup> per gram in surface soils. The organisms like bacteria, fungi etc., play a very important role in the transformation or alteration (through metabolic or enzymatic action) of the structure of chemicals introduced into the environment by a process named as biodegradation. The term is often used in relation to ecology, waste management, biomedicine, and the natural environment (bioremediation) and is now commonly associated with environmentally friendly products that are capable of decomposing back into natural elements. It is a nature's waste management and recycling system which breaks down everything from yard waste to crude oil and a natural process necessary to keep our planet clean and healthy. But unfortunately, the rate at which we are producing waste far outpaces the rate of natural biodegradation, making our current state unsustainable. As a result, landfills have been filling up at record rates, and air, water and soil pollution is increasing. Hence a process called bioremediation which refers to the process of using microorganisms to remove the environmental pollutants i.e. the toxic wastes found in soil, water, air etc., is



being used these days. The microbes serve as scavengers in this process. The removal of organic wastes by microbes for environmental clean-up is the essence of bioremediation. The other names used for bioremediation are bio-treatment, bio-reclamation and bio-restoration. The use of intrinsic or engineered bioremediation processes offers several potential advantages like low cost, Nonintrusive, potentially allowing for continued site use, Relative ease of implementation etc. that are attractive to site owners, regulatory agencies, and the public.

Keywords: Bioremediation, Biodegradation, Pollution

## PRIME FACTS OF BIODEGRADATION

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Using microbial xenobiotic metabolism to degrade environmental pollutants like hydrocarbons, polychlorinated biphenyls, polyaromatic hydrocarbons, heterocyclic compounds, pharmaceutical substances, radionuclides and metals is called Microbial Biodegradation. All the above mentioned pollutants are getting released into our surroundings by anthropogenic causes, which adversely damage biodiversity and environment as well. Several microorganisms effectively conduct this enzyme mediated process. Fungi, bacteria and yeasts are the major groups of microorganisms and *Marinobacter*, *Pseudomonas*, *Viribacillus*, *Acinetobacter*, *Thalassobacillus* species are some of the important microorganisms, involving in biodegradation. These organisms are rich sources of enzymes like mono, di oxygenases and alkane hydroxylases, which are noted biocatalysts for biodegradation. This microbial activity is mainly based on two processes such as growth and cometabolism. In the growth an organic pollutant is used as primary source of carbon and energy source and in the cometabolism an organic compound is metabolised in the presence of a growth substrate that is used as the primary carbon and energy source. The process of biodegradation is controlled by many external factors like oxygen availability, nutrient availability, temperature, pH, salinity, light etc., Biodegradation is an ecofriendly task of eradicating harmful and potential pollutants from our vicinity.

Keywords: xenobiotic metabolism, cometabolism etc.,

## ROLE OF MICROORGANISMS IN BIODEGRADATION

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In nature, cellulose, lignocellulose and lignin are major sources of plant biomass; therefore, their recycling is indispensable for the carbon cycle. Each polymer is degraded by a variety of microorganisms which produce a battery of enzymes that work synergically. In the near future, processes that use lignocellulolytic enzymes are based on microorganisms could lead to new, environmentally friendly technologies. Bacterial treatment significantly and drastically reduced the toxicity associated with dispersed oil. Hydrocarbon-degrading bacterial species demonstrate a unique response to dispersed oil compared to their response to crude oil, with potentially opposing effects on toxicity. While some species have the potential to enhance the toxicity of crude oil by producing biosurfactants, the same bacteria may reduce the toxicity associated with dispersed oil through degradation. Some biodegradation processes originating from the activity of microorganisms in the



black crusts of historic buildings. The crusts are mainly composed of gypsum, carbonaceous particles, and polycyclic aromatic hydrocarbons. The slowly dissolving gypsum from black crusts represents a continuous source of sulphur for microbial growth., there is a continuous deposition of pollutants on the environment and at the same time a biodegradation of pollutants by microorganisms leading to their removal.

**Key Words:** Cellulose, Lignin, Biodegradation, biosurfactants, hydrocarbons.

## GEOBACTERIA CONSUMES RADIOACTIVE CONTAMINATION

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The nano wires grown by certain types of bacteria can also be used to immobilize harmful materials—like uranium – and keep them from spreading. A research team at Michigan State University has learned that Geobacter bacteria, which is found naturally in soil, essentially electroplates uranium, rendering it insoluble so it can't dissolve and contaminate groundwater. These bacteria can be brought into uranium contamination sites like mines and nuclear plants in order to contain the radiation, potentially limiting the disastrous consequences of these types of spill. The poster presents different kinds of microbes that can be used for preventing radioactive contamination and its process.

Key words: Uranium; Geobacter; Nano wires.

## GULF OIL SPILL GASES EATEN BY BACTERIA

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Certain types of bacteria can actually clean up troublesome environmental pollutants like spilled petroleum. In fact, a specific strain called Alcanivorax drastically increases in population when an oil spill provides them with large amounts of food, so that they're able to remove much of the oil. They're at work on the Deepwater Horizon spill in the Gulf of Mexico right now, and while they certainly can't undo the vast damage that has been done to this region as a result, they definitely provide a beneficial effect.

Key words: oil spillage; Alcanivorax; gulf.



## BACTERIA EAT POLLUTION AND GENERATE ELECTRICITY

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Bacteria with tiny wire-like appendages called nanowires not only digest toxic waste – including PCBs and chemical solvents – they produce electricity while they're at it. One type in particular, called Shewanella, is a deep-sea bacteria that grows these oxygen-seeking nanowires when placed in low-oxygen environments. Researchers discovered that when the microbes' nanowires are pricked with platinum electrodes, they can carry a current. If these capabilities can be harnessed effectively, they could one day be used in sewage treatment plants to simultaneously digest waste and power the facilities. The poster presents the method by which these bacteria can be used to produce electricity.

Key words: Nanowires; Shewanella; Power

## BIODEGRADATION AND BIOREMEDIATION

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Biodegradation or biological degradation is the phenomenon of biological transformation of organic compounds by living organisms, particularly the microorganisms. Biodegradation basically involves the conversion of complex organic molecules to simpler (and mostly non-toxic) ones. Several factors influence biodegradation. These include the chemical nature of the xenobiotic, the capability of the individual microorganism, nutrient and O<sub>2</sub> supply, temperature, pH and redox potential. Among these, the chemical nature of the substrate that has to be degraded is very important. Bioremediation refers to the process of using microorganisms to remove the environmental pollutants i.e. the toxic wastes found in soil, water, air etc. The microbes serve as scavengers in bioremediation. The removal of organic wastes by microbes for environmental clean-up is the essence of bioremediation. The other names used (by some authors) for bioremediation are bio-treatment, bio-reclamation and bio-restoration

Key words: Biodegradation, Bioremediation, Microorganisms.

## BIO-PLASTICS - AN ALTERNATIVE FOR FOSSIL FUELS

**P. KAMANL TEJA, S.T.V.RAGHAVAMMA, RAMA RAO NADENDLA**

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There is a common in life: large and complex molecules result from the synthesis of units that are later joined together. Mankind learned this principle and employed it to develop language, culture, technology. This same principle is applied in the petrochemical industry by fractionating the fossilized carbon chains into small molecules and then polymerizing them in order to develop synthetic polymers, which are much more flexible, resistant and durable than natural polymers. "Over increasing population causing rapid industrialization and more use of petroleum based product which makes our environment unsuitable, less balanced and more toxic. The increasing use of plastics and their accumulation has further more contributed to eco-pollution due to its non biodegradability." Bioplastics constitute an emerging and innovative industrial segment, characterized by new synergies and collaborations among chemical, biotechnical, agricultural and consumer sectors.

Key words: Bio-plastics; microorganisms; eco- friendly.



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## MICRO ORGANISMS: A BOON FOR DEGRADATION OF PLASTICS

**P.V. SAI SUMANTH**

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One of the major pollutants in the environment for centuries is plastic. Their accumulation is hazardous and causes various environmental problems. Biodegradation refers to changes in a material due to environmental factors like light, moisture, heat, cold along with biological agents like bacteria and fungi. Plastics can be degraded effectively using various strains of bacteria. A large number of microorganisms are being used for degradation of plastics. In future, degradation using microorganisms will be a promising tool for cleaning the environment.

Key words: micro organisms; environment; degradation.

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## BEHAVIOR OF BACTERIA DESIGNED FOR BIOREMEDIATION AND BIODEGRADATION

**B. SAI SPANDANA, S.T.V.RAGHAVAMMA, RAMA RAO NADENDLA**

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Bioremediation is the process of using biological agents to remove toxic wastes from environment. It is the most effective management tool to manage the polluted environment and recover contaminated soil. Bioremediation is an innovative technology that has the potential to alleviate the toxic contamination. Biodegradation is the decay or breakdown of materials, when microorganisms use an organic substance as a source of carbon and energy. The recombinant microbes function in microcosms according to their design. The survival and fate of recombinant microbes in different ecological niches under laboratory conditions is observed to be same as unmodified parental strain. The behavior of genetically engineered microbes can be predicted more accurately through the coupling of regulatory circuits that control the expression of catabolic pathways to killing genes, so that the genetically engineered microbes survive in polluted environments, but die when the target chemical is eliminated.

Key words: bioremediation; biodegradation; recombinant microbes



## MICROBIAL FUEL CELLS FOR SUSTAINABLE WATER RESOURCES MANAGEMENT

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### ABSTRACT

This paper focuses on the renewable energy production which is a longer-term prospect that requires substantial technical and manufacturing advances. Microbial fuel cells (MFCs) systems have been of great interest as a potential candidate for future alternative energy production. The technology represents one of the oldest sources of microbial energy today. However, due to recent discoveries and improvements in fuel cell design, it has been the recent focus of MFCs usage in wastewater application regarded as very useful and promising. MFC technology is an emerging biotechnology that utilizes bacteria to generate electricity from the degradation of organic substances, for instance, acetate and glucose. MFCs are as diverse chemically as the bacteria that power them. In MFCs, the oxidation reactions occur inside the bacteria, and electrons must be transferred to the extracellular anode, and then flow through an external circuit to react with an electron acceptor such as oxygen. MFCs use biomass as the substrate (metabolic oxidation-reduction reactions) and microorganisms as the catalyst, exploiting whole living cells in an aim to gain energy.

**KEYWORDS:**MFC technology, oxidation-reduction reactions, degradation of organic substances, energy production.

### INTRODUCTION

Microorganisms such as Geobacteraceae can be responsible for this oxidation, using Fe<sup>3+</sup>, Mn<sup>4+</sup> or humic acids as electron acceptor in natural conditions. Geobacter species have novel electron transfer capabilities and harvest electricity from waste organic matter and renewable biomass; besides that, their impact on the natural environment and their application to the bioremediation of contaminated environments also attract great interests. For instance, they can destroy petroleum contaminants in polluted groundwater by oxidizing these compounds to harmless CO<sub>2</sub>. Base on improved understanding of the functioning of Geobacter species, it has been possible to modify environmental conditions in order to accelerate the rate of contaminant degradation.

Besides, numerous species of microorganisms are capable of metabolizing glucose in the anaerobic anodic chamber of MFCs, for instance, *Bacillus subtilis*, *Escherichia coli* and *Lactobacillus plantarum*. For aerobic cathodic chamber, Bacillus species are thermophilic and alkaliphilic bacteria that can thrive in aerobic conditions to power a MFC and be used in fuel cells to investigate the effect of temperature and pH value. According to this microorganism, it is found that temperature does not have a significant effect on the fuel cell efficiency (between 20-70 degrees).

Manganese oxidizing bacteria belong to the species beta Proteobacteria. They are filamentous; chemoorganotrophs and gram negative. They can live in freshwater and saltwater ecosystems. The oxidation of Mn(II) to Mn(IV) is thermodynamically favored under aerobic conditions, with a negative free energy of approximately 16 kcal/mol. Mn<sup>2+</sup> causes problems of corrosion and leave color residue in sinks in drinking water systems. By the oxidation of manganese oxidizing bacteria, Mn<sup>2+</sup> will become MnO<sub>2</sub> as precipitation

Numerous species of microorganisms are capable of metabolizing glucose in the anaerobic anodic chamber of MFCs, for instance, *Bacillus subtilis*, *Escherichia coli* and *Lactobacillus plantarum*. For aerobic cathodic chamber, Bacillus species are thermophilic and alkaliphilic bacteria that can thrive in aerobic conditions to power a MFC and be used in fuel cells to investigate the effect of temperature and pH value. According to this



microorganism, it is found that temperature does not have a significant effect on the fuel cell efficiency (between 20-70 degrees)

#### **Biological principles of MFCs:**

The MFCs require microorganisms to switch from their natural electron acceptors such as oxygen and nitrate, to an insoluble electron acceptor, the anode. In MFC, as shown in Figure 6, each biological degradation process of organic matter is an oxidation process. Under anaerobic conditions, it is possible to exploit the degradation process for electron recovery. In normal microbial metabolism, usually the glucose is oxidized by anaerobic conversion when its electrons are released by enzymatic reactions. The electrons are stored as intermediates to provide the living cell with energy to fuel the reactions for maintenance and growth. When a substrate is anaerobically oxidized in the anodic compartment to produce electrons the potential decreases. At the same time, the potential increases in the cathodic compartment due to the reduction of a reagent. The difference in potential caused by the oxidation of a substrate at the anode and reduction of a substrate at the cathode produces the current. A typical MFC consists of two separate chambers, anaerobic anode and aerobic cathode compartments,

#### **METHODOLOGY**

Microorganisms performances In MFC design In MFC design, bacteria gain energy by transferring electrons from an electron donor, such as glucose or acetate, to an electron acceptor, such as oxygen. The larger the difference in potential between donor and acceptor, the larger the energetic gain from the bacterium, and generally the higher the growth yield will be. In a MFC, bacteria do not directly transfer their electrons to their characteristic terminal electrons acceptor, but these electrons are diverted towards an electrode, i.e. an anode. The electrons are subsequently conducted over a resistance or power user towards a cathode and thus, bacterial energy is directly converted to electrical energy. So, a typical MFC consists of two separate chambers, anaerobic anode and aerobic cathode compartments.

#### **DISCUSSION**

During recent years, microbial fuel cell (MFC) has been proved to become a promising biotechnology utilizing bacteria that are capable of recovering electrical energy from organic matter. It can effectively convert organic contaminants to clean energy (electricity) at normal temperatures and pressures. Previous studies demonstrated that MFCs can be used to harvest biologically generated electricity from organic wastes such as wastewater, sediments and even rhizodeposits. Due to this, MFC has great potential for its application in wastewater treatment plants to simultaneously remove organic pollutants in wastewater and produce energy.

#### **CONCLUSION AND PURPOSE OF PRESENT STUDY**

The idea of using bacteria in MFCs to capture electricity has been around for some time, however, power production was very low and required the addition of extracellular mediators to shuttle electrons from inside to outside of the bacterial cell. Known newer systems adopt mediator-less MFCs that employ biofilms due to the designation of lower internal resistance. Maximizing power generation in MFCs will require innovative flow pattern and increased electrode-electrolyte interaction that minimize internal resistance. Additionally, finding methods to increase the cathode potential, with oxygen as the electron acceptor, could have a substantial impact on power generation.

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## BIO-ENERGY

### MARIYAMA & PADMA BAI

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Bio-energy is renewable energy made available from materials derived from biological sources. Biomass is any organic material which has stored sunlight in the form of chemical energy. As a fuel it may include wood, wood waste, straw, manure, sugarcane, and many other by-products from a variety of agricultural processes.

In its most narrow sense it is a synonym to bio-fuel, which is fuel derived from biological sources. In its broader sense it includes biomass, the biological material used as a bio-fuel, as well as the social, economic, scientific and technical fields associated with using biological sources for energy. This is a common misconception, as bio-energy is the energy extracted from the biomass, as the biomass is the fuel and the bio-energy is the energy contained in the fuel.

One of the advantages of biomass fuel is that it is often a by-product, residue or waste-product of other processes, such as farming, animal husbandry and forestry. Biomass can be converted to other usable forms of energy like methane gas or transportation fuels like ethanol and biodiesel. Rotting garbage, and agricultural and human waste, all release methane gas—also called "landfill gas" or "biogas." Crops, such as corn and sugar cane, can be fermented to produce the transportation fuel, ethanol. Biodiesel, another transportation fuel, can be produced from left-over food products like vegetable oils and animal fats. Also, Biomass to liquids (BTLs) and cellulosic ethanol are still under research.

Keywords: bio-energy, bio-mass, bio-fuel, energy, fuel, bio-diesel, bio-gas, by-product, agricultural processes & wastes.

## BIOFUELS FROM MICROALGAE—A REVIEW OF TECHNOLOGIES FOR PRODUCTION, PROCESSING, AND EXTRACTIONS OF BIOFUELS

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Microalgae are photosynthetic microorganisms with simple growing requirements (light, sugars, CO<sub>2</sub>, N, P, and K) that can produce lipids, proteins and carbohydrates in large amounts over short periods of time. It is obvious that continued dependence on fossil fuel energy resources is unsustainable, leading to both depleting world reserves and the green house gas emissions. Therefore, there are vigorous research initiatives aimed at developing alternative renewable and potentially carbon neutral solid, liquid and gaseous biofuels as alternative energy resources. However, first generation biofuels derived from terrestrial crops such as sugarcane, sugar beet, maize and rapeseed plays an enormous strain on world food markets, contribute to



water shortages and precipitate the destruction of the world's forests. Second generation biofuels derived from lignocellulosic agriculture and forest residues and from nonfood crop feedstocks address some of the above problems; however there is concern over competing land use or required land use changes. Therefore, based on current knowledge and technology projections, third generation biofuels specifically derived from microalgae are considered to be a technically viable alternative energy resource that is devoid of the major drawbacks associated with first and second generation biofuels. These microalgae can be processed into both biofuels and valuable co-products. This paper focuses on the biomass production, harvesting, conversion technologies, and the extraction of useful co-products. It was found that , microalgae-derived biofuels could progressively substitute a significant proportion of the fossil fuels required to meet the growing energy demand.

**Key words :** microalgae, fossil fuel, biofuels.

## BIOPLASTICS AND BIOENERGY

**P. SWARUPA**

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Bioplastics are plastics derived from renewable biomass sources,such as vegetables fats and oils,corn starch or microbes.bioplastic can be made from agricultural by products and also from used plastic bottles and other containers using microorganisms common plastics,such as fossil fuel plastics are derived from petroleum or natural gas.Bioplastics can break down in either anaerobic or aerobic environments,depending on how they are manufactured bioplastic can be composed of starches,cellulose,biopolymers and a variety of other materials.

Bioenergy is renewable energy made available from materials derived from biological sources biomass is any organic material which has stored sunlight in the form of chemical energy.As a fuel it may include wood,wood waste,straw,manure,sugarcane and many other by products from a variety of agricultural process.By 2010,there was 35gw[47,000,000hp] of globally installed bioenergy capacity for electricity generation of which 7gw[9,4000,00hp] was in the united states.in its most narrow sense.it is a synonym to biofuel,which is fuel derived from biological sources.in its broader sense it includes biomass,the biological material used as a biofuel as well as the social,economic,scientific and technical fields associated with using biological sources for energy.This is a common misconception,as bioenergy is the extracted from the biomass,as the biomass is the fuel and the bioenergy is the energy contained in the fuel.There is a slight tendency for the word bioenergy to be favoured in escape compared with biofuel in america.

**Key words:**

Bioplastics, bioenergy, environment,renewable,aerobic.

## BIOENERGY AND BIOPLASTICS FOR SUSUTAINABLE ENVIRONMENT

**K. SAI SRIKAR, S.T.V.RAGHAVAMMA, RAMA RAO NADENDLA**

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Bio energy and Bio plastics produced by microbial technology decreases the damage to the environment. Bio energy is the renewable energy produced by the living organisms by the natural sources. Most of them are finding new ways to convert biomass into bio fuels used as bio-energy. Bio-plastics are plastics derived from renewable biomass source, such as vegetables, fats and oils from micro biota. These bio plastics are mainly used as opposite of polymers derived from fossil resources. The usage of these bio energy and bio-plastics are easily degradable and do not cause much harm to the environment with the help of the microbes and to maintain sustainable environment

**Key words:** Bio mass; fossil resources; natural polymers; microbiota.



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## ENRICHMENT OF STERILIZED BIOGAS MANURES WITH BENEFICIAL MICROORGANISMS

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The beneficial microorganisms used in the enrichment studies (*Rhizobium*, *Pseudomonas*, *Azotobacter*, *Azospirillum*) were collected from the department of Agricultural Microbiology, College of Agriculture, Rajendranagar, Hyderabad. Biogas manure samples were collected from biogas digesters set with six different substrates (cow dung, press mud, poultry litter, kitchen wastes, maize stalks and fruit wastes) after the gas production stopped and the manures were sterilized. Beneficial microorganisms were added individually to the sterilized biogas manure samples. The beneficial microorganisms viability in the enriched biogas manures was monitored upto the end of tenth week and the increase in population was observed in all the biogas manures samples indicating that the biogas manure samples from different substrates support the beneficial microorganisms population for atleast 4 weeks duration.

**Key words:** Cow dung, Enrichment, Kitchen wastes, Maize stalks, Poultry litter, Press mud, Viability.

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## BIOMINING – A USEFUL APPROACH FOR METAL EXTRACTION TO LIMIT ENVIRONMENT POLLUTION

**P. GAYATHREE DEVI<sup>1</sup>, T. NAGA RAMYA KRISHNA<sup>2</sup>, P.LAKSHMI THANMAI<sup>3</sup>**

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Biomining is the extraction of specific metals from their ores through biological means usually bacteria. Although it is a new technique used by the mining industry to extract minerals such as copper, uranium and gold from their ores but nowadays biomining occupies an increasingly important place among the available mining technologies due to environment pollution. Traditional extractions involve many expensive steps such as roasting and smelting, which requires sufficient concentrations of elements in ores while low concentrations are not a problem for bacteria because they simply ignore the waste which surrounds the metals, attaining extraction yields of over 90% in some cases. Biomining is a general term used to describe the use of microorganisms to facilitate the extraction of metals from sulfide or iron-containing ores or concentrate. The future of biomining is challenging, as it offers advantages of operational simplicity, low capital and operating cost and shorter construction times that no other alternative process can provide. In addition, minimum environmental impact and the use of this technology in the mining industry is set to increase. Increased concern regarding the effect of mining on the environment is likely to improve the competitive advantage of microbially based metal recovery processes. The enforcement of more stringent legislation to limit environmental pollution would make biomining more attractive.

Keywords: Biomining, Minerals, microbes

## MICROBES AND THEIR ROLE IN MINING

**K.LAKSHMI SAROJA, S.T.V.RAGHAVAMMA, RAMA RAO NADENDLA**

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Bio-mining is one of the biotechnological procedures used to recover various types of minerals from the ores by using microorganisms. It can also be explained in terms of bioleaching or bio-oxidation. Bioleaching is defined as conversion of insoluble valuable materials into soluble forms by means of micro organisms. In industry bioleaching takes place in three processes-1) Heap or dump bioleaching 2) Stirred tank bioleaching 3) In-situ or in place bioleaching. Heap bioleaching is most widely used process. The metal leaching organisms use iron and sulfur compounds as electron donors and fix carbon dioxide. Many of these produce sulfuric acid. Some of the organisms used bio-mining are *Sulfolobus* in bioleaching of Molybdenite, *Actinomyces*, *Pseudomonas*, and *Neisseria* in extraction of Gold, *Thiobacillus* in copper bioleaching. Fungi also used in bio-mining. Bio-mining is cheap, efficient and also environment friendly method of mineral recovery. Microbial leaching is process by which metals are dissolved from ore bearing rocks using micro organisms It is unaffected by low concentrations of the metals and reduces air pollution and little damage to the environment.

Key Words: Bio-mining; Bioleaching; Mineral recovery; *Pseudomonas*; *Thiobacillus*

## BIO-MINING

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Bio-mining is the process of extracting metals from ores and concentrates by using biological means like bacteria, fungi. Though it is a new process followed by mining industry for extraction of minerals like copper, uranium and gold from their ores but nowadays bio-mining plays an important among the available mining techniques. Bio-mining attains extraction yields of 90% of minerals from ores. This presentation deals with the process of bio-mining, fundamentals of bio-mining including bioleaching mechanisms, how bacteria are used in these processes, applications of bio-mining, advantages of bio-mining over other extraction techniques.



## BIOMINING – A USEFUL APPROACH FOR METAL EXTRACTION TO LIMIT ENVIRONMENT POLLUTION

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Keywords: Biomining, Minerals, microbes

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Key Words: Bio-mining; Bioleaching; Mineral recovery; *Pseudomonas*; *Thiobacillus*



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**BIO-MINING**

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**CARBON SEQUESTRATION****Y.LAKSHMI PRASANNA***Lecturer in chemistry, Government college for women(A) Guntur.*[yangalasettyprassu@gmail.com](mailto:yangalasettyprassu@gmail.com)**ABSTRACT**

Human activities, especially the burning of fossil fuels such as coal, oil, and gas, have caused a substantial increase in the concentration of carbon dioxide (CO<sub>2</sub>) in the atmosphere. This increase in atmospheric CO<sub>2</sub> — from about 280 to more than 380 parts per million (ppm) over the last 250 years—is causing measurable global warming. Potential adverse impacts include sea-level rise; increased frequency and intensity of wildfires, floods, droughts, and tropical storms; changes in the amount, timing, and distribution of rain, snow, and runoff; and disturbance of coastal marine and other ecosystems. Carbon sequestration is the process through which agricultural and forestry practices remove carbon dioxide (CO<sub>2</sub>) from the atmosphere. The term “sinks” is also used to describe agricultural and forestry lands that absorb CO<sub>2</sub>, the most important global warming gas emitted by human activities. Agricultural and forestry practices can also release CO<sub>2</sub> and other greenhouse gases to the atmosphere. Sequestration activities can help prevent global climate change by enhancing carbon storage in trees and soils, preserving existing tree and soil carbon, and by reducing emissions of CO<sub>2</sub>, methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O).

Key words: carbon sequestration, nitrogen fertilizer, Agricultural Practices, rhizosphere.

**Introduction**

Rising atmospheric CO<sub>2</sub> is also increasing the absorption of CO<sub>2</sub> by seawater, causing the ocean to become more acidic, with potentially disruptive effects on marine plankton and coral reefs. Technically and economically feasible strategies are needed to mitigate the consequences of increased atmospheric CO<sub>2</sub>. The United States needs scientific information to develop ways to reduce human-caused CO<sub>2</sub> emissions and to remove CO<sub>2</sub> from the atmosphere. Abstract. This paper reviews progress on understanding biological carbon sequestration in the ocean with special reference to the microbial formation and transformation of recalcitrant dissolved organic carbon (RDOC), the microbial carbon pump (MCP).

**What is carbon sequestration?**

Carbon sequestration is the process involved in carbon capture and the long-term storage of atmospheric carbon dioxide.<sup>[1]</sup> Carbon sequestration involves long-term storage of carbon dioxide or other forms of carbon to either mitigate or defer global warming and avoid dangerous climate change. It has been proposed as a way to slow the atmospheric and marine accumulation of greenhouse gases, which are released by burning fossil fuels.<sup>[2]</sup>

Carbon dioxide (CO<sub>2</sub>) is naturally captured from the atmosphere through biological, chemical, and physical processes.<sup>[3]</sup> Artificial processes have been devised to produce similar effects,<sup>[3]</sup> including large-scale, artificial capture and sequestration of industrially produced CO<sub>2</sub> using subsurface saline aquifers, reservoirs, ocean water, aging oil fields, or other carbon sinks.

Carbon Sequestration is capturing and securely storing carbon dioxide emitted from the global energy system.

**Types of Carbon Sequestration:**

There are number of technologies under investigation for sequestering carbon from the atmosphere. These can be discussed under three main categories:

- Ocean Sequestration: Carbon stored in oceans through direct injection or fertilization.
- Geologic Sequestration: Natural pore spaces in geologic formations serve as reservoirs for long-term carbon dioxide storage.



- Terrestrial Sequestration: A large amount of carbon is stored in soils and vegetation, which are our natural carbon sinks. Increasing carbon fixation through photosynthesis, slowing down or reducing decomposition of organic matter, and changing land use practices can enhance carbon uptake in these natural sinks.

**Geologic Sequestration Trapping Mechanisms:**

- Hydrodynamic Trapping: Carbon dioxide can be trapped as a gas under low-permeability cap rock (much like natural gas is stored in gas reservoirs).
- Solubility Trapping: Carbon dioxide can be dissolved into a liquid, such as water or oil.
- Mineral Carbonation: Carbon dioxide can react with the minerals, fluids, and organic matter in a geologic formation to form stable compounds/minerals; largely calcium, iron, and magnesium carbonates.

While research continues on mineral carbonation, early results indicate reaction times to be too slow for this technology to have near-term widespread applicability. Carbon dioxide can be effectively stored in the earth's subsurface by hydrodynamic trapping and solubility trapping - usually a combination of the two is most effective.

**How is carbon sequestered in soils?**

Through the process of photosynthesis, plants assimilate carbon and return some of it to the atmosphere through respiration. The carbon that remains as plant tissue is then consumed by animals or added to the soil as litter when plants die and decompose. The primary way that carbon is stored in the soil is as soil organic matter (SOM). SOM is a complex mixture of carbon compounds, consisting of decomposing plant and animal tissue, microbes (protozoa, nematodes, fungi, and bacteria), and humus – carbon associated with soil minerals. Carbon can remain stored in soils for millennia, or be quickly released back into the atmosphere through respiration by soil microbes. Climatic conditions, natural vegetation, soil texture, drainage, and human land use all affect the amount and length of time carbon is stored in soil.

**Agricultural Practices that Increase Soil Carbon Sequestration;**

In agricultural systems, the amount and length of time carbon is stored in the soil is largely determined by how the soil resource is managed. A variety of agricultural practices that can enhance carbon storage have been proposed. The benefits of these practices as well as their potential hidden costs must be considered when management decisions are made. Though not discussed here, there may also be direct or indirect costs and benefits to farmers implementing these techniques. Removing CO<sub>2</sub> from the atmosphere is only one significant benefit of enhanced carbon storage in soils. Improved soil and water quality, decreased nutrient loss, reduced soil erosion, increased water conservation, and greater crop production may result from increasing the amount of carbon stored in agricultural soils. Management techniques that sequester carbon in soils include. Conservation tillage - minimizes or eliminates plowing of soil for crop production through practices such as no-till (no plow) farming or mulch tillage. These procedures generally reduce soil erosion, improve water use efficiency, and increase carbon concentrations in the topsoil, leading to a significant increase in soil CO<sub>2</sub> sequestration. Conservation tillage can also lower the amount of fossil fuel consumed since it reduces the operation of farm machinery. Cover cropping - uses crops such as clover and small grains for protection and soil improvement between periods of regular crop production. Cover crops improve carbon sequestration by enhancing soil structure and adding organic matter to the soil. Crop rotation - sequences of crops grown in regularly recurring succession on the same area of land. It mimics the diversity of natural ecosystems more closely than intensive mono cropping practices. Varying the type of crops grown can increase the level of soil organic matter. Effectiveness of crop rotating varies by region, crop type, and crop rotation timing. Mulch left on the soil to reduce erosion Photo courtesy of University of Nebraska-Lincoln Extension. Alfalfa, a cover crop



Photo courtesy of National Renewable Energy Laboratory 3 What is soil carbon sequestration? Great Lakes Bio energy Research Center - [www.glbrc.org/education](http://www.glbrc.org/education) Hidden Costs of Some Proposed Agricultural

**Other Strategies to Reduce CO<sub>2</sub> Emissions Some agricultural practices proposed as methods for sequestering carbon may have hidden costs:** Increased use of nitrogen fertilizer may temporarily increase soil organic matter because nitrogen is often limited in agro ecosystems. The CO<sub>2</sub> released from fossil fuel combustion during the production, transport and application of nitrogen fertilizer, however, can reduce the net amount of carbon sequestered. Nitrogen from fertilization can also run off from agricultural lands into nearby waterways where it may have serious ecological consequences by stimulating excessive algal growth. Growing plants on semiarid lands has been suggested as a way to increase carbon storage in soils. The fossil fuel costs of supplying irrigation for these lands, however, may exceed any net gain in carbon sequestration. Additionally, in many semi-arid regions surface and groundwater contain high concentrations of dissolved calcium and bicarbonate ions. As these are deposited in the soil they release CO<sub>2</sub> into the atmosphere.

**There is still much to learn about carbon sequestration:** Current scientific research is examining: The full impacts of land use and land management on soil carbon sequestration and ways to increase the storage time of carbon in soil. The relationship between underlying mechanisms controlling soil structure and the storage of carbon. These include various chemical, physical, biological, mineralogical, and ecological processes.

**Carbon sequestration using microbial technologies :** Plants transfer carbon through root material and root exudates from the atmosphere into the soil .The chemical and physical processes by which the carbon is cycled or sequestered is depended on microbial processes in the rhizosphere. A better understanding of feed backs linking the rhizosphere and climate change will allow for a better picture of global carbon cycling and the ability of the biosphere to offset rising anthropogenic increases in CO<sub>2</sub> emission.

**Conclusion:** There is no question that global warming will have a significant impact on already existing problems such as malaria, malnutrition, and water shortages. But this doesn't mean the best way to solve them is to cut carbon emissions. Carbon The experiment has been placed on hold until it can go back to the drawing board and a better process is created. While sound in theory the actual implementation of the process requires a level of human precision making it very difficult to complete without contamination and even more difficult to reproduce results. As a result I bounced around to a few other projects to fill the remaining time.

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## CARBON ELIMINATOR

Carbon sequestration in lakh tons for a tree with girth between 10-30 cm

Scientific name	Local name	Carbon sequestration
Tectona grandis	Sagwaan	3.70
Eucalyptus globulus	Nilgiri	2.47
Prosopis juliflora	Gando baval	1.67
Azadirachta indica	Limdo	1.45
Casuarina equisetifolia	Sharu	1.28
Acacia tortilis	Israeli baval	1.04





## BIOLOGICAL CARBON SEQUESTRATION

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Carbon sequestration is a natural or artificial process by which carbon dioxide is removed from the atmosphere and held in solid or liquid form. It involves in carbon capture and the long-term storage of atmospheric carbon dioxide or other forms of carbon to either mitigate or defer global warming and avoid dangerous climate change. Carbon dioxide (CO<sub>2</sub>) is naturally captured from the atmosphere through biological, chemical, and physical processes.

Carbon moves in natural processes called the carbon cycle between the atmosphere (air), the biosphere (living things), and the geosphere (land and water). Biological carbon sequestration is a more specific part of the carbon cycle in which plants naturally take in CO<sub>2</sub> from the atmosphere by way of photosynthesis.

They also accumulate, or sequester, carbon in various ways: in living biomass, in litter on the ground when leaves fall, and in soils when organic material decomposes. At the same time, plants also release carbon as carbon dioxide to the atmosphere. While plants live, these carbon releases occur through natural respiration and, when they die, either by natural means (e.g. fire or decomposition) or by human causes (e.g. clear cutting).

Key words: Carbon sequestration, Global warming, Climate change, Atmosphere, Biosphere, Geosphere, Carbon cycle

## CARBON SEQUESTRATION IN SOIL-THE POTENTIAL UNDERFOOT

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Rising levels of atmospheric CO<sub>2</sub> and associated global warming- center stage of climate change discussion in the past two decades. Carbon is accumulating in the atmosphere at a rate of 3.5 Pg (Pg = 10<sup>15</sup> g or billion tons) per annum, - resulting from the burning of fossil fuels and the conversion of forests to agricultural production. Carbon sequestration is the solution to reduce the negative impacts of climate change and to achieve the food demand of 300 m t of cereals by 2050. To check degradation of soil and water resources and overall enhancements of soil quality. To improve sustainable development. To minimize climate change which is among the major global issues of the 21<sup>st</sup> century. Through carbon sequestration atmospheric CO<sub>2</sub> levels are reduced as soil organic carbon levels increase. By managing the crop land we can stored the 0.107 tonnes of carbon/acre'. If the soil organic carbon is undisturbed, it can remain in the soil for many years as stable organic matter. This process reduces CO<sub>2</sub> levels in the atmosphere, reducing the chances of global warming.

**Key words:** Carbon sequestration, Agricultural production



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## ADVANCES IN CARBONDIOXIDE CAPTURE TECHNOLOGIES

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There is growing concern that anthropogenic carbon dioxide (CO) emissions are contributing to global climate change. Therefore, it is critical to develop technologies to mitigate this problem. One very promising approach to reducing CO emissions is CO capture at a power plant, transport to an injection site, and sequestration for long-term storage in any of a variety of suitable geologic formations. However, if the promise of this approach is to come to fruition, capture costs will have to be reduced. CO<sub>2</sub> capture from coal-derived power generation can be achieved by various approaches: post-combustion capture, pre-combustion capture, and oxy-combustion. All three of these pathways are under investigation, some at an early stage of development. A wide variety of separation techniques is being pursued, including gas phase separation, absorption into a liquid, and adsorption on a solid, as well as hybrid processes, such as adsorption/membrane systems. Current efforts cover not only improvements to state-of-the-art technologies but also development of several innovative concepts, such as metal organic frameworks, ionic liquids, an enzyme-based systems. This paper discusses the current status of the development of CO capture technology.

Key words: Anthropogenic CO, sequestration, phase separation, pre-combustion capture, oxy-combustion.

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## STUDIES ON POST HARVEST MANAGEMENT OF COPRA QUALITY

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## ABSTRACT

Coconut (*Cocos nucifera*. L.) is an important plantation crop in India. The crop is being grown in an extent of 18,39,800 ha with a production of 12,597.3 nuts annually. Among different States, Andhra Pradesh ranks fourth in area and in production with respect to coconut cultivation in our country (Directorate of Economics & Statistics, AP). The crop is often called 'Kalpavriksha' due to its multifarious uses. However, the economic end product *i.e.*, copra is the source of coconut trade in the country. Copra is a source for oil, cake and other edible products. The copra is usually dried by air-drying, forced air-drying and by kiln drying methods. However, improper handling of any of these methods may lead to contamination with mycoflora that deteriorate the quality of copra and thus trade. Isolation studies revealed that *Aspergillus flavus*, *A. niger*, *Rhizopus* spp, *Drechslera* spp, *Botryodiplodia* spp and *Penicillium* spp are the commonly associated mycoflora on copra during storage. *Aspergillus flavus* was predominant among the mycoflora with percent colonies ranging from 68 to 92 per each sample. This is followed by *Penicillium* spp with a range of 61 to 69 per cent colonies. *Aspergillus niger* was recorded to a tune of 46 to 64 per cent colonies whereas other species of *Aspergillus* were recorded to an extent of 47 to 57 per cent. It is a known fact that copra stored under different moisture conditions show different mycoflora on their surface. Dual culture studies carried out with the three species of *Trichoderma* isolated from soils of coconut gardens revealed that *T.viride*, *T.harzianum* and *T.hamatum* were found very effective in inhibiting the mycelial growth of *A.flavus* under in vitro conditions. Of them, *T.hamatum* was found very effective in controlling both the isolates. This was followed by *T.harzianum* and *T.viride* with insignificant differences in percent of inhibitions. A clear inhibition zone was noticed with all the three *Trichoderma* species and the inhibition zone prevailed up to 1 week. This was followed by mycoparasitism. *Trichoderma* spp are well-known biocontrol agents against several soil borne plant pathogens and are widely used under field conditions to combat aflatoxin problems in coconut. Among the different chemical preservatives assayed, maximum inhibition of *A.flavus* strains was obtained by Menadione (100%) followed by Potassium meta bisulphate and Benzoic acid with inhibition of 77.23% and 63.33% respectively. The preservatives, Sodium Benzoate and Ascorbic acid also performed well in inhibiting the *A.flavus* strains by more than 50% *i.e.* 57.78% and 53.89% respectively. However, the efficacy of Propionic acid is also notable with an inhibition of 43.33% on *A.flavus* strains. On the other hand, Glacial acetic acid had a mild inhibitory effect with an inhibition of 6.84% on *A.flavus* strains. The same chemical preservative even did prove ineffective against both the aflatoxin producing molds at 100ppm with no inhibitory effect. Compatibility studies between *a.flavus* and the commonly used preservatives in food industry gives an idea of the nature of chemical preservatives to be used. preservatives that have negative impact on the survival, growth and multiplication of *a.flavus* in the soil and on copra to be used in order to combat the aflatoxin problem in copra. however, the preservatives that are to be recommended should not hinder the multiplication of native *trichoderma* spp are antagonist to *a.flavus* especially when applied to soil.

Key words: *Aspergillus flavus*, Aflatoxin, *Trichoderma* spp, Copra and Coconut

## INTRODUCTION

Coconut (*Cocos nucifera*. L.) is an important plantation crop in India. The crop is being grown in an extent of 18,39,800 ha with a production of 12,597.3 nuts annually. Among different States, Andhra Pradesh ranks fourth in area and in production with respect to coconut cultivation in our country (Directorate of Economics & Statistics, AP). The crop is often called 'Kalpavriksha' due to its multifarious uses. However, the economic end



product *i.e.*, copra is the source of coconut trade in the country. Copra is a source for oil, cake and other edible products (Table-1). The copra is usually dried by air-drying, forced air-drying and by kiln drying methods. However, improper handling of any of these methods may lead to contamination with mycoflora that deteriorate the quality of copra and thus trade.

**Table-1: Threat of aflatoxin contamination among various food & feed stuffs of copra**

Food products	
a. Wet meat or Kernel:	c. Coconut water:
⇒ Coconut milk (CM)	
⇒ Coconut jam (CJ) & Coconut syrup (CS)	
⇒ Coconut skim milk (CSM)	
⇒ Coconut flour (CF)	d. Coconut toddy:
⇒ Desiccated coconut (DC)	⇒ Coconut Jaggery
b. Dry Kernel or Copra:	⇒ Coconut vinegar
⇒ Coconut oil	⇒ Treacle
⇒ Coconut cake	

\* a& b are prone to aflatoxin contamination.

Copra quality is found to be reduced by many fungi and important of them attacking copra are *Aspergillus niger*, *A.glaucus*, *A.flavus*, *A.fumigatus* and *A.ochraceous* (Cooke, 1932; Ward, 1937), *Ceratostomella adiposum* (Thompson, 1933), *Botryodiplodia theobromae*, *Aspergillus spp*, and *Rhizopus oryzae* (Rao *et.al.*, 1971) and *Penicillium frequentas* (Nair and Nathan, 1971). *Endosporostilbe sp.* (Patil and Kelkar, 1975) has been observed recently on the copra and shell of coconut. *Penicillium citrinum* (Susamma and Menon, 1983) and *Drechslera hawaiiensis* (Sharma *et.al.*, 1985) are also known to attack copra. The fungus *D.hawaiiensis* enters the fruit while attached to the bunch itself. The fungus, *Aspergillus spp* enters into the copra meat under high moisture conditions and thereby causing discoloration and also an increase in free fatty acid levels. Susamma and Menon (1983) reported that copra having 5 per cent moisture and kept in gunny bags lined with polythene sheet remained moderately free from infection. The fungi, *B.theobromae*, *Aspergillus spp* and *R.oryzae* enhanced the cellulolytic enzyme activity in the affected copra (Susamma, 1980). They also found that monsoon periods favored the development of both bacterial and fungal populations. Subramanyam 1965 and Paul *et.al* (1980) observed frequent association of *Bacillus spp* with copra spoilage while others (Paul *et.al.*, 1980) noted that bacterial population was replaced by fungi when the temperature rose and moisture content of copra comes down. The present chapter reviewed the research work on post harvest spoilage of copra and the possible aflatoxin contamination and a detailed account on the non pesticidal approaches of managing the post harvest spoilage of copra with emphasis on biocontrol of *A.flavus*, the incitant of aflatoxin contamination of copra and also the possible role of chemical preservatives in checking the aflatoxin inducing *A.flavus* mold infestation in copra.

#### Awareness on aflatoxin contamination

Opinion survey on traders' awareness regarding mycoflora responsible for copra rotting and possible aflatoxin contamination in East Godavari district of Andhra Pradesh revealed that all the traders (100%) were aware of the fact that mycoflora play a vital role in rotting of copra. Of them, 72% of the traders are unaware that copra can be contaminated by aflatoxin. They mostly opined that lack of visual indication on the copra is the sole reason for their being unaware of aflatoxin contamination. On the other hand, the remaining (28%) traders attributed the bitterness in taste of copra to the possible aflatoxin contamination due to molds. The dialogue



on copra deterioration due to molds revealed that all the traders are well aware of the fact that copra quality is dictated by the extent of mold infection under improper storage conditions. Most of the traders felt that extraction of copra will be carried out at farmers' level and so the chances of mold infestation will be more since the farmers are unaware of the fact that improper drying would lead to fungal contamination.

#### ASSOCIATION OF MYCOFLORA WITH COPRA

Isolation studies revealed that *Aspergillus flavus*, *A. niger*, *Rhizopus* spp, *Drechslera* spp, *Botryodiplodia* spp and *Penicillium* spp are the commonly associated mycoflora on copra during storage (Table-2 & Plate-1). *Aspergillus flavus* was predominant among the mycoflora with percent colonies ranging from 68 to 92 per each sample. This is followed by *Penicillium* spp with a range of 61 to 69 per cent colonies. *Aspergillus niger* was recorded to a tune of 46 to 64 per cent colonies whereas other species of *Aspergillus* were recorded to an extent of 47 to 57 per cent. It is a known fact that copra stored under different moisture conditions show different mycoflora on their surface.

Under high moisture conditions, *Aspergillus* spp rapidly penetrate into the copra meat causing its discoloration, thereby resulting an increase in free fatty acid levels. On the other hand, copra stored at 5% moisture levels remained moderately free from infection (Susamma & Menon, 1983). Monsoon periods favor the development of fungal population in copra (Susamma *et.al.*, 1980). The ill effects of consuming aflatoxin tainted cake and oil have been mainly observed in poultry and milch cattle (Bhat *et. al.* 1978). It is even recognized that when animals consume feed contaminated with aflatoxins, the toxin is metabolized in the body and possibly get into the milk as aflatoxin M1, or may be found in animal products such as meat, eggs etc, which are consumed by man. So, building up of awareness among traders, retailers' regarding aflatoxin contamination as a negative common property resource is essential. Stringent legislative measures have to be enforced so as to make the traders store their collected copra under proper conditions thereby preventing the rot from these aflatoxin producing molds.

**Table-2: Nature and extent of fungal infection on copra collected from traders, East Godavari district, Andhra Pradesh**

Fungus	Number of colonies /sample (%)
<i>Aspergillus flavus</i>	68 – 92
<i>Penicillium</i> spp	61 – 69
<i>Aspergillus niger</i>	46 – 64
<i>Aspergillus</i> spp	47 – 67
<i>Rhizopus</i> spp	33 – 44
<i>Drechslera</i> spp	20 – 31
<i>Botryodiplodia</i> spp	11 – 18



**Plate-1: Mycoflora associated with copra during improper storage condition**



### Biocontrol of *A.flavus*, the incitant of aflatoxin contamination of copra

**Isolation of *Trichoderma* spp:** Isolation studies carried out from soil samples collected from coconut gardens revealed the presence of three *Trichoderma* species and were subsequently identified as *T.viride*, *T.harzianum* and *T.hamatum* (Rifai, 1969).

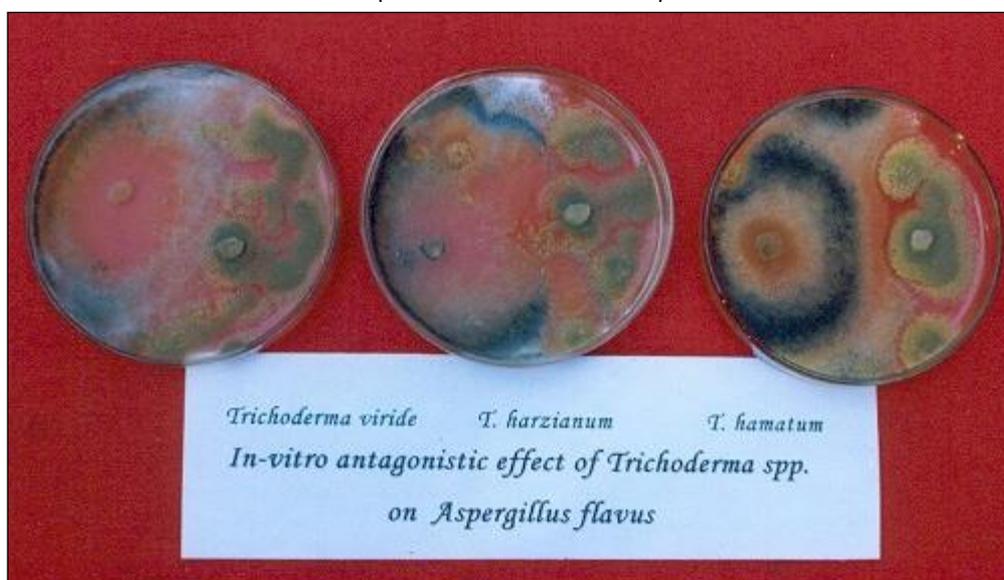
***In vitro* antagonistic studies:** Dual culture studies carried out with the three species of *Trichoderma* isolated from soils of coconut gardens revealed that *T.viride*, *T.harzianum* and *T.hamatum* were found very effective in inhibiting the mycelial growth of *A.flavus* under *in vitro* conditions (Plate-2). Of them, *T.hamatum* was found very effective in controlling both the isolates. This was followed by *T.harzianum* and *T.viride* with insignificant differences in percent of inhibitions (Table-3). A clear inhibition zone was noticed with all the three *Trichoderma* species and the inhibition zone prevailed up to 1 week. This was followed by mycoparasitism. *Trichoderma* spp are well-known biocontrol agents against several soil borne plant pathogens and are widely used under field conditions to combat aflatoxin problems in groundnut.

**Table-3: Dual culture studies between *Trichoderma* spp and *A.flavus***

Antagonist	Per cent inhibition of <i>A.flavus</i> copra isolate	Remarks
<i>Trichoderma viride</i>	84.4 <sup>b</sup>	Inhibition zone was observed after 4 days followed by mycoparasitism.
<i>T.harzianum</i>	83.3 <sup>b</sup>	
<i>T.hamatum</i>	88.8 <sup>a</sup>	

\* Numbers in each column followed by the different letter are significantly different.

Values represent the means of 6 replicates.



**Plate-2: *In vitro* inhibition of linear spread of *A.flavus* by *Trichoderma* spp.**

Further *in vitro* studies carried out to determine the efficacy of the *Trichoderma* spp against *A.flavus* isolates revealed that volatile metabolites of 30 day old cultures of *T.viride*, *T.harzianum* and *T.hamatum* were inhibitory to *A.flavus*. While, 0 and 15 day old cultures of all the three *Trichoderma* spp were infective in inhibiting the mycelial growth of *A.flavus* through volatile metabolites (Plate-3). Among the *Trichoderma* spp of 30 days old, maximum inhibition of *A.flavus* isolates was obtained with *T.viride* (66.67%), followed by *T.harzianum* and *T.hamatum* with an inhibition of 61.11% (Table-4).

In case of non-volatile metabolites, an increasing trend of inhibition of *A.flavus* was noticed with an increase in the concentration of culture filtrate of the *Trichoderma* spp. Among the *Trichoderma* spp, maximum inhibition



of the mycelial growth of *A.flavus* was obtained by *T.hamatum* (57.6%) (Plate-4), followed by *T.harzianum* (42.3%) and *T.viride* (30.7%) at 100% concentration of the culture filtrate (Table-5 ). However, inhibition of the test fungus to a notable extent was also achieved by all the three *Trichoderma* spp at culture filtrate concentrations of 75%, 50% and 20% respectively.



Plate-3: Antagonistic activity of *Trichoderma* spp volatile metabolites on *A.flavus* f



Plate-4: Antagonistic activity of non-volatile metabolites *Trichoderma* spp on *A.flavus*

Table-4: *In vitro* inhibition effect of volatile metabolites of *Trichoderma* spp on *A.flavus*

Antagonist	Per cent inhibition of <i>A.flavus</i>		
	Age of <i>Trichoderma</i> spp		
	0 days	15 days	30 days
<i>T.viride</i>	0	0	66.67 <sup>a</sup>
<i>T.harzianum</i>	0	0	61.11 <sup>b</sup>
<i>T.hamatum</i>	0	0	61.11 <sup>b</sup>

\* Numbers in each column followed by the different letters are significantly different.

Values represent the mean of 6 replicates.

Table-5: *In vitro* inhibition effect of non-volatile metabolites of *Trichoderma* spp on *A.flavus*

Antagonist	Per cent inhibition of <i>A.flavus</i>				
	Concentration of culture filtrate of antagonist				
	10%	20%	50%	75%	100%
<i>T.viride</i>	3.81 <sup>b</sup>	7.6 <sup>c</sup>	11.5 <sup>c</sup>	30.7 <sup>c</sup>	30.7 <sup>c</sup>
<i>T.harzianum</i>	3.84 <sup>b</sup>	23.0 <sup>b</sup>	23.0 <sup>b</sup>	38.4 <sup>b</sup>	42.3 <sup>b</sup>
<i>T.hamatum</i>	19.2 <sup>a</sup>	34.6 <sup>a</sup>	46.1 <sup>a</sup>	48.0 <sup>a</sup>	57.6 <sup>a</sup>

\* Numbers in each column followed by the different letters are significantly different. Values represent the mean of 6 replicates.

Srilakshmi *et al.*, (2001), while working on aflatoxin problem of groundnut, reported that *T.viride*, *T.harzianum* showed clear hyphal coiling with *A.flavus* strain 11-4 mycelia in dual culture studies. In vitro studies carried out to establish the production of volatile metabolites by *Trichoderma* spp against *A.flavus* strain of groundnut revealed that among different isolates, *T.longibrachiatum* produced maximum volatile metabolites thereby inhibiting *A.flavus* to a maximum extent (Srilakshmi *et al.*, 2001). *Trichoderma* spp has the ability to inhibit the growth of *A.flavus* *in vitro* by production of non volatile metabolites (Desai *et al.*, 2000).



Aflatoxin contamination in food and feed stuffs is a common problem when the copra is stored under improper conditions. Ill effects of consuming aflatoxin tainted cake and oil have been observed in poultry and milch cattle (Bhat *et.al.*, 1978). Since the conidia of *Aspergillus flavus* is almost universally present, and with the ambient moisture content favoring the conidial germination and multiplication of the fungus, there is every chance of *A.flavus* infecting copra when dried improperly. Awareness among traders has to be built up with regard to the aflatoxin accumulation in food and feed stuffs as a consequence of bad handling of copra.

Studies on the inhibition effect of chemical preservatives *viz.*, Menadione, Potassium meta bisulphite, Benzoic acid, Sodium benzoate, L-Ascorbic acid, Propionic acid and Acetic acid (glacial) on *A.flavus* strains (AF<sub>2</sub> --- Highly virulent and Aggressive strain, AF<sub>3</sub> ----- Moderately virulent strain) revealed that all the assayed chemicals reduced the linear growth of the aflatoxin producing molds from moderate to significant levels at 500ppm concentration and also to a certain extent at 100ppm concentration (Table-6). A significant positive correlation was noticed among majority of the chemicals with respect to increase in dosage from 100ppm to 500ppm with respect to *A.flavus* strain inhibition in terms of linear growth (Plate-5). The inhibition of linear growth of the virulent, aggressive strain of *A.flavus* (AF<sub>2</sub>) ranged from 6.67 % to 100%, whereas, the other moderately virulent strain, AF<sub>3</sub> of *A.flavus* was inhibited to an extent of 7.00% to 100%. Since, chemicals are targeted against all the strains *i.e.* right from aggressive to moderately to less aggressive strains, the current discussion on the average percent inhibition of *A.flavus* is apt in the present study. Among the different chemicals assayed, maximum inhibition of *A.flavus* strains was obtained by Menadione (100%) followed by Potassium meta bisulphate and Benzoic acid with inhibition of 77.23% and 63.33% respectively. The preservatives, Sodium Benzoate and Ascorbic acid also performed well in inhibiting the *A.flavus* strains by more than 50% *i.e.* 57.78% and 53.89% respectively. However, the efficacy of Propionic acid is also notable with an inhibition of 43.33% on *A.flavus* strains. On the other hand, Glacial acetic acid had a mild inhibitory effect with an inhibition of 6.84% on *A.flavus* strains. The same chemical preservative even did prove ineffective against both the aflatoxin producing molds at 100ppm with no inhibitory effect (Table-6).



Plate-5: *In vitro* inhibition of chemical preservation on the linear growth of *A.flavus* at 500 ppm concentration



**Table-6: Effect of chemical preservatives on the linear growth of *Aspergillus flavus* strains on PDA incubated at 28°C for 96 hrs.**

Chemical	AF <sub>2</sub> ( <i>A.flavus</i> )		AF <sub>3</sub> ( <i>A.flavus</i> )		Average inhibition (%) of <i>A.flavus</i> strains at 500 ppm
	% Inhibition at		% Inhibition at		
	100 ppm	500 ppm	100 ppm	500 ppm	
Menadione	77.78(20 <sup>a</sup> )	100(0 <sup>a</sup> )	85.56(13 <sup>a</sup> )	100(0 <sup>a</sup> )	100
Potassium meta bisulphite	60.00(36 <sup>b</sup> )	77.78(20 <sup>b</sup> )	64.44(32 <sup>b</sup> )	76.67(21 <sup>b</sup> )	77.23
Benzoic acid	54.44(41 <sup>c</sup> )	64.44(32 <sup>c</sup> )	58.89(37 <sup>c</sup> )	62.22(34 <sup>c</sup> )	63.33
Sodium benzoate	54.44(41 <sup>c</sup> )	53.33(42 <sup>d</sup> )	54.44(41 <sup>d</sup> )	62.22(34 <sup>c</sup> )	57.78
L-Ascorbic acid	55.55(40 <sup>c</sup> )	53.33(42 <sup>d</sup> )	53.33(42 <sup>d</sup> )	54.44(41 <sup>d</sup> )	53.89
Propionic acid	0.00(90 <sup>d</sup> )	22.22(70 <sup>e</sup> )	0(90 <sup>e</sup> )	64.44(32 <sup>c</sup> )	43.33
Acetic acid glacial	0.00(90 <sup>d</sup> )	6.67(84 <sup>f</sup> )	0(90 <sup>e</sup> )	7.00(83.7 <sup>e</sup> )	6.84
<b>Control</b>	<b>(90mm)</b>				

\* Means followed by similar letters are not different statistically (P = 0.05) by Duncan's multiple range test. \*\* Values in parentheses are the linear growths of fungi in millimeters.

Post harvest management of copra quality is a challenging task owing to the improper handling of copra by farmers/ traders, lack of awareness on mycotoxin contamination and also to the consequent health hazards due to metabolites produced by the harbored molds on copra. Improper handling of copra after harvest is of concern in the present day coconut scenario due to the threat posed by aflatoxin producing molds that infest copra, thus spoiling the healthy food chain and subsequently the coconut trade. Among the mycoflora that invade copra, *Aspergillus flavus* Link contamination is a potential hazard to the coconut industry and trade because of its secondary metabolite production, the aflatoxins. Aflatoxins are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* Speare that are carcinogenic, hepato-toxic and teratogenic in nature (Coulibaly, 1987). There are several reports of aflatoxin contamination of copra. Phillipine coconut industry which exports copra cake used as feed ingredient in Europe, identified Aflatoxin B<sub>1</sub> level to a tune of 7-70 ppb and therefore gave a regulation that any move by European country to restrict the level of Aflatoxin B<sub>1</sub> in feed stuffs would pose difficulty for the local coconut product given the climatic conditions in the country. Entry of aflatoxins into food chain is considered to be the most devastating problem not only through coconut, but also through cereal grains, spices, dry fruits, pulses, vegetables, cheese, bread and oil seeds; which are the prominent aflatoxin producing potential sources of *A.flavus* and *A.parasiticus* (Mc Donald, 1976).

Detoxification of coconut products that were prone and contaminated by aflatoxins viz., coconut milk, coconut jam, coconut syrup, coconut skim milk, coconut flour, dessicated coconut (products from kernel) and coconut oil, coconut cake (products of copra) is a futile exercise as none of the detoxification procedures available can detoxify to the fullest extent. Detoxification methods of the present day include Ammoniation, Photolysis (Shantha and Sreenivasamurthy, 1977), extraction with sodium chloride (Shantha and Sreenivasamurthy, 1975), filtration (Basappa and Sreenivasamurthy, 1979), by hydrogen peroxide, by sunlight (Shantha *et.al.*, 1986), urea and formaldehyde (Codifer *et.al.*, 1976) are widely used against groundnut aflatoxin contamination (Read, 1989). However, detoxification of copra products after aflatoxin contamination should be the last resort and efforts to tame the aflatoxin problem in food and feedstuffs of copra should be directed towards managing the problem in the initial stages of contamination of the copra.



Copra preservation by chemical preservatives is generally accomplished by a mechanism of drying *i.e.* employing a relatively dry environment against microbes that are responsible for spoilage. A microbe in a non-saline environment is able to exchange water through its membrane easily, but in a saline environment, which is induced by adding chemical preservatives, an isotonic situation is attained thereby resulting in slower microbe growth and probably even death (Jay, J.M.2002). In the present study, Menadione (Vitamin K<sub>3</sub>) effectively checked the *A.flavus* growth (100% inhibition) probably due to its auto-oxidation effect. Addition of Menadione to the rapeseed and soybean oils resulted in the accelerated process of auto-oxidation of these oils. Further, Menadione when added to vegetable oils, influences the dissolution of natural tocopherols as well as their dimerization (Kupczyk and Gogolewski, 2001). The microbial spoilage by yeasts such as *Zygosaccharomyces bailii* and *Saccharomyces cerevisiae* can usually be retarded by a weak organic acid like Benzoic acid which acts by cytostatic action which is a consequence of inhibition of macroautophagy coupled with nitrogen starvation (Reut Hazan *et.al.*, 2004).

Salts in general when dissolved in water, act as weak acids, In solution, sodium benzoate, propionates of sodium and calcium transform into benzoic acid and propionic acid respectively and finally check the mold growth. The chemicals benzoic acid and sodium benzoate are the most common food preservatives in fruit juices, syrups, jams, jellies, pickles and fruit cocktails etc. Yeasts are also inhibited by benzoates to a greater extent than are molds and bacteria. Ascorbic acid or Vitamin C and their salts are highly soluble in water and safe to use in foods. On the other hand, Acetic acid is a general preservative inhibiting many species of bacteria, yeasts and to a lesser extent molds (Sareen, 2003). The present study also supports the same with Acetic acid showing no efficacy in checking the linear growth of *A.flavus* strains, the aflatoxin producing molds. Microbial spoilage of food is reported to be upto a tune of 40% worldwide and inhibition of these microbes often requires levels of preservatives that are near or greater than legal limits (Reut Hazan *et.al.*, 2004). Although, many chemicals kill microbes, all are not permitted in food. However, chemical preservatives are those which are added in very low quantities (up to 0.2%) and which do not alter the organoleptic and physico chemical properties of the foods at or only very little. Among the chemical preservatives that are in use in the preservation of foods, Sulfites, Dehydroacetic acid, Sodium nitrite, Ethyl formate, propionic acid, sorbic acid and Benzoic acid are most common and these are deemed as GRAS (Generally Regarded as Safe) in the specified concentrations (Jay, J.M, 2002). Efforts to minimize the copra spoilage by *A.flavus* group and aflatoxin threat can further be reduced by treating the copra with chemical preservatives that are commercially available and also ensures as a next step in reducing the *A.flavus* and other mycoflora entry into the copra after *Trichoderma* spp antagonism in the soil against these common copra inhabitants. Compatibility studies between *A.flavus* and the commonly used preservatives in food industry gives an idea of the nature of chemical preservatives to be used. Preservatives that have negative impact on the survival, growth and multiplication of *A.flavus* in soil and on copra have to be used in order to combat the aflatoxin problem in copra. However, the preservatives that are to be recommended should not hinder the multiplication of the native *Trichoderma* spp which are antagonistic to *A.flavus* especially when applied to soil. Our earlier research on post harvest contamination of copra revealed the presence of *Aspergillus flavus*, *A.niger*, *Penicillium* spp, *Rhizopus* spp, *Drechslera* spp and *Botryodiplodia* spp as common inhabitants of copra during post harvest storage (Srinivasulu *et.al.*, 2003).

Compatibility studies between *Trichoderma* spp that are isolated *viz.*, *T.viride*, *T.harzianum* and *T.hamatum* from copra godowns and chemical preservatives that inhibit growth of *A.flavus* revealed that Sodium benzoate, Ascorbic acid and Potassium meta bisulphate are safe with regard to the growth and multiplication of *Trichoderma* spp and can be used in conjunction with biocontrol management of copra spoilage. On the other hand, Menadione, Propionic acid, Benzoic Acid and acetic acid reduced the growth of *Trichoderma* spp at 500-



ppm concentration (Plate-6). All these chemicals are relatively safe at 100 ppm with respect to *Trichoderma* spp growth inhibition (Table-7).

**Table-7: Effect of chemical preservatives on the linear growth of *Trichoderma* strains on PDA incubated at 28°C for 96 hrs.**

Chemical	<i>T.viride</i>		<i>T.harzianum</i>		<i>T.hamatum</i>		Average inhibition (%) of <i>Trichoderma</i> spp at 500 ppm
	% inhibition at		% inhibition at		% inhibition at		
	100ppm	500ppm	100ppm	500ppm	100ppm	500ppm	
Menadione	68.89(28 <sup>a</sup> )	100(0 <sup>a</sup> )	68.89(28 <sup>a</sup> )	100(0 <sup>a</sup> )	65.56(31 <sup>a</sup> )	100(0 <sup>a</sup> )	100
Potassium meta bisulphate	0.00(90 <sup>d</sup> )	0.00(90 <sup>e</sup> )	0(90 <sup>c</sup> )	0(90 <sup>e</sup> )	0(90 <sup>c</sup> )	0(90 <sup>e</sup> )	0
Benzoic acid	0.00(90 <sup>d</sup> )	11.11(80 <sup>c</sup> )	0(90 <sup>c</sup> )	30.0(63 <sup>c</sup> )	0(90 <sup>c</sup> )	27.77(65 <sup>c</sup> )	22.96
Sodium benzoate	0.00(90 <sup>d</sup> )	0(90 <sup>e</sup> )	0(90 <sup>c</sup> )	0(90 <sup>e</sup> )	0(90 <sup>c</sup> )	0(90 <sup>e</sup> )	0
L-Ascorbic acid	0.00(90 <sup>d</sup> )	0(90 <sup>e</sup> )	0(90 <sup>c</sup> )	0(90 <sup>e</sup> )	0(90 <sup>c</sup> )	0(90 <sup>e</sup> )	0
Propionic acid	18.89(73 <sup>b</sup> )	53.33(42 <sup>b</sup> )	5.55(85 <sup>b</sup> )	44.44(50 <sup>b</sup> )	11.11(80 <sup>b</sup> )	53.33(42 <sup>b</sup> )	50.36
Acetic acid glacial	0.67(89.4 <sup>c</sup> )	7.11(83.6 <sup>d</sup> )	0(90 <sup>c</sup> )	6.66(84 <sup>d</sup> )	0.28(89.74 <sup>c</sup> )	7.66(83.10 <sup>d</sup> )	7.14
Control	(90mm)						

\* Means followed by similar letters are not different statistically ( $P = 0.05$ ) by Duncan's multiple range test. \*\* Values in parentheses are the linear growths of fungi in millimeters.

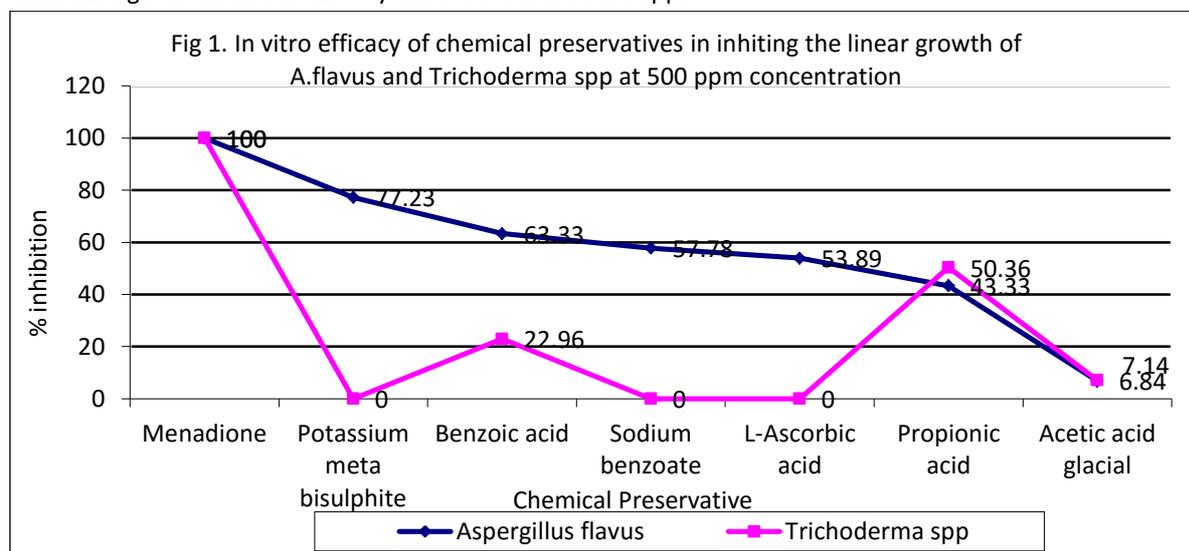


**Plate-6: In vitro inhibition of chemical preservation on the linear growth of *T.viride* at 500 ppm concentration**

Comparison was drawn with respect to chemicals that inhibited *A.flavus* growth and *Trichoderma* spp growth at 500 ppm concentration under *in vitro* conditions. The results indicated that Menadione though effective in controlling *A.flavus* population, is also adverse in terms of *Trichoderma* spp growth. The results with respect to Potassium meta bisulphate, Benzoic acid, Sodium benzoate and Ascorbic acid are encouraging in the sense that only *A.flavus* growth was reduced whereas *Trichoderma* spp growth is almost unaffected under in-vitro



conditions (Fig-1). However, the efficacy of Propionic acid in terms of reduction of both *A.flavus* strains and *Trichoderma* spp is almost on par. With regard to Acetic acid, a poor mold inhibitor is also doubly disadvantageous with its inhibitory effect on *Trichoderma* spp.



The compatibility studies of chemical preservatives on *Trichoderma* spp is only a study taken up keeping in view the precautions to be adopted while applying preservatives to the copra. Potassium meta bisulphate, Benzoic acid, Sodium benzoate and Ascorbic acid can be recommended to be applied on copra as well as in godowns where copra is stored, the soils of which may inhibit *Trichoderma* spp. Whereas, in order to have a dual check of mold growth by biocontrol agents as well, the chemical Menadione has to be applied only to the copra and not to the godowns as a general spray.

Preservation of food products containing chemical food preservatives is usually based on the combined synergistic activity of several additives, intrinsic product parameters like composition, acidity, water, processing temperature, storage atmosphere and temperature. Moreover, by the application of chemical preservatives like Ascorbic acid, Menadione, the copra can also be fortified with vitamins, which are essential to human and animal life. From the present studies, it can be inferred that chemical preservatives offer a feasible, and an ecofriendly approach in managing the post harvest spoilage of copra especially the aflatoxin problem.

Post harvest management of copra quality is a challenging task owing to the improper handling of copra by farmers/ traders, lack of awareness on mycotoxin contamination and also to the consequent health hazards due to metabolites produced by the harbored molds on copra. Improper handling of copra after harvest is of concern in the present day coconut scenario due to the threat posed by aflatoxin producing molds that infest copra, thus spoiling the healthy food chain and subsequently the coconut trade. Among the mycoflora that invade copra, *Aspergillus flavus* Link contamination is a potential hazard to the coconut industry and trade because of its secondary metabolite production, the aflatoxins. Aflatoxins are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* Speare that are carcinogenic, hepato-toxic and teratogenic in nature (Coulibaly, 1987). There are several reports of aflatoxin contamination of copra. Phillipine coconut industry which exports copra cake used as feed ingredient in Europe, identified Aflatoxin B<sub>1</sub> level to a tune of 7-70 ppb and therefore gave a regulation that any move by European country to restrict the level of Aflatoxin B<sub>1</sub> in feed stuffs would pose difficulty for the local coconut product given the climatic conditions in the country. Entry of aflatoxins into food chain is considered to be the most devastating problem not only through coconut, but also through cereal grains, spices, dry fruits, pulses, vegetables, cheese, bread



and oil seeds; which are the prominent aflatoxin producing potential sources of *A.flavus* and *A.parasiticus* (Mc Donald, 1976).

Detoxification of coconut products that were prone and contaminated by aflatoxins viz., coconut milk, coconut jam, coconut syrup, coconut skim milk, coconut flour, desiccated coconut (products from kernel) and coconut oil, coconut cake( products of copra) is a futile exercise as none of the detoxification procedures available can detoxify to the fullest extent. Detoxification methods of the present day include Ammoniation, Photolysis (Shantha and Sreenivasamurthy, 1977), extraction with sodium chloride (Shantha and Sreenivasamurthy, 1975), filtration (Basappa and Sreenivasamurthy, 1979), by hydrogen peroxide, by sunlight (Shantha *et.al.*, 1986), urea and formaldehyde (Codifer *et.al.*, 1976) are widely used against groundnut aflatoxin contamination (Read, 1987). However, detoxification of copra products after aflatoxin contamination should be the last resort and efforts to tame the aflatoxin problem in food and feedstuffs of copra should be directed towards managing the problem in the initial stages of contamination of the copra.

Microbial spoilage of food is reported to be upto a tune of 40% worldwide and inhibition of these microbes often requires levels of preservatives that are near or greater than legal limits (Reut Hazan *et.al.*,2004). Although, many chemicals kill microbes, all are not permitted in food. However, chemical preservatives are those which are added in very low quantities (upto 0.2%) and which do not alter the organoleptic and physico chemical properties of the foods at or only very little. Among the chemical preservatives that are in use in the preservation of foods, Sulfites, Dehydroacetic acid, Sodium nitrite, Ethyl formate, propionic acid, sorbic acid and Benzoic acid are most common and these are deemed as GRAS (Generally Regarded as Safe) in the specified concentrations (Jay, J.M, 2002). Efforts to minimize the copra spoilage by *A.flavus* group and aflatoxin threat can further be reduced by treating the copra with chemical preservatives that are commercially available and also ensures as a next step in reducing the *A.flavus* and other mycoflora entry into the copra after *Trichoderma* spp antagonism in the soil against these common copra inhabitants. Compatibility studies between *A.flavus* and the commonly used preservatives in food industry gives an idea of the nature of chemical preservatives to be used. Preservatives that have negative impact on the survival, growth and multiplication of *A.flavus* in soil and on copra have to be used in order to combat the aflatoxin problem in copra. However, the preservatives that are to be recommended should not hinder the multiplication of the native *Trichoderma* spp which are antagonistic to *A.flavus* especially when applied to soil.

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## EFFECT OF AMINO ACIDS ON ANTIFUNGAL ACTIVITY OF *PSEUDOMONAS* AND *BACILLUS* SPECIES AGAINST *FUSARIUM* AND *PYTHIUM* SPECIES

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### ABSTRACT

Four isolates of *Pseudomonas* and three isolates of *Bacillus* with high antifungal potency against *Fusarium* and *Pythium* species were screened by dual culture (Co-culture) method. These seven isolates were studied for morphological, cultural and biochemical characters as well as 16S r-RNA gene sequencing and identified as *Pseudomonas aeruginosa*13, *P. aeruginosa* 58, *P. putida*71, *P. fluorescens*106, *Bacillus thuringiensis*184, *B. cereus* 220 and *B. subtilis*252. These isolates were used to prepare biocontrol formulations against phytopathogenic fungi using dried fecal pellets of sheep and goats.

Effect of seven amino acids viz. glycine, tryptophan, glutamic acid, methionine, valine, proline and asparagine on *in-vitro* antifungal activity of *Pseudomonas* and *Bacillus* isolates against *Fusarium* and *Pythium* species was studied by co-culture method using potato dextrose broth. Tryptophan, methionine and asparagine found to enhance the antifungal activity of *P. aeruginosa*13 and *P. aeruginosa*58. The methionine also enhanced the antifungal activity of *P. putida*71 and *B. subtilis*252. Glycine enhanced the antifungal activity of *P. putida*71, *B. cereus* 220 and *B. subtilis*252 by more than 10%. Only valine enhanced the antifungal activity of *P. fluorescens*106. In general, the effect of amino acids on antifungal activity was similar for both *Pythium* and *Fusarium* species. Antifungal activity of *Bacillus thuringiensis*184 did not enhanced by any of the seven amino acids. Glutamic acid and proline did not show considerable increase in antifungal activity of any of the seven isolates. This study provided an idea about the mechanisms of antifungal activity of *Pseudomonas* and *Bacillus* isolates.

**Key words-** *Pseudomonas*, *Bacillus*, Antifungal activity, Amino acids, *Fusarium*, *Pythium*.

### Introduction

The amino acids play important role in growth of microorganisms as well as all other living beings. Twenty basic amino acids are used for synthesis of structural and functional proteins. Some of the amino acids are used as precursors for synthesis of antibiotics, hydrogen cyanide, plant growth promoting substances and other secondary metabolites which are involved in biocontrol and growth promotion of crop plants [1]. Glycine was found to be the direct precursor of microbial production of hydrogen cyanide which play important role in biological control of phytopathogenic fungi in soil [2,3]. Proline also stimulates cyanide production but to a lesser extent. The amino acids have been found in the root exudates of crops and enhance the colonization and growth of rhizobacteria [2].

Production of plant growth promoting substances is stimulated by availability of amino acids. Tryptophan is the precursor of indole acetic acid, a plant growth promoting hormone [4,5,6]. The enzymes chitinases (EC-3.2.1.14) and cellulases (EC- 3.2.1.4) are involved in lysis of cell wall of phytopathogenic fungi [7,8,9] whose synthesis needs amino acids. Induced systemic resistance (ISR) is one the important mechanisms of biocontrol in which the natural defense responses of plants are induced by increased levels of enzymes such as chitinase, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase [10]. Synthesis of these and many other metabolically important enzymes is stimulated by extra availability of amino acids.

### Materials and Methods

#### Isolation and identification of phytopathogenic fungi and antifungal *Pseudomonas* and *Bacillus* cultures-

Phytopathogenic *Fusarium* and *Pythium* species were isolated by tissue segment method using infected plant material and identified [11]. *Pseudomonas* and *Bacillus* species were isolated from rhizosphere of healthy crop



plants and screened for efficient antifungal activity by co-culture method and identified on the basis of morphological, cultural and biochemical characters as well as 16S-rRNA gene sequencing [12].

#### Study of the effect of amino acids on antifungal activity of selected *Pseudomonas* and *Bacillus* cultures-

Effect of six amino acids (glycine, tryptophan, glutamic acid, methionine, valine and asparagine) on *in-vitro* antifungal activity of *Pseudomonas* and *Bacillus* isolates against *Fusarium* and *Pythium* species was studied by using potato dextrose broth (PDB) amended with amino acid @ 100 µl/100ml. Eighteen PDB (100ml) flasks amended with amino acid (3 for each amino acid) and three control flasks containing 100ml PDB (without any amendment) were inoculated with 100 µl of each of the seven bacterial cultures and 100 µl of each fungal culture in PDB and incubated at 28°C for 7 days. Percent growth inhibition of *Fusarium* and *Pythium* species was calculated as-  $P. I. = [(C-T)/C \times 100]$ . Where C is wet weight of fungal growth in control and T is the wet weight of fungal growth in test (13,14,15).

#### Results and Discussion-

**Table-1. Effect of amino acids on antifungal activity of *Pseudomonas* and *Bacillus* species against *Pythium* species**

Antifungal isolates	Percent growth inhibition* of <i>Pythium</i> species							
	Control	Glycine	Trypt	G. A.	Meth.	Valine	Aspg	Proline
<i>P. aeruginosa</i> 13	55.00	57.00	67.00	56.50	67.50	58.00	66.00	56.00
<i>P. aeruginosa</i> 58	60.50	62.00	71.00	60.00	70.00	63.00	70.50	62.00
<i>P. putida</i> 71	55.00	67.00	56.00	58.00	65.00	55.50	58.00	55.00
<i>P. fluorescens</i> 106	52.50	52.00	52.00	55.00	55.00	61.00	55.00	54.50
<i>B. thuringiensis</i> 184	57.31	56.50	60.00	60.00	60.00	57.00	60.00	56.50
<i>B. cereus</i> 220	56.00	68.00	58.00	58.00	56.50	57.50	57.00	57.00
<i>B. subtilis</i> 252	58.50	69.00	61.00	58.00	70.00	60.00	60.00	66.00

\*The values mentioned are average of triplicates.

Trypt- Tryptophan; GA- Glutamic acid; Meth- Methionine; Aspg.- Asparagine.

**Table-2. Effect of amino acids on antifungal activity of *Pseudomonas* and *Bacillus* species against *Fusarium* species**

Antifungal isolates	Percent growth inhibition* of <i>Fusarium</i> species							
	Control	Glycine	Tryp	G. A.	Meth.	Valine	Aspg	Proline
<i>P. aeruginosa</i> 13	52.19	51.50	65.00	54.00	63.00	52.00	64.00	55.00
<i>P. aeruginosa</i> 58	57.50	62.00	71.00	61.50	70.00	58.00	70.00	60.00
<i>P. putida</i> 71	53.19	64.00	54.00	54.00	62.50	54.50	54.00	55.50
<i>P. fluorescens</i> 106	53.84	55.50	55.00	53.00	55.00	63.50	55.00	55.00
<i>B. thuringiensis</i> 184	50.00	53.00	52.50	50.50	52.00	52.50	50.00	52.50
<i>B. cereus</i> 220	55.50	65.00	55.00	56.00	55.007	64.50	57.50	56.00
<i>B. subtilis</i> 252	62.00	73.00	61.50	63.00	1.00	63.50	65.00	69.50

\*The values mentioned are average of triplicates.

Tryp- Tryptophan; GA- Glutamic acid; Meth- Methionine; Aspg.- Asparagine.



Fig.1. Screening of antifungal isolates of *Pseudomonas* and *Bacillus* species

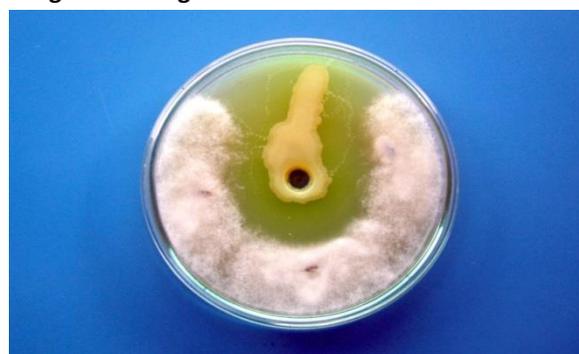


Fig.2. Inhibition of fungal growth by *Pseudomonas* species

#### Effect of amino acids on antifungal activity of *Pseudomonas* isolates-

Antifungal activity of *P. aeruginosa*13 and *P. aeruginosa*58 was found to be enhanced by provision of tryptophan, methionine and asparagine; *P. putida*71 by methionine and glycine and *P. fluorescens*106 by valine, with about more than 10%. Glutamic acid and proline did not show considerable effect on antifungal activity of *Pseudomonas* isolates. The valine found to be enhanced the antifungal activity of only *P. fluorescens*106 and none of the other isolates.

#### Effect of amino acids on antifungal activity of *Bacillus* isolates-

Glycine found to enhance the antifungal activity of *B. cereus*220 and *B. subtilis*252; methionine and proline both enhanced antifungal activity of *B. subtilis*252. Antifungal activity of *B. subtilis*252 was found to be enhanced by all the three amino acids i.e. glycine, methionine and proline while antifungal activity of *B. cereus*220 was found to be enhanced by only glycine. Proline enhanced the antifungal activity of *B. subtilis*252 by about 8%. It is surprising that, the antifungal activity of *B. thuringiensis*184 was not enhanced by any of the seven amino acids, in case of both the phytopathogenic fungi *Fusarium* and *Pythium*. In general, effect of antibiotics on the antifungal activity of the isolates did not show much variation in case of *Fusarium* and *Pythium* species.

Production of antibiotics, competition for nutrients such as iron by production of siderophores (iron chelating compounds), production of hydrogen cyanide (HCN), and breaking of fungal cell wall by degradation of chitin and cellulose are the important mechanisms of antifungal activity of *Pseudomonas* and *Bacillus* species. HCN can be produced directly from glycine and proline. Root exudates of crop plants found to contain essential amino acids [16].

The amino acids play important role in metabolic activities and growth of microorganisms. They act as precursors of many proteinaceous metabolic products [17]. The glutamic acid enhanced the biomass production and antifungal activity of all the isolates tested. Methionine, valine and tryptophan enhanced the



antifungal activity of *Pseudomonas* and *Bacillus* species [18,19]. The root exudates of plants generally contain amino acids, which favor the growth and activity of antagonists [2]. Patten and Glick (2002) observed that, tryptophan was not only involved in siderophore production but also in production of plant growth promoting substances such as indole acetic acid [20]. Bultreys and Gheysen (2000) observed the presence of amino acids especially asparagine favored siderophore production by *Pseudomonas syringae* and *P. viridiflava* LMG 2352 [21]. Voisard *et al.*, (1989) observed that, glycine and FeCl<sub>3</sub> stimulated HCN production [22].

The present study provided an idea about the probable mechanisms of antifungal activity of *Pseudomonas* and *Bacillus* isolates. Increase in antifungal activity by glycine and proline in case of *P. putida*71, *B. cereus*220 and *B. subtilis*252 indicated the HCN production. Increase in antifungal activity by tryptophan, methionine and asparagine in case of *P. aeruginosa*13 and *P. aeruginosa*58 indicated the production of siderophores, the iron chelating agents. Tryptophan production also indicates the production of indole acetic acid, the plant growth promoting hormones.

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## INSECTS AS VECTORS OF MICROBES

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**ABSTRACT**

Insects hamper the public health directly by miasis, dermatitis and allergies due to urticating hairs and by their painful venomous bites and indirectly by transmission of disease causing pathogens. Parasitic diseases vectored by insects have long plagued humankind and constitute a major global health problem. Vectors are the living organisms that can transmit infectious diseases between humans or from animals to humans. The transmission of diseases by insects is either mechanical or biological. Mosquitoes are the best known disease vectors. Others include certain species of ticks, flies, sandflies, fleas, bugs and freshwater snails. Integrated Vector Management (IVM) which is now being endorsed as the recommended strategy to exploit the preventive power of vector control is cost-effective, sustainable and ecologically sensitive.

**Keywords:** Vectors, Pathogens, IVM

**Introduction**

Vector-borne infections, diseases caused by pathogens transmitted by insects and ticks, have long impacted human affairs. Alexander the Great, conqueror of many nations, was vanquished by the bite of a tiny mosquito bearing malaria parasites. The Black Death, decimator of Europe, killer of tens of millions worldwide is the work of a tiny flea vectoring the bacilli that cause bubonic plague from rats to people. Every year more than one billion people are infected and more than one million people die from vector-borne diseases.

**Direct effects**

Insects may directly injure an animal or man in many ways. Some types of injury may be caused by insect feeding, however, other insect activities may also be damaging. These effects frequently have recognizable economic consequences.

**Annoyance** (and blood loss)

**Dermatosis (and dermatitis)** - Dermatitis is a disease of the skin, dermatitis an inflammation of the skin.

**Myiasis** – It is the invasion and feeding on living tissues of humans or animals by dipterous larvae. Fortunately, myiasis is a rare condition in humans, but it commonly occurs in livestock.

**Envenomization** – It is the introduction of a poison into the body of humans and animals.

**Allergic reactions (anaphylaxis)** - A hypersensitive response to insect proteins. **Entomophobia** - An irrational fear of insects.

**Indirect Effects**

The primary indirect effect of medical and veterinary insects is disease transmission. Vector-borne infections (VBI) were first described in 1877 when lymphatic filariasis was found to be transmitted by mosquitoes from human to human (Gubler, 1998). For the past 128 years several VBI have been described involving a wide variety of infectious pathogens, together with a wide range of arthropods. Vector-Borne Diseases are transmitted typically by the bite of infected arthropods like mosquitoes, ticks, mites, fleas, sandflies or black fly. These arthropods that carry and transmit diseases are known as *vectors* in public health.

Vector transmission is of two types; Mechanical Transmission and Biological Transmission

- 1. Mechanical Transmission:** There is no specific biological association between the pathogens and the arthropods. In the case of flies, transmission may be by regurgitation. Biting flies, such as horse flies and deer flies, may transmit pathogens mechanically in the course of biting with contaminated mouthparts.
- 2. Biological transmission:** When there is a strong biological association between the arthropod vector and the pathogen, the term biological transmission is applied.



Table 1: Important insect vectors of microbes causing diseases in humans

Vector	Pathogen	Disease	Distribution	Victim
<b>INSECTS</b>				
Anoplura (lice)	Bacteria			
<i>Pediculus humanus</i>	<i>Borrelia recurrentis</i>	epidemic relapsing fever	Africa	humans
<i>Aedes</i> spp. especially <i>A. aegypti</i>	YF virus	yellow fever	Africa, Central & South America	humans
<i>Culex</i> spp.	SLE virus	St. Louis encephalitis	North & South America	humans, horses
<i>Phlebotomus papatasi</i> (& other species)	sand fly fever virus	Sand fly fever	Africa, Asia, Europe	Humans
<i>Anopheles</i> spp.	<i>Plasmodium falciparum</i> , <i>P. malariae</i> , <i>P. ovale</i> , <i>P. vivax</i>	Malaria	world (tropics)	Humans
<i>Glossina</i> spp.	<i>Trypanosoma brucei gambiense</i>	Sleeping sickness	tropical Africa	Humans
<i>Phlebotomus</i> spp. (Old World); <i>Lutzomyia</i> spp. (New World)	<i>Leishmania</i> spp.	Leishmaniasis, Kala-azar, dumdum fever	world (New World: forested and arid tropics) (Old Worl savanna, steppe, subtropical)	humans, dogs
<i>Aedes</i> , <i>Anopheles</i> , & <i>Mansonia</i> spp.	<i>Brugia malayi</i>	Brugian filariasis	Southeast Asia	Humans
<i>Simulium</i> spp.	<i>Onchocerca volvulus</i>	Onchocerciasis	Africa, tropical South America	Humans
<i>Chrysops</i> spp.	<i>Loa loa</i>	Loiasis	Africa	Humans
Hemiptera (true bugs)	Protozoa			
<i>Triatominae</i> spp.	<i>Trypanosoma cruzi</i>	Chagas' disease	South America	Humans
Siphonaptera (fleas)	Bacteria			

(adapted from Higley, L. G., Karr, L. L., and Pedigo, L. P. 1989. *Manual of Entomology and Pest Management*. Macmillan, New York)

### Integrated Vector Control

#### Anti-larval measures

**Environmental control** -The most important step in reducing the number of mosquitoes (Success depends on Community involvement & multisectoral coordination)

- i. Elimination of breeding places (source reduction) like Filling and leveling, Drainage of breeding places.
- ii. Proper disposal of refuse other wastes.
- iii. Cleanliness in and around the houses .
- iv. Observing 'dry day'- intermittent irrigation.

**Chemical Control**

Larvicide	Formulation	Duration	Toxicity of ingredient
Petroleum oil-Diesel oil and larvicidal oil	Suspension	1-2 weeks	-
Paris green	Granules	2 weeks	High
<b>OP Compounds</b>			
Fenthion	EC	2-11 weeks	High
Fenitrothion	EC	1-3 weeks	Moderate
Malathion	EC, Suspension	1-2 weeks	Slight
Temephos	EC, Suspension	2-4 weeks	-
Insect growth regulators- Diflubenzuron, Methoriprene, Pyriproxyfen	Suspension/Granules	4-8 weeks	-
Bacterial Larvicides- <b>Bacillus thuringiensis H-14</b>	EC/Granules	1-2 weeks	-

**Insect Growth Regulators:** they are highly toxic to insect larvae or pupae, interfering with development into adults. Used in large scales in USA. Costly & have restricted availability -not used in India. Particularly useful if insects have developed resistance to insecticides or because of their environmental effect. Safe to human beings and environment.

- I. Juvenile hormone analogues: prevent development of larvae into pupa or pupae into adult. Ex. Methoprene
- II. Chitin synthesis inhibitors: interfere with moulting process, killing larvae when they moult.

Act more rapidly. Ex. Diflubenzuron and Triflumoron.

**Biological Control**

**Larvivorous fish:** Feed on mosquito larvae. Easy, practical, cheap; they have no food value. Two species are widely used- Gambusia - prefer clean water, tolerate wide range of temperature, pH water salinities. Guppy- can survive in polluted water, cannot withstand temperature <10 degree C. Useful in ornamental tanks, wells, Garden ponds, fountains, swimming pools, large water collection. Approximately 5 fish per sq.m of water surface.

**Bio-larvicides:** Bacteria are used for the control of mosquito larvae. *Bacillus thuringiensis H 14* and *Bacillus sphaericus*. Available as wettable powder and granules which contain bacteria, spores and toxic crystals. Safe to environment, human being and animals but are expensive.

**Others Bio-larvicides-** Fungi- Coelomyces, Culicinomyces, Nematodes - *Romanomermis cluicivorax* and *R. iyengari*.

**A. Anti-adult measures:**

1. Space sprays: Space sprays are those where the insecticidal formulations is sprayed into the atmosphere in the form of mist or fog.

Done where immediate results are needed like during outbreak of disease to bring down mosquito population drastically. Useful where exophilic vector species needs to be controlled.

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MICROBIAL PESTICIDES &amp; FERTILIZERS

S. No	Name of the insecticide	Formulation	Equipment used	Remarks
1	Pyrethrum extract	2.0%	Flit pump or hand operated fogging machine	Used for indoor spray
2	Technical Malathion	5 parts of technical Malathion in 95 parts of kerosene	Outdoor thermal fogging	Used for outdoor spray
3	Deltamethrin 1.25ULV	1 litre in 199 litre diesel	Outdoor thermal fogging	Used for outdoor spray

#### Indoor Residual sprays

Application of insecticides to surfaces so that the insecticide particles remain on the surface in the form, size and quantity suitable for insects to pick up on contact and sufficient to exert a lethal effect over a long period. Helpful to control **endophilic** species.

#### Indoor residual sprays

S.NO	Name of the Insecticide	Dosage	Residual effect in weeks
1	DDT 50% WP	1g	10-2
2	BHC 50% WP	200 mg	6-8
3	Malathion 25% WP	2g	6-8
4	Deltamethrin 2.5% WP	20 mg	10-12
5	Alpha Cypermethrin 5.0% WP	25 mg	10-12
6	Cyfluthrin 10% WP	25 mg	10-12
7	Lambdacyhalothrin 10%WP	25 mg	10-12
8	Fenitrothion 40% WP	1g	6-8
9	Primiphos- methyl 25% WP	2g	6-8

#### Genetic control

Today we are mainly depending on insecticides for control of mosquitoes. The problem with this are development of Resistance and environmental Pollution. Therefore it is desirable to have alternate strategy which overcomes above problem. This lead to the think of genetic control of mosquito.

1. Sterile Insect Technique
  - a. Conventional SIT
  - b. Translocation Heterozygotes
  - c. Genetic Sexing
  - d. Cytoplasmic Incompatibility

#### Personal Propylaxis

**Insecticides Treated Mosquito Nets:** They provide better and effective protection by not only preventing mosquito bites & also killing them. Thus it reduces Mosquito population. They are prepared by soaking the net in insecticidal solution & dried. Synthetic Pyrethroids are used for impregnation. Permethrin, Deltamethrin, Cyfluthrin and lambdacyhalothrin are used.

**Dosage of insecticides:** 25 mg per sq. m in case of deltamethrin (2.5%) and lambdacyhalothrin (10%), 50 mg per sq. m in case of cyfluthrin (10%) , They have effect up to 6 months when not washed. Under the programme Long lasting insecticide treated bed nets are provided free of cost in endemic areas.

1. **Repellents:** They are mainly used for application on exposed parts of skin. They act by preventing human-insect contact and do not knock down or kill. Chief advantage is short duration of protection.



So it is useful for plantation workers, Army people, labors who work outdoor at night and travelers. N,N-Diethyl-3-toluamide (DEET), indalone, dimethyl phthalate, dimethyl carbate. DEET is best available product; the repellent effect last for 4-6 hours. Some people can develop allergy on application.

2. **Screening:** Screening of buildings with copper or bronze having 16 meshes to the inch is recommended. They are costly.
3. **Others-** Mosquito hitting swatter, full cover of hands and legs, use of fan at high speed.

**Insecticide Vaporizers:** They are used in the form of coils, mats, liquid vaporizers and aerosols/spray. They protect against mosquito and biting flies by,

1. Irritating and disturbing them after contact and preventing them from biting and also preventing them entering room.
2. Paralyzing or killing them.

Considering huge loss of lives in mankind across the globe due to insect vectoring diseases, management of vectors play a key role in curtaling the outbreak of diseases. Integrated Vector Management(IVM) which is now being endorsed as the recommended strategy to exploit the preventive power of vector control is cost-effective, sustainable and ecologically sensitive.

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**IMPACT OF BIOFERTILIZERS ON AGRICULTURE CROPS****P. RAVI TEJA\*, M. GANGA DEVI, CH. ANIL KUMAR**

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Biofertilizers are the new cost effective renewable source of plant nutrients to supplement chemical fertilizers. Now a days the practices of using bio fertilizers as a partial substitute for chemical fertilizers, is gaining much momentum. Biofertilizers reduce the environmental pollution, cost of cultivation crops and also improves the soil physical properties soil tilth and health. They improve soil fertility and soil productivity. The Rhizobium bacteria penetrate into the root where it fixes atmospheric nitrogen. Through nodulation the microorganism with soluble carbohydrates. Azotobacter is other beneficiary in habitual for soil fixes of nitrogen. These bacteria synthesize slime that helps to aggregation of soil. Azospirillum species are found in root cortex of *Graminae* and leads symbiotic relationship with host plants. These gram negative bacteria fix nitrogen and proliferation both in aerobic and anaerobic conditions. VAM (Vesicular Arbuscular Mycorrhizae) mobilize the phosphorus and make it available to the plants mostly in P deficient soils. These features of bio fertilizers help in better utilization of added inorganic fertilizers and help in reducing its level of application and will reduce the deleterious effects of harmful chemicals residues. Bio fertilizers can be effectively used in cultivation of rice and other staple crops, commercial crops such as sugarcane vegetable and fruit crops cultivation. Bio fertilizers also give protection to crop against diseases and insect pest hence reduces the application of pesticide in the field. Use of bio fertilizer not only increase yield but also improves physical properties of soil by increasing the activity of soil micro organisms which ultimately increases the sustainability of soil and efficiency of applied bio fertilizers.

Key words: Biofertilizers, Azospirillum, Azotobacter, Rhizobium, Rice, Sugar cane

**MICROBIAL PESTICIDES AND FERTILIZERS****P. SRI LAKSHMI, D. MANUSHA**

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A fertilizer is any material of natural or synthetic origin that is applied to soils or to plant tissues (usually leaves) to supply one or more plant nutrients essential to the growth of plants. Fertilizers are commonly used for growing all crops, with application rate depending on the soil fertility, usually as measured by a soil test and according to the particular crop. Whereas a bio-fertilizer is substance which contains living microorganisms. Bio-fertilizers can be expected to reduce the use of chemical fertilizers and pesticides. The microorganisms in bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of bio-fertilizers healthy plants can be grown, while enhancing the sustainability and the health of the soil. They provide "eco – friendly" organic agro – input. Bio-fertilizers such as Rhizobium, Azotobacter, Azospirillum and blue – green algae (BGA) have been in use for a long time. A pesticide is a substance meant for attracting and destroying the pests. The pesticides are used in protecting the crops from diseases. A pesticide is used as a herbicide, insecticide, fungicide bactericide and many more. Bio-pesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. The use of bio-fertilizers & pesticides (microbial) increases the soil fertility and replenishes the nutrients in the soil which are lost by the use of chemical fertilizers and pesticides which are not only harmful for the soil but also to the plants which we grow in the soil and to the humans who consume those plants. So it is advisable to use *organic or microbial or bio- fertilizers and pesticides.*

**BIO-FERTILIZERS - A GATEWAY TO SUSTAINABLE AGRICULTURE****\*T. LAKSHMI TIRUPATHAMMA, C.VENKATA RAMANA<sup>2</sup>, L. NARAM NAIDU<sup>2</sup>**<sup>1</sup>Department of vegetable science, College of Horticulture, Dr. Y.S.R.H.U, Tadepalligudem, West Godavari, A.P., India.<sup>2</sup>Horticultural Research Station, Dr. Y.S.R.H.U, Lam Farm, Guntur-34, India.**\*Email: [tanneerulakshmi46@gmail.com](mailto:tanneerulakshmi46@gmail.com)**

Bio-fertilizers are one of the best modern tools for agriculture. It is a gift of our modern agricultural science and are used to improve the fertility and quality of the soil. Bio-fertilizers are defined as biologically active products or microbial inoculants of bacteria, algae and fungi (separately or in combination), which may help biological nitrogen fixation for the benefit of plants. Bio-fertilizer contains microorganisms which promote the adequate supply of nutrients to the host plants and ensure their proper development of growth and regulation in their physiology. It offers an economically attractive and ecologically sound route for augmenting nutrient supply. Leguminous crops have the ability to fix nitrogen (N) biologically from the atmosphere. This can benefit not only the legumes themselves but also any intercropped or subsequent crops.

Bio-fertilizers are applied in the agricultural field as a replacement to our conventional fertilizers. Conventional fertilizers contain compost; household wastes and green manure. Those are not as effective as chemical fertilizers. So, farmers often try to use chemical fertilizers in the field for crop development. But obviously the chemical fertilizers are not environment friendly. (Mishra *et al.*, 2013) They are responsible for water, air and soil pollution and can spread cancer causing agents. Moreover, they may destroy the fertility of the soil in a long run. Scientists have developed Bio-fertilizers to prevent pollution and to make this world healthy for everybody in a natural way.

Bio-fertilizers play a very significant role in improving soil fertility by fixing atmospheric nitrogen, both, in association with plant roots and without it, solubilize insoluble soil phosphates and produces plant growth substances in the soil. They are in fact being promoted to harvest the naturally available, biological system of nutrient mobilization (Venkateshwarlu, 2008). Bio-fertilizers application in agriculture will have greater impact on organic agriculture and also on the control of environmental pollution, soil health improvement and reduction in input use. Therefore, clean agriculture recently depends upon using bio-fertilizers as well as organic in order to produce high yields with the best commodity quality without contamination and less accumulation with heavy metals (Mallikarjuna Rao *et al.*, 2013).

As a boon for farmers, Bio-fertilizers being essential components of organic farming play vital role in maintaining long term soil fertility and sustainability. Bio-fertilizers would be the viable option for farmers to increase productivity per unit area in organic farming for an era of prosperity and clean environment. Further they are eco-friendly and pose no danger to the environment can be replaced with chemical fertilizers.

**BIO FERTILIZERS & PESTICIDES****K. VIJAYA LAKSHMI, G. JYOTHI,***M.Sc. Zoology, Government College for Women, Guntur*

'Bio-fertilizer' is a substance which contains living microorganism which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers are not fertilizers. Fertilizers directly increase soil fertility by adding nutrients. Bio-fertilizers add nutrients through the natural processes of fixing atmospheric nitrogen, solubilizing Phosphorus, and stimulating plant growth through the synthesis of growth promoting substances.

Bio-pesticides are the type of pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. For example, baking soda has pesticidal applications and is considered bio-pesticides. Bio-pesticides are usually less toxic than conventional pesticides. Bio-pesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects and mammals. Bio-pesticides often are effective in very small quantities and often decompose quickly, resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.

Key words: bio-fertilizer, fertilizer, bio-pesticide, pesticide, growth, micro organisms, conventional, soil fertility

**EFFECT OF TILLAGE AND NITROGEN FERTILIZATION ON SOIL ORGANIC CARBON STORAGE****P.LAKSHMI THANMAI \* P.GAYATREE DEVI<sup>2</sup> T. NAGA RAMYA KRISHNA<sup>3</sup>**

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Cropping has led to a decrease in soil organic carbon (SOC) content worldwide during the last two centuries, with a net flux of carbon to the atmosphere (Houghton et al. 1983). Nitrogen fertilization and conservation tillage systems (no-till, chisel till, disc till, sweep till, etc.) are management practices that counteract soil degradation. Fertilizers contribute to soil nutrient levels depleted by crop production and sustain productivity (Sheldrick et al.2002), while conservation tillage – defined as tillage systems that leave at least 30% of soil surface covered by residues – decreases erosion (Unger & McCalla 1980). Another effect of fertilizer and conservation tillage on soils is their impact on SOC storage. Soils can be used as sinks for carbon in the global carbon cycle, thereby mitigating the greenhouse effect (Lal et al. 1999; Follett 2001; Sauer-beck 2001). Nitrogen fertilizer can increase SOC levels (Paustian et al. 1997); nevertheless, the influence of this effect depends on management (Glendinning & Powlson 1991), and the impact of soil type and climate are also important. In agricultural ecosystems specifically, tillage has been found to promote soil C loss at a faster rate and store less total SOC than NT systems primarily because mechanical soil mixing increases soil respiration rates (Gregorich et al. 1998, Stavi et al. 2011, Duiker and Beegle 2006).



## A COMPARATIVE STUDY ON EFFECT OF BIOINOCULANTS, BIOFERTILIZERS AND CHEMICAL FERTILIZERS ON GROWTH OF PADDY AND THEIR ABILITIES TO SUPPRESS PHYTOPATHOGENS

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The present project work was conducted to test plant growth parameters of paddy plants grown in plant growth chamber, that were subjected to treatments of biofertilizers, chemical fertilizers commonly employed in East Godavari district for paddy cultivation and bioinoculant that was isolated and tested for Plant growth promoting traits in *in vitro* conditions. The abilities of paddy plants to resist phytopathogen effect of *F.udum* and *Rhizoctonia solani*, were also tested from the above treatments.

Soil plant microbe interactions are complex and there are many ways the outcome can influence plant health and productivity. These interactions may be detrimental, beneficial, or neutral to the plants. However, the focus of this work is to exploit the beneficial bacteria to enhance plant growth by bioinoculant bacteria (PGPR). *Fusarium udum* and *Rhizoctonia solani* are soil borne fungal phytopathogens that affect paddy and pigeon pea crops in the early stages of cropping practices. Soil borne bacteria are well known for their antagonism, predation, and competition towards other microbes in consortia. These bacterial traits of antagonism and competition could suppress fungal phytopathogens enabling a sustainable method of crop protection and forfeits the classical chemical crop protection methods. Bacterial species present in enormous percentage in agriculture soils were isolated and tested for Phosphate solubilization, IAA production, ACC deaminase etc. They were further tested for fungal antagonistic traits such as volatile HCN and Ammonia production, enzymes such as cellulase, proteases, chitinase, siderophore production. The prominent bacteria among these is used as bioinoculant in the present work influencing the plant growth and simultaneously with biocontrol ability for fungal phytopathogens.

## UNDERUTILIZED VEGETABLES FOR NUTRITIONAL SECURITY

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Nature has provided different sources of life forms on which human survived on planet Earth. Primitive man ate all types of fruits, leaves, roots and tubers of plants collected from wild; before he learnt to grow plants. Many wild edible plants are nutritionally rich and supplement nutritional requirements of human beings, especially the vitamins and micronutrients. Underutilized plant species have great potential for contribution to nutritional security, health, income generation and environmental services, but these have remained underexploited. Due to modernization of agricultural practices many of these have become neglected and some have been so neglected that genetic erosion of their gene pools has become so severe that they are often regarded as lost crops. There is a need to broaden the food basket with lesser known crops. Large number of vegetable crops like air potato, lesser yam, pointed gourd, spine gourd, teasel gourd, sword bean, winged bean, broad bean, basella, chekurmanis, drumstick leaf, colacasia leaf etc., are underutilized. Underutilized vegetables are found in numerous agricultural ecosystems and often survive mainly in marginal areas. It is time to review their status and to focus on lesser-known cultivated species. These underutilized



plant species hold a great genetic diversity with a vast heritage of indigenous knowledge. With the systematic promotion of underutilised vegetable crops, it is possible to enhance nutritional and food security.

Key words : Underutilized vegetables, genetic diversity, teasel gourd

### **BIOFERTILIZERS AND BIOPESTICIDES FOR BETTER ENVIRONMENT**

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The availability and affordability of fossil fuel based chemical fertilizer at the farm level have been ensured through imports and subsidies. The exploitation of beneficial microbes as a biofertilizers (Examples: nitrogen, compost, phosphorus biofertilizers) and biopesticides (Examples: neem and bacillus based pesticides) has become paramount importance in agriculture sector for their potential role in food safety and sustainable environment and crop production. The demand for biofertilizers and biopesticides is on the increase since last decade owing to its eco-friendly characteristics and a worldwide trend to reduce the reliability on chemical derived fertilizers. And these environmental friendly and protect the environment against pollutants.

Key words: biofertilizers; biopesticides; environment

### **IMPORTANCE OF MICROORGANISMS IN AGRICULTURE**

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Microorganisms are minute living things that are usually unable to be viewed with the naked eye. Bacteria, fungi, protozoa, algae, viruses are examples. In the last century, chemical fertilizers were used in agriculture. Farmers were happy of getting increased yield in agriculture in the beginning. But slowly chemical fertilizers started displaying their ill-effects such as: Polluting water, destroying micro-organisms and friendly insects and making the crop more able to the attack of diseases .Microbes are harmful but some microbes are useful for our day to life. Biological control agents like Bacteria, Fungi, Nematodes, Protozoans, Nosema, Grasshoppers, Caterpillars, Crickets, Viruses NPV(Nuclear Polyhedrosis Virus) Gypsy moths and Caterpillars to control a number of pests. Beauveria bassiana can be used as a biological insecticide to control a number of pests. Biofertilizers increase the fertility of soil. Heterocyst's are cells that are terminally specialized for nitrogen fixation. Microorganism improves physical, chemical and biological environments of the soil and destroy soil born pathogens and pests, develops resistance of plants to pests and disease and enhances the photosynthetic capacity of crops. Biofertilizers are cheap, safe and renewable resources rather than chemical fertilizers. Bio-control agents are expensive, safe than chemical pesticides. Microbes in Agriculture are recommended

Key words: Microorganisms, Biological control, biofertilizer, Agriculture



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**EFFECT OF HERBICIDES ON MICROBIAL POPULATION IN RABI BLACKGRAM****SHAIK RAZIA<sup>1\*</sup>, PRAMILA RANI B.<sup>2</sup>, TRIMURTHULU.N.<sup>3</sup>**<sup>1</sup> PG student,<sup>2,3</sup>Principal Scientists, Department of Agronomy

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Herbicides are biologically active compounds, and an unintended consequence of its application may lead to significant changes in microbial populations and activities influencing microbial ecological balance affecting soil fertility. The fate of herbicides applied in agricultural ecosystems is governed by the transfer and degradation processes, and their interaction with soil microorganisms. Keeping in view, an attempt was made to study the effect of pre and post emergence herbicide combinations like Pendimethalin, Alachlor, Imazethapyr, Quizalofop ethyl and Aciflurfen + Clodinothop propargyl on rhizosphere soil microflora of Blackgram field at RARS, LAM. A rise in population of Microflora (Bacteria and fungi) was recorded with application of Pre emergence herbicides (Pendimethalin and Alachlor) in the rhizosphere of Blackgram over control. The drop in microbial mass was observed with application of Post emergence herbicides at 20 DAS. There is gradual increase in microbial counts was noticed in later stages of crop growth at 30 and 65 DAS upto harvest. The highest yield was recorded in application of Pendimethalin (P.e) *fb* Imazethapyr (Po.e) at 20DAS with effective control over weed flora.

Keywords: Pre emergence herbicides, Post emergence herbicides, Soil microflora, Blackgram

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**PLANT BENEFICIAL MICROBES AND THEIR FUTURE PROSPECTS****D.UMA**

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Agriculture is the oldest economic sector in the world, and is more dependent on fertile soils and a stable climate than any other trade. It has a huge influence on the ecological balance, water and soil quality, and on the preservation of biological diversity. Agricultural techniques and economic framework conditions worldwide have undergone a radical transformation that agriculture has become a major source of environmental pollution. The investigation about ecologically compatible techniques in agriculture and environmental sciences can take essential advantage from the use of beneficial microorganisms as plant microbe interactions fulfil important ecosystem functions. Plants are involved in a complex network of interactions with microorganisms, some of those are beneficial and others are detrimental. There is a growing worldwide demand for sound and ecologically compatible environmentally friendly techniques in agriculture, providing the quality agricultural products. The application of beneficial microorganisms is an important alternative to some of the traditional agricultural techniques which, very often severely alter the agro ecosystem balance and cause serious damage to health. The use of beneficial microorganisms in the replacement or the reduction of chemicals has been so far attested. Beneficial microorganisms such as diazotrophs bacteria, biological control agents (BCAs), plant growth promoting rhizobacteria (PGPRs) and fungi (PGPFs), can play a key role in this major challenge, as they fulfil important ecosystem functions for plants and soil. Plant growth stimulation and crop protection may be improved by the direct application of a number of microorganisms known to act as bio-fertilizers and/or bio-protectors. In this paper author presents the status of development and application of beneficial microbes that provide an option for future prospects.

Key Words : Biological diversity, Microorganisms, Ecologically compatible, Agroecosystem, Bio-protectors.



## SLOW RELEASE "ORGANIC FERTILIZER" - VERMICULTURE K. MOUNIKA<sup>1\*</sup>, CH. SANTHI PRIYA<sup>1</sup> AND CH. HARI PRIYA<sup>2</sup>

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Chemical agriculture is proving destructive in every way – agronomically, socially, economically and environmentally. Organic farming by vermiculture will be supportive in every way. Vermicompost works as a 'slow-release fertilizer' whereas chemical fertilizers release their nutrients rather quickly in soil and soon get depleted. Significant amount of chemical nitrogen is lost from soil due to oxidation in sunlight. Somany experimental results calculated that upon application of 100 kg urea (N) in farm soil, 40-50 kg gets oxidised and escapes as 'ammonia' (NH<sub>3</sub>) and 'nitrous oxides' (N<sub>2</sub>O) into the air, about 20-25 kg leaches underground polluting the groundwater, while only 20-25 kg is available to plants. N<sub>2</sub>O is a powerful 'greenhouse gas' nearly 312 times as compared to CO<sub>2</sub>. With continued application of vermicompost the organic nitrogen tends to be released at constant rate from the accumulated 'humus' and the net overall efficiency of nitrogen over a period of years is considerably greater than 50% of that of chemical fertilizers. Availability of phosphorus is sometimes much greater than that from inorganic fertilizers.

## YIELD AND YIELD ATTRIBUTES OF MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK] AS INFLUENCED BY PHOSPHORUS AND BIOFERTILIZERS

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A field experiment was conducted during *kharif* season of 2015 on loamy sand soil at the Agronomy farm, S.K.N. College of Agriculture, Jobner (Rajasthan). The experiment consisted of four levels of phosphorus (Control, 20, 40 and 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and four levels of biofertilizers (Control, PSB, *Aspergillus awamori* and PSB + *Aspergillus awamori*) thereby, making sixteen treatment combinations and replicated thrice. The results indicated that application of phosphorus upto 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> recorded highest grain yield (1221 kg ha<sup>-1</sup>) and straw yield (2988 kg ha<sup>-1</sup>) which was at par with 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Results further indicated that seed inoculation with the PSB and *Aspergillus awamori* significantly increased grain yield (1260 kg ha<sup>-1</sup>) and straw yield (3140 kg ha<sup>-1</sup>) over the rest of the treatments.

**Key words** :Mungbean, Phosphorus, PSB, *Aspergillus awamori*

## ANTIFUNGAL ACTIVITY OF BACTERIAL SPECIES AGAINST FUNGAL PHYTOPATHOGENS AFFECTING PADDY AND PIGEON PEA CROPS

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The present research was conducted to test antifungal properties of soil bacteria against fungal phytopathogens infecting crop plants. Soil microbes and the plant interactions is complex and there are many ways the outcome can influence plant health and productivity. These interactions may be detrimental,



beneficial, or neutral to the plants. However, the focus of this work is to exploit the beneficial bacteria to enhance plant growth by biocontrol of fungal phytopathogens by different mechanisms.

*Fusarium udum* and *Rhizoctonia solani* are soil borne fungal phytopathogens that affect paddy and pigeon pea crops in the early stages of cropping practices. Soil borne bacteria are well known for their antagonism, predation, and competition towards other microbes in consortia. These bacterial traits of antagonism and competition could suppress fungal phytopathogens enabling a sustainable method of crop protection and forfeits the classical chemical crop protection methods. Bacterial isolates such as *Bacillus* species present in enormous percentage in agriculture soils were isolated and tested for PGPR and fungal antagonistic traits. Eight bacillus species were tested for volatile HCN and Ammonia production, enzymes such as cellulase, proteases, chitinase productions, siderophore production. These bacillus species were further tested for fungal phytopathogen suppression in both qualitative and semi- quantitative method using dual plate assay methods. *Bacillus thuringensis* and *Bacillus cereus* were found to suppress phytopathogens by inhibiting their growth both *in vitro* and *in vivo* methods. Hence these species of *Bacillus* enable the biocontrol methods of plant disease suppression.

**Key words:** Biocontrol, Antagonism, phytopathogens, chitinase, siderophores.

## MICROBIAL FERTILIZERS- A BIOREMEDY

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Biofertilizers, more commonly known as microbial inoculants, are select cultures of soil organisms that can improve soil fertility and crop productivity. They add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. The microorganisms in bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of bio-fertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts. The root site is known to be a unique microsite for the association of symbiotic and non-symbiotic organisms. Infecting crop roots with vesicular-arbuscular mycorrhizal (VAM) fungi can improve their uptake of phosphorus and other nutrients, resulting in an increase in yield. Mineral nutrients are known to circulate through a chain of plants, soil and animals, but it is soil microorganisms that change these nutrients into a form available to plants. In sustainable agriculture, it is important to make full use of all microorganisms in order to promote the circulation of plant nutrients and reduce the use of chemical fertilizers as much as possible. Phosphate Solubilizing (PSB)/Mobilizing Biofertilizer in which Phosphorus, both native in the soil and applied by inorganic fertilizers, becomes mostly unavailable to crops because of low levels of mobility and solubility, and its tendency to become fixed in the soil. PSB provides life forms that help to improve the phosphate uptake of plants. Biofertilizers have various benefits. Besides accessing nutrients, different biofertilizers also provide growth-promoting factors to plants, and some have been successfully facilitating composting and effective recycling of solid wastes. They control soil borne diseases and improve the soil health.



## MICROBIAL PESTICIDES –SAFETY TOOL FOR ENVIRONMENTAL SECURITY

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Microbial pesticides are products derived from microbial pathogens of insects, usually contain micro organism as active ingredients. This include bacteria ,fungus, virus, nematodes. They rely for their effect primarily on infection and multiplication in the host and therefore by definition they will contain viable infective propagules of the chosen pathogen. In microbial bacterial substance *Bacillus thuringiensis* products are the most widely sold; Bacteria cause damage in one of two ways. Some produce toxins that are poisonous to other living organisms. These toxins may cause illness, paralysis, or death. Other bacteria produce enzymes that dissolve living cells or cause undesirable changes in nonliving materials such as adhesives and plastics other pathogens which are under development for pest control include viruses, other bacteria, protozoa and fungi. chemical stomach poisons. By contrast, fungi infect by penetrating the cuticle and can be used as contact pesticides. Most attention has focused on the mitosporic genera *Metarrhizium*, *Beauveria*, *Verticillium*, *Puccinomyces* and *Hirsutella*, although probably more than 700 species in a very wide range of genera are pathogenic to arthropods. Other arthropods such as mites Other arthropods such as mites and ticks and also weeds and plant diseases are targets for microbial pesticides. Hanel and Watson (1983) achieved some control of termites in Australia by artificially infesting nests with a strain of the fungus *Metarhizium anisopliae*. in case of viral control mostly NPV viruses are used. The strain chosen was from a heterologous host. nematodes are not microorganisms, but the Entomopathogenic species in the *Steinernematidae* and *Heterorhabditidae* act as vectors for entomopathogenic bacteria in the genus *Xenorhabdus* and are usually included in reviews of microbial pesticides. Most biological pesticides act slowly compared to chemical poisons. BT kills within 24 h, most fungi and viruses take a week. There are no biological equivalents to conventional insecticides which kill within minutes. However, the requirement for rapid kill may not be an impossible perception to change among many clients. Microbial control, which makes use of naturally occurring microbes in control of insect pests, pathogens, and weeds, is less harmful to non-target organisms and the environment than the chemical pesticides. The rationale for the development and deployment of microbial insecticides for pest management is their environmental safety, specificity, and biodegradability.

**KEYWORDS:** microbialpesticides, bacillusthurengensis, stomachpoison, metarhizium, npv, entomopathogenic nematodes, environmental safety.

## EFFECT OF NITROGEN LEVELS, BIO-FERTILIZERS AND FYM ON SOIL HEALTH AND YIELD OF RICE-FALLOW SORGHUM

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A field experiment was conducted at Agricultural College Farm, Bapatla during 2012 to study the influence of nutrient management on soil properties and yield of rice-fallow sorghum. The test soil was non-saline, sandy clay loam in texture, neutral in reaction, low in available nitrogen, medium in phosphorus and high in potassium status. The available Cu, Mn and Fe were above their respective critical limits while Zn was deficient.

The experiment was laid out in RBD with thirteen treatments replicated thrice. The treatments comprised of T<sub>1</sub>-90 kg N ha<sup>-1</sup>; T<sub>2</sub>- 120 kg N ha<sup>-1</sup>; T<sub>3</sub>- 150 kg N ha<sup>-1</sup>; T<sub>4</sub> - 90 kg N ha<sup>-1</sup> + Bio-fertilizer consortium ; T<sub>5</sub>- 120 kg N



ha<sup>-1</sup> + Bio-fertilizer consortium ; T<sub>6</sub>-150 kg N ha<sup>-1</sup> + Bio-fertilizer consortium; T<sub>7</sub>- 90 kg N ha<sup>-1</sup> + FYM ; T<sub>8</sub>- 120 kg N ha<sup>-1</sup> + FYM ; T<sub>9</sub>- 150 kg N ha<sup>-1</sup> + FYM ; T<sub>10</sub>- 90 kg N ha<sup>-1</sup> + FYM + Bio-fertilizer consortium; T<sub>11</sub>- 120 kg N ha<sup>-1</sup> + FYM + Bio-fertilizer consortium; T<sub>12</sub>- 150 kg N ha<sup>-1</sup> + FYM + Bio-fertilizer consortium ; T<sub>13</sub>- No nitrogen. Well decomposed FYM @ 10 t ha<sup>-1</sup> was applied one week before sowing. Bio-fertilizer consortium containing *Azotobacter*, PSB and PGPR each @ 5 kg ha<sup>-1</sup> was applied one day before sowing.

The results revealed that the soil properties viz., bulk density, particle density, porosity, pH and EC were not markedly influenced by the imposed treatments, while significantly high organic carbon was recorded in FYM treated plots. There was a significant influence of the treatments on available nitrogen and phosphorus, but not on potassium. The available nitrogen content was markedly influenced by levels of nitrogen as well as components of integration at all the crop growth stages. Among micronutrients (Cu, Zn, Mn and Fe), the treatment influence was significant related to Fe only. Application of inorganics in combination with organics and bio-fertilizers proved to be more efficient in improving the bacterial and fungal population and enzyme activities (urease and dehydrogenase) significantly.

An increase of 23, 19 and 4 per cent in grain sorghum yield was recorded by the treatments that received maximum inorganic nitrogen (150 kg N ha<sup>-1</sup>) along with FYM and bio-fertilizers, with FYM and with bio-fertilizers, respectively over only inorganic treatment. The grain and stover yields of sorghum were markedly influenced by the levels of nitrogen and application of FYM and bio-fertilizers.

Even though application of nutrient inputs other than fertilizer is uncommon in rice-fallows, the present attempt of integrated use of nutrients in rice -fallows aiming at soil health management under cereal-cereal sequence has resulted in not only a significant increase in yield but also improved the soil health.

## SUCCESS STORY OF BT COTTON BETTER THAN MICROBIAL PESTICIDE (THURICIDE)

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Among the microbial pesticides, Thuricide (*Bacillus thuringiensis kurstaki*) is toxic to Lepidoptera larvae and was applied as liquids, wettable powders, and dusts to plant since 1960. It has limitations such as: Short residual on plants (degraded by ultraviolet light); Must be ingested by larvae to kill them; and Insects that feed only a little or not at all before tunneling into stalks, fruit, etc. usually are not controlled. Bt gene was inserted into *Pseudomonas syringae*, followed by heat-killing, resulting in a thicker wall that better protected the toxin from U-V degradation which led to 3 to 5 days residual stability instead of 1-2 days for earlier formulations. Then it was incorporated by means of molecular techniques into cotton for tobacco budworm and cotton bollworm resistance, so that seeds carry the instructions for plants to produce Bt toxins for insect resistance (host plant resistance to insects). Although crop yields and effectiveness of insect control vary among Bt hybrids and transgenic technologies, Bt plants generally are very effective for controlling target insects. In India, Bt cotton was introduced a decade back and occupied nearly 100% cotton growing area as farmers are satisfied with their yield performance coupled with resistance to insect pests.



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**A REVIEW OF MICROBIAL ENHANCED OIL RECOVERY****G. AMULYA \* N. BHAVYA<sup>2</sup> P. LAKSHMI THANMAI<sup>3</sup>***\* M.Sc. (Ag.), (Pathology) (2) M.Sc.(Ag.), (Ag. Statistics) (3)M.Sc.(Ag.), (Agronomy, Agricultural college, Bapatla.**ANGRAU**Author e- mail: Amulya3gummalla@gmail.com*

It is anticipated that over 2 trillion barrels of conventional oil will remain in reservoirs worldwide after conventional recovery methods have been exhausted. Other oil recovery methods depend on many economic and technological limitations. Microbial Enhanced Oil Recovery (MEOR), is a biological based technology consisting in manipulating function or structure, or both, of microbial environments existing in oil reservoirs. The ultimate aim of MEOR is to improve the recovery of oil entrapped in porous media through inoculation of microorganism in a reservoir, while increasing economic profits ( Petrisor *et.al.*,2007). Microbial technologies are becoming accepted worldwide as cost-effective and environmentally friendly approaches to improve oil production (Sarkar *et al.*, 1989). It has several advantages compared to conventional EOR processes where it does not consume large amounts of energy as do thermal processes, nor does it depend on the oil price as do many chemical processes. The negative perception on the use of bacteria and handling them in the field for MEOR processes although it was verified by tests conducted by public health laboratories which reported that the mixed cultures of bacteria are safe to handle and pose no threat to the environment, plants, animals or human beings. Besides, the reservoir's environment is not favorable for the pathogenic organisms to grow. It is difficult to extrapolate the results from one microbial field trial to other reservoirs as each reservoir has its unique properties and microbial population for indigenous MEOR cases.

**Key words:** Bacteria, Conventional EOR, Microbes, MEOR(Microbial enhanced oil recovery), Oil production.

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**MICROBIAL ENHANCED OIL RECOVERY****K. DEEPIKA & D. YAMINI***M.Sc. Zoology, Government College for Women , Guntur*

The MEOR (microbial enhanced oil recovery) mechanism of extracting oil from less productive wells has solved an age-old problem that perplexed the oil industry the world over. It extracts over three times the oil than any other conventional process.

After these microbes are injected into an oil well, they take close to a fortnight to do their job. Oil, being viscous, is trapped in these pores. These microbes produce carbon dioxide and methane, gases that enter the pores and squeeze out every ounce of oil. They also produce bio-surfactants (detergent-like compounds) that reduce the tension between oil and the rock surface and help release the oil. The reaction of these microbes in oil also releases alcohol and volatile fatty acids. The alcohol reduces the viscosity of oil, making it light enough to flow out. The fatty acids solubilize the rock surface and thus push oil off them. The MEOR process of oil recovery actually offers more than the advantages of conventional methods of oil recovery plus the added strengths of the microbes.

*Key words:* extract – microbes – oil – pores – alcohol – volatile – viscosity - conventional – oil recovery



## A STUDY ON WASTEWATER TREATMENT AND IT'S SAFE DISPOSAL

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### ABSTRACT

Water, which is one of the most vital resources for all kinds of life and is also the resource which is most adversely affected both qualitatively and quantitatively by all kinds of human activities. The micro and macro communities in nature are orderly and play an important role in keeping the water healthy and acceptable for various uses. Of various types of human activities, sewage disposal continues to be most ominous one, especially in developing countries.

The word 'sewage' is a older term and is in replaced by "waste water". Sewage may be defined as a cloudy or dark fluid with very foul smell, arising out of domestic wastes containing mineral and organic matter either in solution or heavy particles of solid matter floating or in suspension form. The direct disposal of sewage into fresh waters possess a variety of hazards. So the sewage is handled by well developed methods. Treated at various levels and ultimately disposed to fresh water or recycled. The reuse or reclamation of municipal or domestic sewage has become an attractive option to avoid the effects caused by sewage on freshwater ecosystems.

The concept of oxidation ponds in tropical countries has been found to be of utmost utility. By way of solar energy and its use the sewage can be very well degraded and even has been found to be reuse in floriculture, agriculture, aquaculture and other uses. Waste water ponds or oxidation ponds are recognized as effective; and economical units for treatment of domestic sewage as well as biodegradable industrial wastes. The algae and bacteria play an inter-dependent role in these ponds. The algae use the nutrients and carbon dioxide released by bacterial decomposition. The bacteria make use of the oxygen liberated by the algae during photosynthesis. This mechanism of sewage treatment is one of the cheapest methods, where natural sunlight, tropical conditions and biological oxidation are used. Due to these special conditions, the chances of recycling and recovery of nutrients from sewage or waste water are very high.

### DISCUSSION

Water, is a natural resource and is very essential; for all organisms including man. It is known that there is only 5-6 percentage of fresh water on the earth and this little amount of water is also experiencing severe shortage due to the exploiting usage. The water conservationist warned the people that water resource is most adversely affected both qualitatively and quantitatively by all kinds of human activities and a urgent solution is required for efficient water resource management through enhanced reuse of waste water. With rapid increase of population and use of water supply, the quantity of waste water is increasing in the same proportion. The direct and untreated wastewater or sewage disposal in to the environments create a hazardous effect to the mankind (Ref 1-5). So the developed countries gave attention by treating the sewage or waste water since the beginning of the 19<sup>th</sup> century (Ref: 5-10).

Sewage may be defined as a "cloudily or dark fluid with very foul smell arising, out of domestic wastes coming from kitchen and laundry. The sewage contains mainly mineral and organic matter either in solution or heavy particles of solid matter floating or in suspension or in colloidal and Pseudo-colloidal forms. Sewage may vary considerably in composition; and strength from place to place owing to marked differences in the dietary habits and consumption of the people living there (Ref: 11-15) (Modak 1938, Mara 1976 Billore sk et.al 2002, Tehobalglous et.al 2003).

It is estimated by the National Sewage of Pollution Board that in India with 70% of urban population is producing about 90 liters of waste water per day per capita for by the Class-I cities and also class-II towns. This



sewage has to be treated to reduce the pollution loads present in it before discharged into the surrounding environment. In the developed countries only the sewage is handled by various well developed organizations and on the other hand the treatment & safe disposal of sewerage or waste waters still remain a major problem, despite the fact that low cost technologies for safe disposal of domestic and industrial sewage is available. Recently a little attention is being given by the developing countries in sewage treatment processes to save the environment from the hazardous effect of sewage or wastewaters.

**Sewage treatment:** Treatment of sewage waters generally involves three stages called primary, secondary and tertiary treatment.

In the primary treatment heavy solids will be removed and the remaining liquid will be subjected to the secondary treatment. The dissolved and suspended biological matters will be removed in water borne microorganism and algae play an important role in the secondary treatment. In the tertiary treatment the sewage effluents are treated to reduce the infection of water before discharging into the environments.

Recently a new concept of "Oxidation or stabilization ponds" came into existence in sewage waste water treatment systems. The oxidation ponds are the effective and economical units for treatment of domestic sewage as well as biodegradable industrial wastes. The driving force in a waste oxidation pond is solar energy utilized by photosynthesis. The action of sunlight on algae in the pond enables them to grow and consume the nutrients present in the sewage. The algae and bacteria play an interdependent role in the oxidation ponds while the algae use the nutrients and carbon dioxide released by bacterial decomposition. The Bacteria make use of the oxygen liberated by the algae during photosynthesis. This mechanism of sewage treatment and purification is one of the cheapest methods where natural sunlight, tropical conditions and biological processes taking place in special ponds, the chances of recycling and recovery of nutrients from sewage are very high. Thus the treated sewage elements from oxidation ponds are used for various purposes like irrigation, agriculture, aquaculture and other uses worldwide. In India also the farmers are using treated sewage waters for the production of cereals, vegetables, flowers and for agroforestry. The use of treated sewage for fish growth, without giving any supplementary feed was started from 1950's and a high yield of production was recorded. The East Kolkata sewage fisheries are the largest single waste water use systems in aqua culture in the world.

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## MICROBIOLOGY OF WASTE MANAGEMENT

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Until hundred years ago the waste products ago from activities were returned in the environment and underwent the biospheres natural elimination processes without there being any long term charge on the environment. During the last century, the increase in the amounts of refuse has been accompanied by a decrease in its quality, mainly due to the production and dispersal of heavy metals and xenobiotics compounds. Both useful and noxious microbial process has been underestimated in applied research in field of waste management which , until now has, dealt mainly with artificial technologies .This paper present some examples of microbiological process occurring in waste treatment, particularly dumping ,waste incineration, composition and biomethanization .

Key words: waste management; heavy metals; xenobiotics.

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## WASTE MANAGEMENT USING MICROORGANISMS

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Wastewaters are the waters coming from human quarters, particularly from kitchen, laundry, etc. These wastewaters are to be treated before their disposal into the environment; otherwise these wastewaters cause hazardous effects on the mankind and also on the environment. Treatment of these waters or their reuse is a new thought since 19<sup>th</sup> century. Only developed countries are adopting new methods to reuse or reclaim the wastewaters. But in the developing countries the treatment of wastewaters is very less. The treatment is mainly mechanical and biological. In mechanical treatment the wastewaters or sewage waters are flown into the ponds where the heavy solids and other non – biodegradable materials settle down. The treatment of wastewaters biologically is concerned with the microorganisms and algae present in these waters. Algae and microorganisms play an interdependent role and oxidise the waste and biodegradable materials and reduce the toxic levels of the sewage or wastewaters. During this process some energy is released by the photosynthetic activity of algae which is used by the microbes to oxidise the biodegradable materials. This is the most important and cheapest method of waste management using microbiology.

Keywords : wastewaters, treatment, sewage, biodegradable materials, energy release

**APPLICATION OF COMPOSTING TECHNOLOGY IN AGRICULTURE****M. VENKATA LAKSHMI\*, N. GOUTAMI AND H. ARUNA KUMARI***\*Department of Agronomy, Agricultural college, Bapatla-522101**Corresponding author\* [mamidi.venkatalakshmi@gmail.com](mailto:mamidi.venkatalakshmi@gmail.com)*

Composting, as defined as the actively managed process of decomposition of organic residuals in the municipal solid waste stream. A range of composting systems is designed to manage this decomposition process to yield a high quality compost product without creating a public nuisance or negative environmental impact.

Composting is a biological process that is optimized when the starting carbon to nitrogen ratio is in the range of 30:1 and the moisture and oxygen levels and temperatures are closely managed and monitored. When processing household organics, it is of critical importance to have the right starting mix of feed stocks, and to manage moisture, oxygen and temperatures closely in order to minimize the risk of nuisance factors and environmental impacts.

Composting has been regarded as an efficient and effective way to deal with the organic waste and helps work toward achieving the provincial 50 per cent waste reduction goal. It also creates rich organic soil that can enhance lawns and gardens. Therefore, MSW composting has been listed as one of the six new environmental standards applied to new waste management systems in NL. However, NL comprises more than 200 small communities without access to the central composting facility. For those areas, small-scale composting technologies are desired to manage their MSW so as to reduce collection and transport costs and eliminate the other environmental contamination during transportation.

The large amount of municipal, industrial, and agricultural wastes has led increasing environmental, social and economic problems. Stringent environmental regulations for waste disposal and landfills make finding new sites for waste disposal and management a growing challenge. Additionally, landfills use arable lands and soils which can be used for agriculture. The two primary environmental concerns related to landfills are leachate generation and gas emission. The leachate produced from landfills may contain a variety of toxic and polluting components. If managed improperly, leachate can contaminate groundwater and surface water. Landfill gas emissions are a mixture of carbon dioxide and methane, small amounts of nitrogen and oxygen, and trace amounts of various other gases such as benzene, toluene, and vinyl chloride. Some components of landfill gas may be toxic or explosive, other components can include ammonia, hydrogen sulphide and other organosulphur compounds, which produce the characteristic unpleasant odour. The generation of these landfills by-products depends on the constitution of the disposed material.

Key words : Composting, MSW and agricultural wastes

**COMPOSTING FROM A SUSTAINABLE POINT OF VIEW: RESPIROMETRIC INDICES AS KEY PARAMETER****GAYATREE MISHRA<sup>1</sup>, P.GAYATHREE DEVI<sup>2</sup> AND P.LAKSHMI THANMAI<sup>3</sup>**<sup>1,2,3</sup> P. G Students, Department of Agronomy, Agricultural College, Bapatla. ANGRAU

Composting at an industrial scale can be performed using low technology processes, such as windrows, or by implementing more complex technologies such as tunnels or, in general, in-vessel systems. In both cases process control can be done via measurement of oxygen content in the exhaust gases (or as interstitial oxygen in the material) and/or by monitoring the temperature evolution of the material. However, the use of respiration indices (RIs) as a control parameter to obtain reliable information on the actual microbial activity is being increasingly studied. Also RIs are used to determine the biological stability of the final product or the



biodegradability of the wastes intended to be composted. In this case, the RI value can be related to the amount of biodegradable organic matter content. As a new application, RIs can also be used to determine the environmental impact of composting plants. Indeed, emission factors of pollutant gases (ammonia, Volatile Organic Compounds, etc.) or consumption of resources (water, energy, electricity) can be referred to the resulting reduction of RI obtained during the entire composting process. In this case, RI might be a promising parameter for the comparison of composting technologies from the point of view of its sustainability or to define the critical phases of the process in terms of environmental impact (for instance, treatment of exhaust composting gases by biofiltration). In fact, studies on composting sustainability should consider not only the composting process but the equipment used for the treatment of its emissions, which has an important effect on the global environmental impact of waste treatment plants.

Keywords: Composting, respirometric indices, microbial activity

## SUSTAINABLE RESOURCE MANAGEMENT OF AGRICULTURAL WASTE BY COMPOSTING METHOD

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Composting is a sustainable waste management practice that converts any volume of accumulated organic waste into a usable product. When organic wastes are broken down by microorganisms in a heat-generating environment, waste volume is reduced, many harmful organisms are destroyed, and a useful, potentially marketable, product is produced. Organic wastes may include manure from livestock operations, animal bedding, yard wastes, such as leaves and grass clippings, and even kitchen scraps. Composting is a biological process whereby regular introduction of air by mechanical turning stimulates aerobic microorganism to reduce organic materials such as manure to a more stable materials similar to humus. A properly managed compost operation promotes clean and readily marketable finished products, minimizes nuisance potential and is simple to operate. Composting of agricultural waste was conducted in lab scale with four treatments and three replications each and with 250 grams cowdung, 500 grams substrate. Nitrogen % in the substrate was significantly more in T4 (1.84 %). By the end of the process of composting N % was highest in T4 (2.80 %). P % was highest in T5 (1.90 %). K % was highest in T3 and T5 (1.58 %). Organic carbon % in the substrate was significantly more in T6 (40.90 %). By the end of the composting the lowest Organic carbon % was recorded in T2 (23.2 %). This makes it possible to carry out a more reliable and more detailed analysis. Therefore, the concern towards the management of solid waste as an integral part for sustainable development has increased.

Key words: Sustainable, Management, Organic waste, Humus, Agricultural waste, Analysis.



## COMPOST TECHNOLOGY OF MICROBIOLOGY

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**Abstract:** Decomposition is an essential and continuous process where organic, or carbon based materials exposed to the elements of nature, particularly air and water are broken down into smaller compounds by microorganisms. Decomposition produces a nutrient rich organic matter that can be readily digested by soil microbes that make the nutrients available for uptake by plants. Composting is the actively managed process of decomposition of organic residuals in the municipal solid waste stream. The end product, compost, is organic material that can be used as a soil amendment to grow plants. Mature compost contains a stable, carbon-rich material called humus that is dark brown in colour with soil-like, earthy smell. Organic waste undergoes several pre-treatment steps before composting. Materials are screened for contaminants, often chopped or shredded to particle sizes for faster decomposition, and may be blended with other organic streams or bulking agents for optimal density.

There are so many methods of composting useful for mankind. Some of them are:

1.Backyard or on-site composting; 2.Vermicomposting; 3.Windrow composting; 4.Aerated Static Pile (ASP) composting; 5.In-vessel composting

These are different methods, consisting of different factors for the composting purpose but all of them are used for replenishing the soil. Composting is a useful process to eliminate waste materials and decreasing the pollution.

Key words: decomposition, composting, nutrient, organic, waste

## A REVIEW ON COMPOSTING TECHNOLOGY TOWARDS SUSTAINABILITY

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Vermicompost promises to user in the 'Second Green Revolution' by completely replacing the destructive agro-chemicals which did more harm than good to both the farmers and their farmland. Earthworms restore and improve soil fertility and significantly boost crop productivity.'Food Safety and Security' is a major issue everywhere in the world and this urgently needs a change in strategy of farm production. The new concept of farm production against the destructive 'Chemical Agriculture' has been termed as 'Sustainable Agriculture'. This is about growing 'nutritive and protective foods' with the aid of biological based 'organic fertilizers' without recourse to agro-chemicals. Feed material having C-N ratio less than 40:1 can be used successfully for vermicomposting.A temperature of 18-25°C and moisture content Of 40-60% are more congenial. Earthworm castings (worm manure) are rich in microbial activity and plant growth regulators, and fortified with pest repellence attributes as well. In short, earthworms through a type of biological alchemy are capable of transforming garbage into "gold" . Around 600 to 700 million tonnes of wastes and 1800 million tonnes of animal dung are available in the country per annum.Major crops like rice,wheat,sorghum,pearl millet and maize alone yield approximately 236 million tonnes straw per year.Earthworms and its vermicompost can work as the main 'driving force' in sustainable food production for food security while maintaining soil health and fertility. They can 'completely eliminate' the use of chemical fertilizers and 'significantly reduce' the use of chemical pesticides in crop production & also the huge water requirements for crop irrigation which became essential in chemical agriculture.

Key words: Composting, Vermicomposting, organic fertilizer



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**MICROBIAL MODELS FOR RESILIENCE TO CLIMATE CHANGE INDUCED ABIOTIC STRESSES  
AND SUSTAINABLE AGRICULTURE**

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Growing population and changing climate is a major challenge to achieve sustainable food security around the world. Climate change is imposing lesser food production and economy losses to the poor. As global warming continues climate-related crop failures are likely to become more severe. Increased incidences of abiotic stresses are impacting productivity in principal crops. Extreme conditions such as prolonged drought and heat waves are likely to increase further in future due to climate change. Mitigation strategies are required to cope with such impacts. Microorganisms are playing an important role and can be exploited for their unique properties of tolerance to extremities and their interaction with crop plants. Methods are being developed for mitigating abiotic stresses and climate change for agriculture production. Microorganisms can influence plants response to abiotic stresses like drought, chilling injury, salinity, metal toxicity and high temperature, by several mechanisms such as induction of osmo-protectants and heat shock proteins inside the plant cells. They can also influence the physico-chemical properties of rhizospheric soil through production of exopolysaccharides and formation of biofilms. Many microbial species and genera have been reported to provide tolerance to host plants under different abiotic stress environments. The use of microorganisms can alleviate the most common stresses caused due to climate change in crop plants for sustainable agriculture.

**Key words:**Crops - climate change - abiotic stresses - rhizosphere –plant microbe interactions—microbial models.

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## AN APPRAISAL OF BIOTECHNOLOGICAL APPLICATIONS OF DIVERGENT MICROBES OF MANGROVES

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### ABSTRACT

**Biotechnological** potential of divergent microbes always attracts researchers to explore, especially **microbes** in habituating unique ecosystems draws wide range scientific attention. One such unique ecosystem which harbors a wide array of microbes – Fungi, Bacteria and Actinomycetes is **mangroves** which thrive along extreme and unique transitional zone of marine and terrestrial ecosystems of the tropical and sub tropical parts of the globe. Owing to mangroves unique and extreme adaptations microbes in habituating the same evolved in such a way that it comprises a broad basket of bioactive compounds having immense biotechnological applications. The appraisal tried to evaluate different biotechnological applications of divergent microbes of mangroves ranging from cytotoxic, antibacterial, antifungal, industrial enzymes and enzyme inhibition activities having applications in varied biotechnological industries which embody – Pharmaceutical, Food, Fermentation, Textiles to Detergent industries. Finally the appraisal opined that in order to explore the biotechnological applicability of mangroves microbes we have to conserve the mangroves by integrated way spearheading by legal frame work which may leads to sustainable development of both microbes and mangroves.

Key words: Mangroves, Microbes, Biotechnology, Sustainable development

### INTRODUCTION

Mangroves are productive and extreme woody plants sprawling along the extreme transitional zone of marine and terrestrial ecosystems. Evolution of adaptive characters of mangroves is such that no other plants would evolve to that extent of surviving and sustaining along the extreme transitional zone of sea and land, Mangroves evolutionary extremeness has been envisaged in it's coping ability with respect to salinity variations vis-à-vis to regular tidal fluctuations and adaptations to poor oxygenic conditions (Miththapala.S.2008)<sup>1</sup>. Mangroves sprawls along different landforms of coast at the juncture of sea – land transitional zone ranging from estuaries, lagoons, salt marshes, backwaters, mudflats to coral reefs (MoEF – 2014-15)<sup>2</sup>. Indian mangroves extends to 4,662.56 km<sup>2</sup> amounting 0.14% of India's geographical entity (MoEF – 2011-12)<sup>3</sup> and bagging 4<sup>th</sup> largest mangrove size in world (Naskar and Mandal, 1999 as cited in Singh A.K. et al.2012)<sup>4</sup>. Across the globe mangroves extends for about 15.2 million hectares flourishing in 124 countries (Mathew.G. et al., 2010)<sup>5</sup>, Where in mangroves significantly developed along the tropical coastlines (Anonymous, n.d.)<sup>6</sup>.

In the midst of divergent pathogenic challenges to the mankind search for a promising source is imperative, In this regards divergent microbial community of mangroves may become a credible hub of bioactive compounds possessing immense biotechnological applications (Chand Basha. SK and Sambasiva Rao.KRS, 2017)<sup>7</sup>. Keeping this as background the appraisal discusses about biotechnological applicability of divergent microbes of mangroves – Fungi, Bacteria and Actinomycetes. We shall discuss individual microbial community's bio activity which consolidates our knowledge on biotechnological potentiality of divergent microbial community of mangroves.

Fig.1.Representational image of Mangroves<sup>18</sup>

## Objectives of the Appraisal

- To review the Biotechnological potentiality of Mangroves Fungi
- Appraisal of Biotechnological potentiality of Mangroves Bacteria
- Evaluation of Biotechnological potentiality of Mangroves Actinomycetes
- Broadly Outlook the general characters of Mangroves ecosystem

## Discussion: Biotechnological applications of Mangroves Microbes:

**Fungi:** Different works suggests that fungal community of mangroves could act as a potential source of bioactive substances having wide range biotechnological applications. Shearer et al.2007 (as cited in Thatoi.H et al, 2012)<sup>8</sup> labeled mangroves ecosystem as crux of biodiversity especially for fungi of marine nature. For the first time Vazquez et al. 2000 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated marine *Aspergillus niger* from rhizosphere portion of *Avicennia germinans* (black mangroves) and suggested that it exhibited phosphate – solubilization ability. Polizeli et al, 2005 (as cited in Thatoi.H et al, 2012)<sup>8</sup> extracted xylanolytic enzymes from varied fungal strains – *Aspergillus niger*, *A.awamori*, *A.aculeatus*, *Penicillium brasilianum* and other fungal strains. Xylanolytic enzymes reported to have it's biotechnological applications in industries viz...Paper manufacturing, Juice, wine, bread making, Animal feed and production of xylitol.Xiao et al, 2005; Chen at al, 1893 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated extracts of ethyl acetate and broth from fungal sources and suggests that it exhibited Biopesticide activity. Isaka et al, 2002 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated different aigialomycins (A-E) and hypothermycin from *Aigialus parvus* BCC 5311 which has exhibited antimicrobial activity. Li et al, 2002 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated xylanase and ligninase enzymes from *Aspergillus niger* and *Phlebia sp.* which has industrial applications. Lin et al 2002a (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated Enniating G from *Fusarium sp.* which exhibited Antimicrobial activity. Song et al, 2004 (as cited in Thatoi.H et al, 2012)<sup>8</sup> reported that fungal microbes are the excellent sources of  $\beta$  – Carboline, Andenosine, 8-hydroxyl-3,5-dimethyl-isochroman-1- one which exhibited anti tumor activity. Huang et al,2011 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated 8 secondary metabolites from *Penicillium chermesinum* (ZH4-E2) culture which is an endophytic fungus of mangroves, of the 8 secondary metabolites 3 Azaphilones and 3p-terphenyls are novel ones, where in different Terphenyls found to exhibit enzyme inhibition activity against  $\alpha$ -glucosidase and acetyl cholinesterase. Xu et al,2007 (as cited in Thatoi.H et al, 2012)<sup>8</sup> extracted 8 novel indole triterpenes – Shearinines from an endophytic fungus *Pencillium sp.* and suggested that Shearinines exhibited in vitro blocking activity on potassium channels activating by calcium ions. Sindhu.R et al, (2009)<sup>9</sup> isolated *Pencillium goddewskii* SBSS 25 from southern kerala, India and suggests that the strain was a potential alkaline protease candidate with it's applications in detergent industry. Arun sisi et al,(2012)<sup>10</sup> isolated *Aspergillus flavus* from Muthupettai mangroves of tamilnadu, India and reported that the strains was an excellent source



of cellulose which has applications in different industries – medical, food, pharmaceutical and chemical processing industries.

**Bacteria:** Bacterial communities flourishing well in mangroves coming just after trees (kathiresan and qasim 2005 as cited in Thatoi.H et al, 2012)<sup>8</sup>. Bacterial population exceeds those of fungal populations in mangrove ecosystem (kathiresan and qasim 2005 as cited in Thatoi.H et al, 2012)<sup>8</sup>. Different works suggests that Mangroves originated bacteria could be a potential hub of biotechnological applications. Gupta et al, 2007 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated bacteria from Bhitarkanika mangrove soil and exhibited phosphate solubilising activity. Ramanathan et al, 2008 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated cellulose degrading, phosphorous solubilizing bacteria and nitrogen fixing from Sunderbans mangroves in India. Venkateswaran and Natarajan, 1983 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated different phosphorous solubilizing bacteria from port novo mangroves of tamilnadu, India. Gayathri et al, 2010 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated endophytic phosphorous solubilizing bacteria from leaf of pichavaram mangroves of tamilnadu, India. Kathiresan and Selvam, 2006 (as cited in Thatoi.H et al, 2012)<sup>8</sup> reported varied bacteria from rhizosphere soils of mangrove plants with a wide range of agricultural benefits which embodies nitrogen fixing, phosphate solubilization, Ammonia production and others. Kathiresan and Selvam, 2006 (as cited in Thatoi.H et al, 2012)<sup>8</sup> reported *Azotobacter vinelandii* and *Bacillus megaterium* from mangroves rhizosphere soil which reported to exhibiting the mangrove seedlings enhancement. Zahran et al, 1995 (as cited in Thatoi.H et al, 2012)<sup>8</sup> reported nitrogen fixing bacteria from marine soils which can enhances the fertility of arid and saline soils. Ventosa and Nieto, 1995 (as cited in Thatoi.H et al, 2012)<sup>8</sup> extracted polyketide synthases from Halophilic bacteria which could be employed for the synthesis of tetracycline, erythromycin, rapamycin and other antibiotics. Mishra.2010 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolates catalase, oxidase, peroxidase, ascorbic acid oxidase and polyphenol oxidase enzymes from *Pseudomonas aeruginosa*, *P.alcaligenes* and *Methylococcus sp.* that has potential industrial applications. Armando et al, 2009 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated proteases, amylases, esterases and lipases from Vibrionales, Bacillales and Actinomycetales which has varied Industrial benefits. Jospheh and Paul Raj, 2007 (as cited in Thatoi.H et al, 2012)<sup>8</sup> reported phytase with varies industrial benefits from *Bacillus criculans*, *B.licheniformis* and *B.pantothenicus*. Pornsunthorntawee et al, 2008 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated lipopeptides and glycolipids which are potential biosurfactants from *Bacillus subtilis* and *Pseudomonas aeruginosa*. Desai et al, 2010; Brito et al, 2006 and others (as cited in Thatoi.H et al, 2012)<sup>8</sup> extracted enzymes of oil degradation and polyaromatic hydrocarbons which could be employed in process of bioremediation from different bacterial groups – *Arthrobacter*, *Pseudomonas*, *Shewanella* and others. Essam H et al, (2000)<sup>11</sup> isolated *Bacillus alcalophilus* (extremophilic bacterium) from mangrove debris of Tubli bay, Bahrain. The bacterial strain produced alkaline thermostable lipase which found it's applications in detergent industry.

**Actinomycetes:** Based on the bioactive potentiality of actinomycetes of mangroves we may designate it as the golden spoon of biotechnological applications different works support this. Hong et al, 2009 (as cited in Thatoi.H et al, 2012)<sup>8</sup> reported different secondary metabolites from actinomycetes having bioactivity ranging from anti infections, anti tumor and anti cancer. Sivakumar et al, 2005 (as cited in Thatoi.H et al, 2012)<sup>8</sup> isolated *Streptomyces albidoflavus* from mangroves of pichavaram, Tamil nadu, India and exhibited cytotoxic activity. Miller et al, 2007 (as cited in Selva kumar.D, 2010)<sup>12</sup> isolated piperazimycins from broth of *Streptomyces sp.* where in piperazimycins exhibited in vitro cytotoxic activity. Maskey et al, 2003 (as cited in Selva kumar.D, 2010)<sup>12</sup> reported *streptomyces sp.* 6921 from Mauritius marine sediments and produced a rare biological quinine which exhibited anti bacterial activity. Sujatha et al, 2005b (as cited in Selva kumar.D, 2010)<sup>12</sup> isolated *Streptomyces psammoticus* and reported to yield SBR – 22 polyketide antibiotic exhibiting anti bacterial property against methicillin resistant bacteria. Jeong et al, 2006 (as cited in Selva kumar.D, 2010)<sup>12</sup> reported that broth of *Streptomyces sp.* KORDI-3238 yielded streptokordin exhibiting cytotoxic properties. Lu et



al, 2009 (as cited in Selva kumar.D, 2010)<sup>12</sup> isolated marine *Streptomyces sp.* GB-2 from china's coastal soil yielding Sisomicin which exhibited antibacterial property. Xie, X.C. et al, 2008 (as cited in Dong – Bo Xu et al, 2014)<sup>13</sup> isolated a new nitrogenous compound from *Streptomyces sp.* 124092 which exhibited cytotoxic activity of moderate intensity against SMMC- 7721. Lin, W et al, 2005 (as cited in Dong – Bo Xu et al, 2014)<sup>13</sup> reported an endophytic *Streptomyces sp.* GT – 20026114 from a mangrove plant leaves – *Aegiceras comiculatum* of Xiamen, Fujian – China but no reports of any possible bioactivity. Suthindhikiran K. and Kannabiran K. (2010)<sup>14</sup> isolated 50 actinomycetal isolates, majority belongs to *Streptomyces sp.* from puducherry coast along Bay of Bengal, India of the total isolates 24% are reported to exhibit cytotoxic, hemolytic and antimicrobial activity. Jagan mohan Y.S.V.V. et al, (2013)<sup>15</sup> isolated 93 actinomycetal isolates along varied stretches of bay of Bengal's marine stations and suggests that 16, 11 and 9 isolates exhibited antibacterial, both antibacterial & antifungal and antifungal bioactive potentialities. Krishnakumar S. et al, (2011)<sup>16</sup> isolated a marine *Streptomyces sp.*- SBU1 from cape comorin coast of India and reported to exhibit significant L – Glutaminase production which has anti cancer properties. Selvam. K et al, (2011)<sup>17</sup> isolated 56 actinomycetal strains along different marine locations of south Indian coast out of which 9 isolates exhibited significant amylase, cellulose and lipase activity all these enzymes found it's applications in different biotechnological industries ranging from food, fermentation, textiles to pharmaceutical and detergent industries. Based on the aforesaid vivid discussion we can decipher that divergent microbial communities of Mangroves embodies the bioactive potentiality which may serve varied biotechnological industries in turn for the welfare of mankind.

#### Conclusion

It is evident that divergent microbes of mangroves may serves as potential contenders of bioactivity having wide range biotechnological applications. The biotechnological exploration of this valuable ecosystem has to be cautiously harnessed without over exploitation which may accompany severe repercussions. The appraisal opined that in order to sustainably tap the biotechnological potentiality of mangroves microbes, there is a need to conserve mangroves by integrated way of legal, civic and research regimes spearheading by legal framework, by doing so not only allows us to explore mangroves for human welfare but also conserving a most valuable ecosystem of the earth which adds to it's beauty.

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**BACOPA MONNIERI SHOWING ANTI-INFLAMMATORY PROPERTIES****D. VIJAYA SREE<sup>1</sup>, K. GEETANJALI<sup>2</sup>**<sup>1</sup>Lecturer in Biochemistry, Govt. College for Women (Autonomous), Guntur<sup>2</sup>Assistant Professor, Mahatma Gandhi University, Nalgonda, Telangana**ABSTRACT**

Enzyme Lipoxygenase (LOX-5) contributes in innate immunity development and progression of excessive and chronic inflammatory responses, allergic and non-allergic reactions. Many LOX – 5 inhibiting drugs were available manufactured by industries using many chemicals which may harm the environment. For the sustainability of environment traditional medicines (phytochemicals) may reduce the effect and is safe for the therapeutics. *Bacopa monnieri* (L., BM) is one of the traditional Ayurvedic herb recognized for its efficacy in relieving acute pain and, by selective inhibition of lipoxygenase (LOX-5). The present study shows oxygraph analysis of lipoxygenase – 5 assay using aqueous, methanolic and ethanolic extracts of *Bacopa monnieri*.

**Key words:** Lipoxygenase, innate immunity, chronic inflammatory responses, inflammation, *Bacopa monnieri*

**INTRODUCTION**

Inflammation (Latin, *inflammatio*, to set on fire) is the complex biological response of vascular tissues to harmful stimuli, viz; pathogens, damaged cells, or irritants.<sup>[1]</sup> It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation can either acute or chronic. In both cases, edema, blush, an increase in local temperature, and pain are present. Several inflammatory disorders are allergy, myopathy, leukocyte defects, pharmacological cancer etc.

Bacterial infections cause an increased number of neutrophils, which produce an oxidative burst at the site of microbial invasion. The uncontrolled release of reactive oxygen species is assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis. Administration of agents, which decrease the neutrophil accumulation in inflamed areas, might be a remedy in these cases.

Leukocytes promote the switch of arachidonic acid derived prostaglandins and leukotrienes to lipoxins, which initiate the termination sequence. Neutrophil recruitment thus ceases and programmed death by apoptosis is engaged.<sup>[2]</sup>

Every plant produces primary and secondary metabolites found in varied amounts during their metabolic activities. Of those compounds secondary metabolites and pigments are the one's that can have therapeutic actions in humans and which can be refined to produce drugs.

The use of herbs to treat disease is almost universal among non-industrialized societies<sup>[3]</sup>. A number of traditions came to dominate the practice of herbal medicine at the end of the twentieth century. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies.

Recent reports indicate that there is an inverse relation between dietary intake of antioxidant rich food and the incidence of human diseases. Hence search for new synthetic and natural antioxidant compounds is essentially important.

*Bacopa monnieri* is the plant rich of medicinal values like antibacterial, antifungal, antiulcer, antidepressant, anti-inflammatory, cognitive, anxiolytic and antioxidant properties. The plant extracts give protection against lipoxygenase thus reduce the production of leukotrienes which are responsible for the inflammation produced from linoleic acid and the oxidation of arachidonic acid followed by dehydration of 5-hydroperoxyeicosatetraenoic acid (5-HPETE).<sup>[4][5]</sup>

**Materials and Method:**Preparation of Aqueous extract: 3 grams of dried stem is grinded using mortar and pestle. The powdered stem is boiled in 150ml deionised water for 20 to 30 minutes. Then the extract was filtered using cheese cloth and with filter paper. Then the filtrate is concentrated using vacuum rotary evaporator.



Preparation of Ethanolic and Methanolic Extracts: 3grams of the stem is powdered using mortar and pestle then it is tied in a cheese cloth and placed in the soxhlet extraction column and extracted with 70 % ethanol / methanol. Using vaccum rotary evaporator these solvents were concentrated.

Concentration of 1ml plant extract was calculated and expressed as µg per g of the dry weight of the stem.

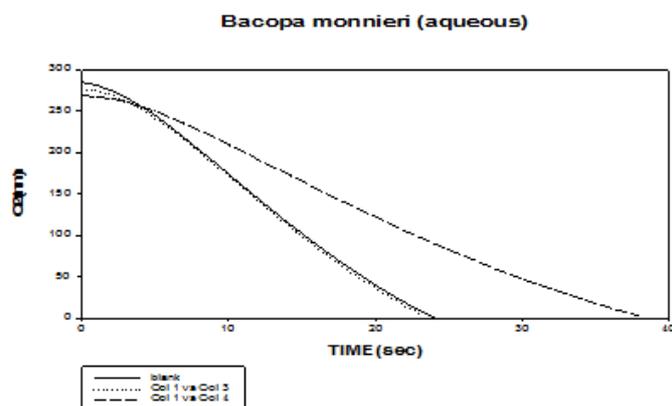
Procedure for 5-Lipoxygenase Extraction and Purification: 500 gm of fresh potatoes were weighed and peeled. It is blended thoroughly using 500ml of the Phosphate buffer (p<sup>H</sup> 6.3). The above homogenate was filtered through a muslin cloth. Filtrate was collected in tubes and was centrifuged at 4°C at 10,000 rpm for 20 min. The supernatant was stored as crude extract. Slowly 0-15% ammonium sulphate was added to the supernatant while it was put on a magnetic stirrer at 4°C for 30 min. The above solution was centrifuged at 4°C at 15000 rpm for 15 min. To the collected supernatant, required amount of ammonium sulphate (15-45%) was added and at this concentration the 5-Lipoxygenase enzyme precipitates. The above solution is centrifuged at 4°C at 15000 rpm for 15 min. The pellet was collected and is dissolved in 40mM phosphate buffer, and the final volume of the enzyme obtained was aliquoted.

**5-Lipoxygenase Assay-Oxygraph Analysis:** Enzyme used is 5-lipoxygenase from Potato (*Solanum tuberosum*) and the substrate is linoleic acid (LA). The reaction volume is 3ml and contains Phosphate buffer, Double distilled water, 5-lipoxygenase enzyme, linoleic acid. For enzyme inhibition studies the 5-lipoxygenase enzyme is incubated for 10min with the plant extract/inhibitor along with other components except the substrate.

Control Reaction: 2 ml of 150mM phosphate buffer was added to the reaction chamber first followed by the addition of required amount of double distilled water. Then 5-lipoxygenase was added to the reaction chamber and was put on stirring, when the air saturation occurred, a straight line (curve) was observed on the computer screen. Then 10 µl of LA was added through the capillary bore and the decrease in the O<sub>2</sub> level was observed on the computer screen. The reaction was stopped after 1 minute and the data was analyzed to get the amount of O<sub>2</sub> utilized per minute. This was directly correlated to the enzyme activity.

Inhibitory Studies: Here the reaction was done in presence of an inhibitor/ plant extract. All the components except LA were added in a tube along with the inhibitor/ plant extract and incubated for 10 minutes. Then the reaction mixture was transferred to the reaction chamber and the reaction was started after the air saturation was reached followed by the addition of LA while the stirrers were on. Then the amount of O<sub>2</sub> utilized per minute was noted down. The amount of O<sub>2</sub> utilized for the reaction with the plant extract was compared to the control reaction and the percentage inhibition was calculated. The following reaction was repeated using different concentrations of the plant extract till a point was reached where the enzyme was 50% inhibited. This kind of study is called dose dependant study.

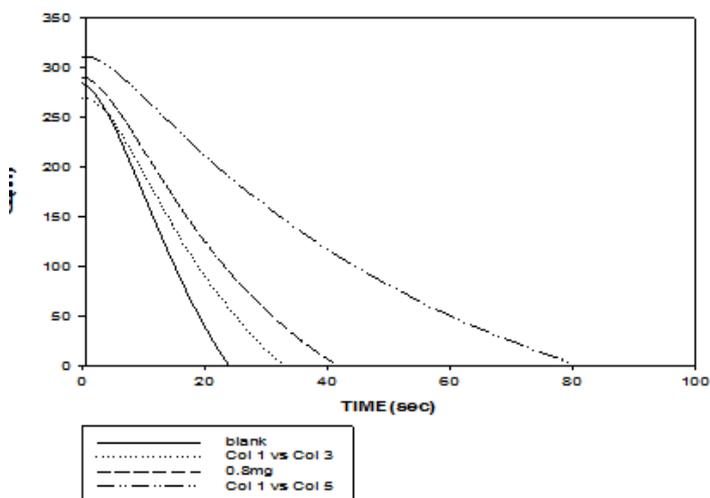
## Results and Discussion



The graph shows that methanolic extract shows 50% inhibition on lox-5 is at >1.8mg

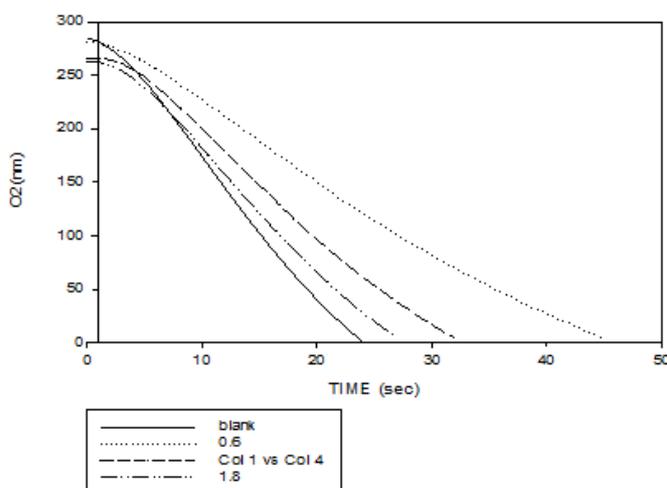


**Bacopa monnieri (ethanolic)**



The graph shows that ethanolic extract shows 50% inhibition on lox-5 at 1.0mg

**Bacopa monnieri (methanolic)**



The graph shows that methanolic extract shows 50% inhibition on lox-5 is at >1.8mg

In LOX-5 assay in aqueous ethanolic extract shows 50% inhibition at 1mg while extract at 1.5mg and methanolic extract at 1.8mg

**Conclusion**

The investigations on Bacopa monnieri leaf and stem extracts were found positive towards the evidence of anti-inflammatory activity. Present results also indicate that the ethanolic extract has shown good anti-inflammatory activity than aqueous and methanolic extracts.

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**“BIO DIVERSITY AND ITS CONSERVATION”, A DOMINANT IDEOLOGY IN INDIAN CHILDREN FICTION**

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**ABSTRACT**

The objective of this research paper is to analyse the children’s fiction published by the National Book Trust of India, Children Book Trust of India and Publications Division under the ministry of Information and Broadcasting as to how these books, with in the setting of a story, in a lively and entertaining way promote positive impressions about the importance of Biodiversity and nurture an idea of living in harmony with others in the young minds. This research article also explores the possibility of using the children’s fiction to instill a spirit of adventure for championing the cause of Biodiversity through their child protagonists and thus as an effective tool in socializing our children environmentally.

**Key-Words.** : Threats, Habitat Loss, unethical treatment, Anthropomorphism, Biodiversity depletion.

The year 2010 was declared by the United Nations as “ The International Year of Biodiversity”. The purpose was to attract the attention of the world towards the biodiversity present on the Earth. Our Earth is politically divided into large number of Nations and Countries. In terms of Environment it is not possible to find such demarcation. Environmental issues like depletion of ozone layer, Green House Effect, and climate change are worrying the entire human race either directly or indirectly. The human impact on Biodiversity is also recognized as an International issue now.

Nobody can deny the powerful role played and is being played by Literature in increasing awareness, transmitting values, altering people’s mind set about Earth, Environment and associated challenges over the years. A Pro-Environmental attitude can be cultivated among the junior reading audience through Children’s literature.

“Writing a children’s story is the best art-form for something you have to say” -C.S. Lewis.

Children’s fiction can be Interdisciplinary and is a lovely way of communicating with unpolluted minds. When the future of our planet is at stake, conveying strong ecological messages can be done through fascinating stories with plants, birds and animals as the characters. Children with their raw imagination can immediately connect the events, descriptions in the stories with their surroundings and will develop a responsible attitude towards the Planet and its Components.

Our earth is the only place in the entire universe where life exists in the form of a large variety of plants, animals and micro organisms. Each organism has its own purpose and importance. We should respect each one. “This ultimate truth is depicted in a marvelous way in the story”. “The Lion and the Hedgehog” written by J.B. Sharma, illustrated by Arya Prabaraj and published by the NBT. (National Book Trust of India).

The Lion, the king of the forest sees some ugly creatures, crawling with body full of thorns. He roars in disgust “Who are you and what the hell one you creatures doing in my den”.

“Your majesty, we are Hedgehogs, Cleaners of your den: The Lion wants the pest to leave his forest at once. As the den has not been properly tended, weeds grow up every where and the den becomes messy with thorny plants growing here and there. The thorny plants hurt the lions legs and body and ultimately he realizes the usefulness of the Hedgehogs.

The diversity among plants, the peculiar adaptations some plants possess comes to the fore front in the story “we stand alone”. Written by Anis Hameed and Ayesha Hamed from the collection of 34 short stories, published by CBT. The writer gives voice to the conifers, the mahogany and the sal trees. The conifers bear the gibes of other trees for having thin needles like prickly leaves. Even the birds did not like to make their nests on



their branches and neither could they give any shade to a tired traveler. They were miserable. Secondly, they had been jeered at and made fun of by the stately trees for not getting a chance to wear new foliage year after year as the even green never shed their leaves in autumn.

However, the conifers were willing to bear these disdainful comments as long as it meant seeing them shiver during the winter after shedding all their leaves.

The writer very delicately weaves the story around the trees with lines like “The more leaves we would have had, the more exposed we would have been to the frost. The conifers heave a sign of relief on seeing the goats nibbling greedily at the tender, green leaves on the lower branches of the trees and the goat herdcutting the higher branches to feed his goats. Nature’s magic comes alive in beautiful words in this story.

The paradigm shift in economic development linked to land use and biodiversity depletion strike poignantly in the story “George” written by W.J. Sarkar again from “A collection of 34 short stories” published by CBT. This story articulates the human impact on Biodiversity leading to extinction of species with habitat loss.

The story runs like this. “The same snake hurting down again from the same palm tree, smashing into the same shrub, winding its way up the same tree and vanishing over the same wall. After the third visit, we decided to call him “George”. The frightened neighbors were informed “He is a rat snake –harm less! We should not kill him for they help farmers to keep down the rats and mice.

George glides on and entering the jungle, is soon lost to sight in the under growth.

At the end the writer nostalgically laments “All those green fields have gone, where the Pampas grass used to wave in the wind, there are now rows and rows of houses and factories belching smoke. Then” where will all these wild creatures go? How will they find food? Where will the birds build their nests? Will they all vanish forever?

**The Man Vs Wild conflict :** when we talk about biodiversity we keep ourselves out of it. Human being is also one group of living organisms. One component of the overall biodiversity present on the earth. A false notion of superiority percolates in our minds that we have the right to use the other living organism in a way that suits us resulting in the misuse of biological resources.

“Hunt for the Golden Langur”. A story from the collection “Man, Beast and Wilderness, written by A.K. Sri Kumar, Published by children book Trust of India is the description of a hunter’s journey through the living, breathing jungles of the North-East. Christopher Broad a hunter comes to India for the trophies of elephant tusks and Rhino horns. In the exemplary show of the man vs wild, Broad gets attacked by a Shot Rhino and lies crumpled in the grass, surrounded by the eerie silence of the jungle. As he recoups, he senses a change coming over him. He still chooses to track the elephant to his haunts but to foster a strange kinship with the wild this time.

He tells Dhiman a jungle child, “ Why should we kill them? Let us leave them to their ruminations. It is enough of wonder and pleasure to watch these splendid creatures in their haven!”.

Most of these stories provide an interesting narration and try to sensitize the little minds towards conserving Biodiversity. The names of these stories are tell-tale like , “The Lapwings Nest”, “Pecky the WoodPecker”, “The Maleo Birds”, “Two Little sparrows etc.. The Night of the Light Birds is yet another story by Indira Anantha Krishnan, a famous children’s writer against the practice of lighting lamps and forcing birds to commit suicide. Our Anthropomorphic thinking has resulted in unethical treatment of some animals.” “My Bhoora darling” is the story of a seven year old girl Sita, and her confrontation with the cruel treatment of animals in the name of sport. She witnesses her pet piglet Bhoora in a cruel fight with buffaloes where the helpless creatures are prodded and pushed with long bamboo sticks, with blood pouring out from every part of their bodies, much to the pleasure of the cheering crowd. She approaches the Judiciary and with their help stops the inhuman show.



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To conclude The Indian Children fiction published by these Governmental publishing agencies holds a great promise of transmitting the knowledge and importance of Biodiversity with realistic pictures both in words and pictures and thus tries to play a major role in sustaining the Biodiversity on the Earth.

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**ANTIBACTERIAL ACTIVITY OF MORINGA AND CARICA LEAVES****D. VIJAYA SREE<sup>1</sup>, Dr. K. SUCHARITA<sup>2</sup>**<sup>1</sup>Lecturer in Biochemistry, Govt. College for Women (Autonomous), Guntur<sup>2</sup>Lecturer in Microbiology, Govt. College for Women (Autonomous), Guntur**Abstract**

The antibacterial activity of leaf extracts of *Carica* and *Moringa* was determined in-vitro by using cup plate method against gram negative bacteria – *Escherichia coli*. Varying concentrations of acetone, ethanol and aqueous extracts were prepared and tested against bacteria. An antibiotic ampicillin is used as standard to compare the effect of antibacterial activity of leaf extracts in same concentrations. Of extracts, ethanolic extracts of *Carica* and *Moringa* leaves are having good inhibitory properties against *E.coli*. Therefore ethanolic extracts of *Carica* and *Moringa* leaves could be seen as a good source for drugs. Now a days use of natural drugs is more effective for sustainable of environment compared to synthetic drugs.

**Key words:** *Carica* leaves, *Moringa* leaves, *Escherichia coli*, antibacterial activity.

**INTRODUCTION**

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries. For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties. Plants have many antimicrobial properties due to secondary metabolites such as alkaloids, phenolic<sup>[1]</sup> compounds,  $\beta$ -carotene, calcium, carpaine, fats, flavonoids and vitamin C<sup>[2]</sup> etc. The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections. Plants are the cheapest and safer alternative sources of antimicrobials.<sup>[3]</sup> The present study is to know the antibacterial activity of *Carica papaya* and *Moringa oleifera* leaves. These leaves are used in traditional medicine as a source of many therapeutic agents in the Indian culture and grow well in the tropical countries.<sup>[4]</sup>

**Preparation of Extract:** Fresh leaves of *Carica papaya* and *Moringa oleifera* were collected from the local gardens and were rinsed properly in sterile distilled water, and then grinded with motor pestle using 25ml solvents like acetone, ethanol and distilled water for respective extracts.

The filtrates were weighed and stored in sterile universal bottles at 4 °C in a refrigerator.

**Antibacterial Activity:** Antibiotic Assay Medium was prepared, autoclaved at 121°C for 5 minutes at 15 Psi pressure. Antimicrobial activity of the *Carica* and *Moringa* leaves solvent extracts was determined by Cup-Plate Method on Antibiotic Assay Medium. Wells were made on Antibiotic Assay Medium after solidification of media by using standard sterile cork borer of 8mm of diameter.<sup>[5]</sup> When well is loaded (100  $\mu$ l) with antibiotics and leaves extracts by the help of micropipette, it diffuses in the medium and inhibits the growth of organism. The plates were left for 1hr for pre-diffusion and incubated in bacteriological incubator at 37°C for 24hrs. At the end of incubation, the zone of inhibition is measured around each well using scale and the susceptibility is determined.

**Results and Discussion:** Depending upon the active ingredient and diffusion capacity into agar medium, *Carica* and *Moringa* leaves extracts showed the antimicrobial activity with respect to ampicillin standard against the tested microorganisms *E.coli*

The ethanolic extracts of *Carica* and *Moringa* leaves showed the maximum antimicrobial activity against the tested microorganism in comparison to acetone and aqueous extracts. The antibacterial activity observed in



form of zone of inhibition measured in mm (millimeter) is shown in Table 1, Table 2, and Table 3. The potency of antimicrobial activity of plant extracts are in following manner:

Ethanol Extracts >> Acetone Extracts > Aqueous Extracts.

Table1. Antibacterial Activity of *Carica* Leaf Extracts against *E.coli*

Conc. (mcg/ml)	Acetone Extract	Aqueous Extract	Ethanol Extract
<b>Zone of Inhibition in mm</b>			
20	10.9	10.6	11.1
40	11.9	11.4	12.4
60	12.7	12.3	13.8
80	13.9	13.5	14.6

Table2. Antibacterial Activity of *Moringa* Leaf Extracts against *E.coli*

Conc. (mcg/ml)	Acetone Extract	Aqueous Extract	Ethanol Extract
<b>Zone of Inhibition in mm</b>			
20	11.9	11.4	12.6
40	12.9	12.3	13.9
60	14.3	13.7	14.8
80	15.1	14.8	15.9

Table3. Antibacterial Activity of Ampicillin against *E.coli*

Conc. (mcg/ml)	Acetone Extract	Aqueous Extract	Ethanol Extract
<b>Zone of Inhibition in mm</b>			
20	13.3	13.1	13.5
40	14.5	14.3	14.8
60	15.3	15.0	15.6
80	16.6	16.4	16.9

**Conclusion:** It was observed that the three extracts were able to inhibit the growth of micro-organisms under test. Among the three extracts, the ethanolic extract showed the maximum antimicrobial activity against *E.coli*. The results revealed the presence of medicinally important constituents in these solvent extracts. Therefore, *Carica* and *Moringa* leaves extracts could be seen as a potential source for useful drugs. The secondary metabolites that involve in antibacterial activity has to be screened.

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**YEAST-MEDIATED STEREOSELECTIVE BIOCATALYSIS****K. SARITHA RANI***Lecturer in Chemistry, Govt. College for Women (A), Guntur***Abstract**

Currently, research is focused on the interaction of small molecules with biological macromolecules. The search for enzyme inhibitors and receptor agonists/antagonists is essential for target-oriented research in the pharmaceutical, agrochemical and food industries. Organic synthesis has made advances and developed practical processes in the synthesis of natural products, drugs, agricultural chemicals, polymers and many classes of functional molecules.

Biocatalysis gives an added dimension, innovative new approaches, and enormous opportunity to prepare industrially useful chiral compounds. The advantages of biocatalysis over chemical catalysis are that enzyme catalysed reactions are stereoselective and regioselective and can be carried out at ambient temperature, at atmospheric pressure and under environmentally friendly conditions.

Biocatalysis minimises the problems of isomerisation, racemisation, epimerisation and rearrangement of molecules that may occur during chemical processes. The present article focuses on the yeast-mediated stereoselective biocatalysis and stereoselective synthesis of terpenoids.

**Keywords:** stereoselective reactions, yeast, biocatalysis, terpenoids, chiral compounds.

**INTRODUCTION**

In chemistry, stereoselectivity is the property of a chemical reaction in which a single reactant forms an unequal mixture of stereoisomers during the non-stereospecific creation of a new stereocenter or during the non-stereospecific transformation of a pre-existing one. The selectivity arises from differences in steric effects and electronic effects in the mechanistic pathways leading to the different products. Stereoselectivity can vary in degree but it can never be total since the activation energy difference between the two pathways is finite. However, in favorable cases, the minor stereoisomer may not be detectable by the analytic methods used.

An enantioselective reaction is one in which one enantiomer is formed in preference to the other, in a reaction that creates an optically active product from an achiral starting material, using either a chiral catalyst, an enzyme or a chiral reagent.

Enantioselective synthesis is a key process in modern chemistry and is particularly important in the field of pharmaceuticals, as the different enantiomers or diastereomers of a molecule often have different biological activity.

A diastereoselective reaction is one in which one diastereomer is formed in preference to another (or in which a subset of all possible diastereomers dominates the product mixture), establishing a preferred relative stereochemistry.

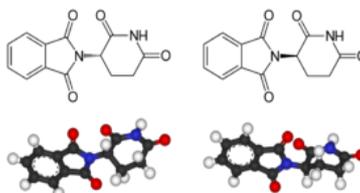
**Thalidomide**

While it had long been known that the different enantiomers of a drug could have different activities, this was not accounted for in early drug design and testing. However following the thalidomide disaster the development and licensing of drugs changed dramatically.

First synthesized in 1953, thalidomide was widely prescribed for morning sickness from 1957 to 1962, but was soon found to be seriously teratogenic, eventually causing birth defects in more than 10,000 babies. The disaster prompted many countries to introduce tougher rules for the testing and licensing of drugs, such as the Kefauver-Harris Amendment (U.S.) and Directive 65/65/EEC1 (E.U.).



Early research into the teratogenic mechanism, using mice, suggested that one enantiomer of thalidomide was teratogenic while the other possessed all the therapeutic activity. Thus it raised the importance of chirality in drug design, leading to increased research into enantioselective synthesis.

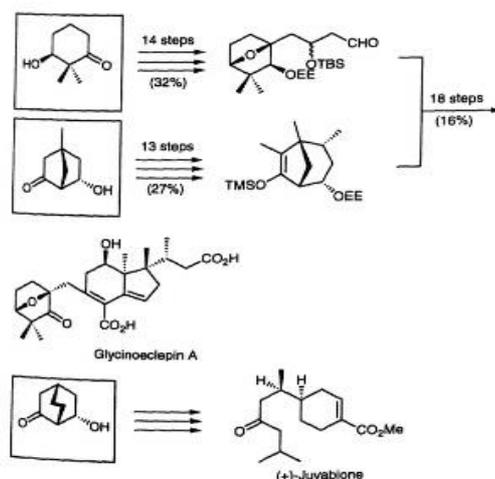


The two enantiomers of thalidomide: Left: (S)-thalidomide Right: (R)-thalidomide

### Yeasts as catalysts for Enantioselective Reduction and Oxidation

#### Asymmetric reduction of $\beta$ -diketones

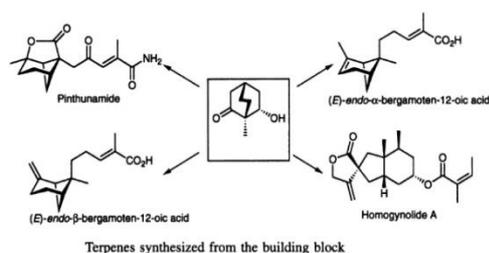
The ability of yeasts such as baker's yeast (*Saccharomyces cerevisiae*) was utilised extensively by chemists to reduce carbonyl compounds to alcohols. The hydroxy ketones are the building blocks for the synthesis of glycinoclepin A, a degraded triterpenoid with remarkable hatch-stimulating activity against the soybean cyst nematode. The hydroxy ketone was converted to (+)-juvabione, a terpene with juvenile hormone activity.



Synthesis of glycinoclepin A and (+)-juvabione.

The hydroxy ketone (A) was the starting material of the following terpenes:

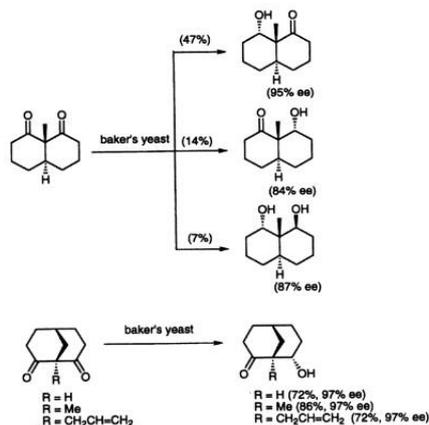
1) pinthunamide, a promoter of root growth of lettuce 2) (E)-endo- $\alpha$ - and  $\beta$ -bergamoten-12-oic acids, sesquiterpenes isolated from wild tomato leaves as the oviposition stimulator for gravid moths, and 3) homogynolide A, an insect antifeedant shows the reduction of 9-methyl-trans-decalin-1,8-dione with baker's yeast. The major product might be useful in isoprenoid synthesis.



Terpenes synthesized from the building block



The reduction of diones by baker's yeast to give various stereoselective products is shown below:



Reduction of 9-methyl-*trans*-decalin-1,8-dione and bicyclo[3.3.1]nonane-2,8-diones with baker's yeast.

### Microbial hydroxylation of Terpenes

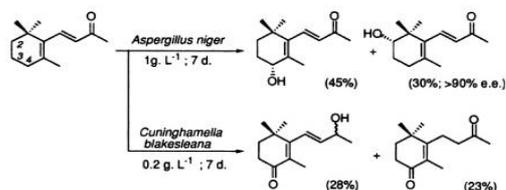
Ionones and their derivatives are other important constituents of various essential oils, with particular relevance in tobacco flavouring. Their oxidative transformation by microorganisms was used to increase their flavouring properties, due to the formation of hydroxylated or keto derivatives.

For example,  $\beta$ -ionone (A) is mainly oxidised by *A. niger* to give in good yields and moderate-to-good optical purities 4(R)-hydroxy and 2(S)-hydroxy  $\beta$ -ionone.

*Cunninghamella blakesleeana* essentially affords 4-keto derivatives, but partial reductions of the side chain are also observed. Up to 10g/ml of  $\beta$ -ionone is rapidly converted by *Lasiodiplodia theobromae* to 2-,3-, and 4-hydroxylated derivatives, but on prolonged incubation, extensive reduction and degradation reactions of the side chain occurred, leading ultimately to complex mixtures.

More recently, efficient methods for quantitative production of hydroxylated species have been described, using an immobilized *A. niger* mycelium without or in the presence of an organic solvent.

#### Microbial Hydroxylation of Terpenes



### Conclusion

The use of microorganisms in the field of terpenoid hydroxylation will certainly become increasingly popular. Despite the current multiplicity of hydroxylated products obtained, the use of whole microorganisms is still justified. Except in a few cases, the predictability of the regio and stereoselectivity of such reactions remains low. However, in the future, expression of individual specific monooxygenases in various host systems would probably become feasible and help in the prediction of hydroxylation reactions.

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## QUALITY EVALUATION OF HEN EGGS TO DETECT ANTIBIOTICS RESIDUES BY REVERSE PHASE (RP) HPLC

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### ABSTRACT

Well the objective of our study is to quantify the antibiotics (Oxytetracycline & Enrofloxacin) residue levels in domestic and layers by RP-HPLC. This study was carried out on one hundred randomly collected domestic and layers hen egg samples (20 of each) to evaluate the antibiotic (oxytetracycline and enrofloxacin) residues level qualitatively by high performance liquid chromatography (HPLC). The separation was carried out using mobile phase composed of 0.1% formic acid: Acetonitrile (50:50, v/v) using C<sub>18</sub> (Hypersil ODS-BPS, 250 x 4.6mm; 5μ) at a flow rate of 1ml/min. The residues were quantified at detection wave length 350 nm. The obtained results revealed a detectable level of oxytetracycline and enrofloxacin residues which confirm widespread misuses of antibiotics in farms and lack of application of recommended withdrawal times. The boiling effect of broiler hen egg results shows the reduction in concentration of antibiotic residues. These findings recommended that restricted measures and harder regulations must be applied to prevent the misuse residues prior to marketing and can help in prolonging the healthy life span of the masses and helps to improve the Quality of Life (QoL) of the Homosapiens

**Key words:** Antibiotic, oxytetracycline, enrofloxacin, Maximum residue limit, HPLC.

### NEED OF THE STUDY

- To determine the MRL concentration of antibiotics in hen eggs which are being regularly used in the poultry industry. This method helps in avoiding antibiotic resistance and other unwanted effects in public health.

### OBJECTIVE

- To measure the amount of maximum allowable residual limit of enrofloxacin and oxytetracycline (MRLs) in hen egg samples by RP-HPLC with UV detection.
- MATERIALS AND METHODS

### Working Reference Standards:

- Enrofloxacin - Hetero drugs Pvt Ltd; Hyderabad
- Percentage purity - 99.65 (as is basis)
- Oxytetracycline - Hetero drugs Pvt Ltd; Hyderabad
- Percentage purity - 99.78 (as is basis)

### Reagents used:

- HPLC grade Water- Merck
- Formic Acid- Merck
- HPLC grade Acetonitrile- Merck

### CHROMATOGRAPHIC CONDITIONS

Column : C18 (Hypersil ODS-BPS; 250 x 3.6mm; 5μ)  
 Mobile phase : 0.1% Formic acid: Acetonitrile (50:50, %V/V)  
 Pump mode : Isocratic  
 Flow rate : 1mL/min



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Detection : 350nm  
Injection volume : 20  $\mu$ L  
Column oven temperature : Ambient  
Run time : 15 min  
Retention time : Enrofloxacin:  $5.23 \pm 0.02$  min  
Oxytetracycline :  $2.77 \pm 0.02$  min

#### CONCLUSION

- The residual level ranged from 0.111 $\mu$ g/g to 0.900 $\mu$ g/g for enrofloxacin and 0.041 to 0.757  $\mu$ g/g for oxytetracycline in "layer hen egg" samples
  - The residual level ranged from 0.011 $\mu$ g/g to 0.051 $\mu$ g/g for enrofloxacin and 0.010 to 0.189 $\mu$ g/g for oxytetracycline in "raw hen egg" samples.
  - Enrofloxacin and oxytetracycline in layer egg samples were exceed MRLs (0.1 $\mu$ g/g), this may be due to unpaid attention and lack of implementation of recommended withdrawal time during withdrawal of layer eggs.
  - This study stress on the need for stricter regulation for the use of antibiotics in poultry industry prior to marketing.
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**AN OUTLOOK ON GEOGRAPHY OF CORINGA MANGROVES****\*SK CHAND BASHA AND N.ANKAMMA**

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Mangroves are distinct woody plant communities thriving along the unequivocally extreme transitional zone of land and sea. The current communication deals with Geography of Coringa Mangroves and narrates that geographical aspects are imperative in understanding the coringa mangroves. Geographically coringa mangroves are a Sand pit, one of the coastal landform and extending for a length of 18km. Fresh water sources of coringa mangroves are by rivers Coringa and Gaderu which are the tributaries of Gautami Godavari, the eastern branch of Godavari river, apart from it numerous creeks also traverse the mangroves region. It has co-ordinational extents of 16°44' to 16° 53'N and 82°14' to 82°22'E and located in Chollangi village of East Godavari district which is 18 km from Kakinada and 150 km from south of Vizag. Coringa mangroves bounded by Kakinada Bay on it's Northern side and on its Western side embodies main land portion comprising of deltaic and flood plains. Coringa mangroves are the second largest mangrove cover in India after Sunderban mangroves of West Bengal recognizing the importance of coringa mangroves it has been designated as a Sanctuary and conserving 24 mangrove species – *Rhizophora mucronata*, *Avicennia marina*, *Excoecaria sp.* *Bruguiera sp.* *Lumnitzera sp.*, *Sonneratia sp.* and others. Coringa wild life sanctuary also conserving some of the critically endangered faunal species like long billed vulture and a hub of numerous Avian species. Near to the coringa mangroves are the Hope islands, marshy islands which has excellent scenic beauty. The paper opined that owing to its distinct geographical aspects coringa mangroves supports great a majority of both floral and faunal sp. particularly mangroves which attracts a wide array of researchers to explore the same for divergent research projects which adds as a credential inputs to the scientific regime.

Key words: 1.Coringa Mangroves, 2. Geography and 3. Sanctuary

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**MICROBIOLOGICAL BIODIVERSITY****B. LAKSHMI DEVI & B. BLESSY***M.Sc. Zoology, Government College for Women , Guntur*

Biodiversity or biological diversity generally refers to the variety and variability of life on Earth. It is a measure of the variety of organisms present in different ecosystems. This can refer to genetic variation, ecosystem variation, or species variation (number of species). The number and variety of plants, animals and other micro organisms that exist is known as biodiversity. The richness of biodiversity depends on the climatic conditions and area of the region. All species of plants are taken together are known as flora and about 70,000 species of plants are known to date. All species of animals taken together are known as fauna which includes birds, mammals, fish, reptiles, insects, crustaceans, molluscs, etc. The microbiological diversity includes algae, fungi, microbes, viruses, etc.

These microorganisms are used in various categories of life for the benefit and welfare of mankind. They can be used for organic, inorganic, scientific, research, medicine, healthcare and many other purposes. The study of microbial diversity of poultry waste and paddy straw based co-composting system. The predominant micro-flora of the poultry manure were bacteria, fungi, enteric bacteria and spore forming bacteria whose population was high at the initiation of composting but decreased significantly. The initial load of enteric groups of bacteria in poultry waste, that also includes some pathogenic ones, is considerably reduced and some new



vital group contributes to compost quality as the microbiological biodiversity sets in the system and becomes stable. In the present study the prevalence of microbiological population has to be a study.

Keywords: biodiversity, plants, animals, species, microorganisms, population, study, microbiological

## BIODIVERSITY MEASURE - GENETIC DIVERSITY

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This is the genetic variability of a species. Genetic diversity can be measured directly by genetic fingerprinting or indirectly by observing differences in the physical features of the organisms within the population (e.g. the different colour and banding patterns of the snail *Cepea nemoralis*). Genetic fingerprinting of individuals within cheetah populations has indicated very little genetic variability. Lack of genetic diversity would be seen as problematic. It would indicate that the species may not have sufficient adaptability and may not be able to survive an environmental hazard. The Irish potato blight of 1846, which killed a million people and forced another million to emigrate, was the result of planting only two potato varieties, both of which were vulnerable to the potato blight fungus, *Phytophthora infestans*.

## A SHORT NOTE ON MICROBIAL INDICATORS OF DRINKING WATER

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The use of indicator organisms, in particular the coliform group, as a means of assessing the potential presence of water-borne pathogens has been paramount to protecting public health. These are based upon the principle of the detection of selected bacteria that are indicative of either contamination or deterioration of water quality through the use of simple bacteriological tests.

Indicator organisms are used to assess the microbiological quality of water. For many pathogens, such as viruses and protozoan parasites, reliable indicators are not available. Even if there were, there is no absolute correlation between the number of indicator organisms and (a) the actual presence or numbers of enteric pathogens or (b) the risk of illness occurring.

The use of indicator bacteria, in particular *Escherichia coli* (*E. coli*) and the coliform bacteria, as a means of assessing the potential presence of water-borne pathogens has been paramount to protecting public health. The analysis of large volumes of sample for faecal indicator bacteria using membrane filtration procedures can be very useful in assessing water treatment efficiency at various points in the treatment process.

Many pathogens are present only under specific conditions and, when present, occur in low numbers compared with other micro-organisms. Whilst the presence of coliform bacteria does not always indicate a public health threat, their detection is a useful indication that treatment operations should be investigated.

The key criteria for ideal bacterial indicators of faecal pollution are that they should be universally present in large numbers in the faeces of human and other warm-blooded animals. They should also be present in sewage effluent, be readily detectable by simple methods and should not grow in natural waters. Ideally, they should also be of exclusive faecal origin and be present in greater numbers than faecally transmitted pathogens. No single indicator organism fulfills all these criteria, but the member of the coliform group that satisfies most of the criteria for the ideal indicator organism in temperate climates is *E. coli*. The presence of



*E. coli* in a sample of drinking water may indicate the presence of intestinal pathogens. However, the absence of *E. coli* cannot be taken as an absolute indication that intestinal pathogens are also absent. *E. coli* bacteria are the only biotype of the family Enterobacteriaceae which can be considered as being exclusively faecal in origin and it can represent up to 95 % of the Enterobacteriaceae found in faeces.

For water quality monitoring and assessment, reliance has been placed on relatively simple and more rapid tests for the detection of faecal indicator bacteria and other coliform bacteria.

Key Words: Indicators, Faecal, *E. coli*, Enterobacteriaceae, Public health, Pathogens.

### STUDY OF ELEMENTS AND MINERALS IN THE ROOT OF *SYZYGIUM SAMARANGENSE*

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*Syzygium samarangense* is a plant species in the family Myrtaceae, native to an area that includes the Greater Sunda Islands, Malay Peninsula and the Andaman and Nicobar Islands, but introduced in prehistoric times to a wider area [Merr. & L.M.Perry (2016), Julia F. Morton (1987)] and now widely cultivated in the tropics. Phytochemical constituents are non-nutritive plant chemicals that have preventive and curative properties of disease. The use of plants and phytochemicals, both with known biological properties, can be of great significance in therapeutic treatments. The aim of present work includes Study of elements and minerals present in the root of *Syzygium samarangense*. The Biochemical compounds like Amino Acids, Proteins, Soluble Sugars, Crude fat and Crude Protein etc present in the root of *S samarangense* was studied. Elements and metals present in the roots were extracted using acid digestion method and the level of Calcium, Magnesium, Iron, Manganese, Zinc, Copper and Cobalt were estimated by using Atomic Absorption Spectrophotometer. Sodium and Potassium were estimated flame photometrically.

### MICROBIAL ANALYSIS OF STREET VENDED FRUIT JUICES

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Fruit juices are popular drinks as they contain antioxidants, vitamins, and minerals that are essential for human being and play important role in prevention of heart diseases, cancer, and diabetes. In the present study, we have conducted a microbiological examination of freshly prepared street vended fruit juices (sapota, pineapple, grape, mixed fruit juices). A total of 12 juice samples from three different stalls of pulivendula were collected and examined for their physico-chemical properties and for their microbiological quality. Physico-chemical properties of fruit juices include pH, Titrable acidity and TSS. The pH of sapota juice (7.26) is high when compared to other fruit juices. The titrable acidity was high for grape juice (0.556) and the TSS of sapota juice (20.3) is high. Microbiological analysis includes the isolation and identification of bacteria. The total colony forming units was found high in mixed fruit juices on an average of  $8.5 \times 10^6$  cfu/ml where as *E. coli* count was found high in grape with  $1.5 \times 10^4$  cfu/ml and pineapple juices with  $1.0 \times 10^6$  cfu/ml and *Staphylococcus* was found high in sapota juice with  $1.9 \times 10^4$  cfu/ml. Isolated bacteria were identified by using biochemical tests and gram staining.

Key words: Fruit juices, pH, Titrable acidity, TSS and Colony forming units



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**IMPORTANCE OF GUT BACTERIA IN HEALTH****\*K.RAJENDRA, D.ESWAR TONY, RAMA RAO NADENDLA, S.T.V.RAGHAVAMMA***Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur 522 034*

All of us carry trillions of bacteria in our gastrointestinal tract which perform many critical functions including digesting food and helping us to absorb vitamins and medications. Most research on the human microbiota focuses on the microorganisms in the gut, as they are thought to influence the health in various ways. In the gut lumen, the microorganisms play a crucial role in the development of robust and balanced immune system. Alterations in an individual's gut microbiota, which can happen when taking certain antibiotics for example can increase the risk of infections with opportunistic pathogens such as *clostridium difficile*. Although animal models provide interesting insights, no direct conclusions can be drawn about such associations in humans. This area of research is fairly new and more studies, in particular in humans, are needed to understand how and to what extent the composition of microorganisms in the gut influences various metabolic functions in the body. Prebiotics are non digestible food components that are selectively used by gut bacteria for fermentation. This means that bacteria associated with beneficial health outcomes can be specifically targeted. There is good evidence that prebiotics can induce changes in the gut microbiota, but it is still unclear exactly how the use of prebiotics can alter the composition and function of the gut microbiota, how stable these changes are, and what those changes mean for human health and this will need to be investigated further.

**Keywords:** Gut microbiota, prebiotics, immune system, clostridium difficile, antibiotics.

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**BIO-DEGRADATION AND BIO-REMEDIATION****G.VINEETHA, S.T.V.RAGHAVAMMA, RAMA RAO NADENDLA***Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur*

Bio-degradation refers to the conversion of complex toxic organic molecules to simple non toxic compounds whereas bio-remediation involves the removal of environmental pollutants by microbes. Bio-remediation involves bio venting , bio leaching , rhizo filtration , bio stimulation , phyto remediation , composting , bio reactors. This bio-degradation is mainly applied in treatment of oil spills .Oil spills are the most dangerous environmental hazards that impinge on many aquatic life and these are cleaned by biological, chemical and mechanical methods .Among all these techniques biological method is efficient one that involves conversion of complex hydrocarbons to simpler fatty acids and carbon- di -oxide. The most vital organisms used in this bio degradation process are the Pseudomonas, corynebacterium, arthobacter , mycobacterium , nocardia . Even though bio-degradation is slow process it has great beneficial effects in case of oil spills, rubber decomposition. Finally we conclude that the most economical and eco-friendly method for reduction of toxic compounds to simpler non toxic compounds in nature is the bio-degradation process.

Key words: oil spill; bio venting; rhizo filtration; pseudomonas; artobacter; mycobacterium; bio reactor; nocardia; bio augmentation;



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## ANTIBIOTIC SENSITIVITY TESTING ON MILK, BUTTERMILK, GLUCOSE WATER, COCONUT WATER

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There are so many bacteria which cause infections in organisms. So it will be essential for us to find out the antibiotics that would be effective against a particular antibiotic. This is done by antibiotic sensitivity testing. There are various methods for this purpose. Antibiotic sensitivity explains the susceptibility of bacteria to various antibiotics

Antibiotics play a major role in the treatment or prophylaxis of any infection. Antibiotics are categorized as bactericidal, if they kill bacteria or bacteriostatic, if they reversibly inhibit the growth of bacteria. The antibiotics for treatment are selected after performing Antibiotic susceptibility Test (AST). This test is usually done by KIRBY-BAUER method. In this method the antibiotic impregnated discs are used to test the susceptibility of any bacterial strain to a specific antibiotic.

Certain bacteria show resistance to one or more antibiotics. It is very important to know the survival of a bacterium in the presence of an antibiotic before suggesting for an infectious disease. Bacterial pathogens are tested for their susceptibility to antibiotics to guide antibiotic treatment. Sensitivity test are generally performed from single pure bacterial colonies on an agar plate.

In Kirby-bauer method, antibiotic diffuses out of a disc placed on the surface of the agar. Upon incubation, if bacteria are sensitive then the test organism is unable to grow immediately around the disc showing a zone of inhibition.

The main objective of our project is to identify the appropriate nutrient solution(s) that can be suggested while using a particular antibiotic.

Keywords: Antibiotic, Microbes, Kirby-bauer, Antibiotic Susceptibility Test

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## ROLE OF INDUSTRIES IN ENVIRONMENTAL PROTECTION & POLLUTION CONTROL

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### INTRODUCTION

The natural environment commonly referred to simply as the **environment**, is all living and non-living things that occur naturally on Earth or some part of it (e.g. the natural environment in a country). This includes complete ecological units that function as natural systems without massive human intervention, including all vegetation, animals, microorganisms, and natural phenomena that occur within their boundaries. And it includes universal natural resource and physical phenomena that lack clear-cut boundaries, such as air, water, and climate, as well as energy, radiation, electric charge, and magnetism not originating from human activity.

### Environmental protection

Environmental protection is a practice of protecting the natural environment on individual, organisation controlled or governmental levels, for the benefit of both the environment and humans.

Due to the pressures of over consumption, population and technology, the biophysical environment is being degraded, sometimes permanently. This has been recognized, and governments have begun placing restraints on activities that cause environmental degradation. Since the 1960s, activity of environmental movements has created awareness of the various environmental issues. Division of the biosphere is the main government body that oversees protection. It does this through the formulation of policy, coordinating and monitoring environmental issues, environmental planning, and policy-oriented environmental research. The National Environment Management Council (NEMC) is an institution that was initiated when the National Environment Management Act was first introduced in year 1983. This council has the role to advise governments and the international community on a range of environmental issues.

The NEMC the following purposes:

- provide technical advice;
- coordinate technical activities;
- develop enforcement guidelines and procedures;
- assess, monitor, and evaluate activities that impact the environment;
- promote and assist environmental information and communication; and
- seek advancement of scientific knowledge

### Environmental Pollution:

Pollution is the introduction of contaminants into the natural environment that cause adverse change. Pollution can take the form of chemical substances or energy such as noise, heat or light, pollutants. The components of pollutants can be either foreign substances/energies or naturally occurring contaminants. Pollution is often classed as point source or nonpoint source pollution.

### Industrial pollution:



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Industrial pollution is the contamination of the environment by businesses, particularly plants and factories, that dump waste products into the air and water. Industrial waste is one of the largest contributors to the global pollution problem endangering people and the environment

#### Causes of industrial pollution:

- 1. Lack of Policies to Control Pollution:** Lack of effective policies and poor enforcement drive allowed many industries to bypass laws made by pollution control board which resulted in mass scale pollution that affected lives of many people.
- 2. Unplanned Industrial Growth:** In most industrial townships, unplanned growth took place wherein those companies flouted rules and norms and polluted the environment with both air and water pollution.
- 3. Use of Outdated Technologies:** Most industries still rely on old technologies to produce products that generate large amount of waste. To avoid high cost and expenditure, many companies still make use of traditional technologies to produce high end products.
- 4. Presence of Large Number of Small Scale Industries:** Many small-scale industries and factories that don't have enough capital and rely on government grants to run their day-to-day businesses often escape environment regulations and release large amount of toxic gases in the atmosphere.
- 5. Inefficient Waste Disposal:** Water pollution and soil pollution are often caused directly due to inefficiency in disposal of waste. Long term exposure to polluted air and water causes chronic health problems, making the issue of industrial pollution into a severe one. It also lowers the air quality in surrounding areas which causes many respiratory disorders.
- 6. Leaching of Resources from Our Natural World:** Industries do require large amount of raw material to make them into finished products. This requires extraction of minerals from beneath the earth. The extracted minerals can cause soil pollution when spilled on the earth. Leaks from vessels can cause oilspills that may prove harmful for marine life.

#### Effects of Industrial Pollution:

- 1. Water Pollution:** The effects of industrial pollution are far reaching and liable to affect the eco-system for many years to come. Most industries require large amounts of water for their work. When involved in a series of processes, the water encounters heavy metals, harmful chemicals, radioactive waste, and even organic sludge.

These are either dumped into open oceans or rivers. Thus, many of our water sources have high amount of industrial waste in them which seriously impacts the health of our eco-system. The same water is then used by farmers for irrigation purpose which affects the quality of food that is produced.

Water pollution has already rendered many ground water resources useless for humans and wildlife. It can at best be recycled for further usage in industries.

- 2. Soil Pollution:** Soil pollution is creating problems in agriculture and destroying local vegetation. It also causes chronic health issues to the people that encounter such soil on a daily basis.
- 3. Air Pollution:** Air pollution has led to a steep increase in various illnesses and it continues to affect us on a daily basis. With so many small, mid and large scale industries coming up, air pollution has taken toll on the health of the people and the environment.
- 4. Wildlife Extinction:** By and large, the issue of industrial pollution shows us that it causes natural rhythms and patterns to fail, meaning that the wildlife is getting affected in a severe manner. Habitats are being lost, species are becoming extinct and it is harder for the environment to recover from each natural disaster. Major industrial accidents like oil spills, fires, leak of radioactive material and damage to property are harder to clean-up as they have a higher impact in a shorter span of time.



**5. Global Warming:** With the rise in industrial pollution, global warming has been increasing at a steady pace. Smoke and greenhouse gases are being released by industries into the air which causes increase in global warming. Melting of glaciers, extinction of polar bears, floods, tsunamis, hurricanes are few of the effects of global warming.

The **issue of industrial pollution** concerns every nation on the planet. As a result, many steps have been taken to seek permanent solutions to the problem. Better technology is being developed for disposal of waste and recycling as much polluted water in the industries as possible. Organic methods are being used to clean the water and soil, such as using microbes that naturally uses heavy metals and waste as feed. Policies are being pushed into place to prevent further misuse of land. However, industrial pollution is still rampant and will take many years to be brought under control.

**Pollution control:**

**Control of Industrial Pollution:**

The ultimate object behind the measures to control pollution to maintain safety of Man, Material and Machinery (Three Ms). The implementation of control measures should be based on the principle of recovery or recycling of the pollutants and must be taken as an integral part of production i.e. never as a liability but always an asset.

**Some important control measures are:**

**1. Control at Source:**

It involves suitable alterations in the choice of raw materials and process in treatment of exhaust gases before finally discharged and increasing stock height upto 38 metres in order to ensure proper mixing of the discharged pollutants.

**2. Selection of Industry Site:**

The industrial site should be properly examined considering the climatic and topographical characteristics before setting of the industry.

**3. Treatment of Industrial Waste:**

The industrial wastes should be subjected to proper treatment before their discharge.

**4. Plantation:**

Intensive plantation in the region, considerably reduces the dust, smoke and other pollutants.

**5. Stringent Government Action:**

Government should take stringent action against industries which discharge higher amount of pollutants into the environment than the level prescribed by Pollution Control Board.

**6. Assessment of the Environmental Impacts:**

Environmental impact assessment should be carried out regularly which intends to identify and evaluate the potential and harmful impacts of the industries on natural eco-system.

**7. Strict Implementation of Environmental Protection Act:**

Environment Protection Act should be strictly followed and the destroyer of the environment should be strictly punished.

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## ROLE OF INDUSTRIES IN ENVIRONMENTAL PROTECTION AND POLLUTION CONTROL

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All the constituents of environment like all organisms such as plants animals and human beings dependent upon each other. Thus they maintain a balance in nature. The environmental imbalance gives rise to various environmental problems such as pollution, soil erosion leading to floods, salt deserts and sea recedes, desertification, landslides, change of river directions, extinction of species, and vulnerable ecosystem in place of more complex and stable ecosystems, depletion of natural resources, waste accumulation, deforestation, thinning of ozone layer and global warming. The environmental problems are visualized in terms of pollution, growth in population, development, industrialization, unplanned urbanization etc. This lead to traffic congestion, water shortages, solid waste, and air, water and noise pollution are common noticeable problems. Environmental protection through pollution control, is also receiving administrative and legislative support. To boost the existing environment-protection movement, greater emphasis is urgently needed for environmental education, peoples' participation, population control, and cost-effective pollution control measures by the industries. Industries play a major role in environmental protection through pollution control.

**Key words:** environment; industries. Protection.

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## ROLE OF INDUSTRIES IN ENVIRONMENTAL PROTECTION AND POLLUTION CONTROL

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Industrial Pollution affects the air, water, and all the environments. Industrial pollution began when our country went through the industrial revolution. All parts of the world are affected in some way by industrial pollution. For the daily needs of the growing population, different types of industries are setup by produce different products.

Till now, there are about 17 industries which are declared to be most polluting. These include caustic soda , cement , distillary , dyes and dye intermediaries , fertilizers, iron and steel , oil refineries , paper and pulp , pesticides and pharmaceuticals , sugar, text tiles , thermal power plants, tanneries and so on.

Due to industrial activities, a variety of poisonous gases like NO, SO<sub>2</sub>, SO<sub>3</sub>, Cl<sub>2</sub>, CO, CO<sub>2</sub>, H<sub>2</sub>SO<sub>4</sub> etc are liberated into the atmosphere causing acute pollution problems. Methyl isocyanate gas leakage from union carbide factory at Bhopal in 1984 is the great tragedy and caused mass killing of many living organisms.

Environmental engineering involves water and air pollution control, recycling, waste disposal etc.

The environment protection act was enacted in 1986 by the Govt. with the objective of providing for protection and improvement of the environment. Anthropogenic activities are important for preservation and conservation of environment protection.

**Key Words:** Pollution , Pollutants , Industries , Environmental acts , Anthropogenic activities.



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**ROLE OF INDUSTRIES IN ENVIRONMENTAL PROTECTION AND POLLUTION CONTROL****K. GIRIDHAR, S.T.V.RAGHAVAMMA, RAMA RAO NADENDLA***Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, Andhra Pradesh, India.*

All the constituents of environment like all organisms such as plants animals and human beings dependent upon each other. Thus they maintain a balance in nature. As we are the only organisms try to modify the environment to fulfill our needs; it is our responsibility to take necessary steps to control the environmental balance. The environmental imbalance gives rise to various environmental problems such as pollution, soil erosion leading to floods, salt deserts and sea recedes, desertification, landslides, change of river directions, extinction of species, and vulnerable ecosystem in place of more complex and stable ecosystems, depletion of natural resources, waste accumulation, deforestation, thinning of ozone layer and global warming. The environmental problems are visualized in terms of pollution, growth in population, development, industrialization, unplanned urbanization etc. This lead to traffic congestion, water shortages, solid waste, and air, water and noise pollution are common noticeable problems. Environmental protection through pollution control, is also receiving administrative and legislative support. To boost the existing environment-protection movement, greater emphasis is urgently needed for environmental education, peoples' participation, population control, and cost-effective pollution control measures by the industries. Industries play a major role in environmental protection through pollution control.

Key words: industry; environment; protection

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**ROLE OF INDUSTRIES IN ENVIRONMENTAL PROTECTION AND POLLUTION CONTROL****M. VIJAYA LAKSHMI***Lecturer in English, Govt. College for Women(A),Guntur*

Environmental protection is a practice of protecting the natural environment on individual, organisation controlled or governmental levels, for the benefit of both the environment and humans. Due to the pressures of over consumption, population and technology, the biophysical environment is being degraded, sometimes permanently. This has been recognized, and governments have begun placing restraints on activities that cause environmental degradation. Since the 1960s, activity of environmental movements has created awareness of the various environmental issues. There is no agreement on the extent of the environmental impact of human activity and even scientific dishonesty occurs, so protection measures are occasionally debated.

In industrial countries, voluntary environmental agreements often provide a platform for companies to be recognized for moving beyond the minimum regulatory standards and thus support the development of best environmental practice. For instance, in India, Environment Improvement Trust (EIT) has been working for environment & forest protection since 1998. A group of Green Volunteers get a goal of Green India Clean India concept.



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## ROLE OF INDUSTRIES IN ENVIRONMENTAL PROTECTION AND POLLUTION CONTROL

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**Abstract:** Environmental pollution is a global environmental problem. In India, it is assumed threatening proportions mainly due to poverty, continuing forest devastation, the negative impact of economic development and sheer greed. Since independence in 1947, the Indian ecosystem has received some major setbacks as a result of haphazard industrial and urban development. Of the country's 304 hectares 50% are subject to ecological degradation. About 80% of the population lives under substandard conditions. The 14 major rivers, including the Ganga, which provide nearly 85% of the country's drinking water are all polluted. City dwellers breathe clean air for about 2 hours in the morning. Human diseases caused by contaminated food have doubled during the last 30 years. Over 80% of all hospital patients are the victims of environmental pollution.

Pollution from industrial installations and processes takes many forms. Reducing pollution is important. Environmental health practitioners work with industry operators to apply the best available techniques to reduce the amount of pollution caused by thousands of 'regulated factors', in order to protect the environment and minimize the risks to public health. The Environmental Agency also plays a part in controlling pollution from larger industrial sources.

**Key words:** environmental pollution, haphazard, health, pollution, industry, human, disease, contaminate

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*In loving memory of Mrs. Nannam Sujatha M.A., B.Ed., M.Phil.,  
Lecturer in Government College for Women (Autonomous), Guntur*



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“**Lady Hope College**” was started in 1942 under the management of Guntur Municipality, for Women Students in and around Guntur with constant encouragement from social reformers. Later it was taken over by the Government of the then composite Madras State in 1947 and was renamed as Government College for Women, Guntur. The College was recognized by UGC on 01-06-1956. Presently the College is affiliated to Acharya Nagarjuna University. The college has been bifurcated into Junior College and Degree College in 1996. Today **The Government College for Women**, with **NAAC “A” grade** caters to the academic needs of 1894 Girl Students, offering 20 Under Graduate Courses and 3 Post Graduate Courses with 82 Faculty Members, and 50 Non Teaching and Support Staff. The College has been conferred with Autonomy by the University Grants Commission from 2013-'14 and recognized as Government College for Women at Guntur as **College with Potential for Excellence**.

The motto of this college is “**Thamasoma Jyothirgamaya**”

Conceptual Promotion of College in Autonomy is to “**Enter:: Empower :: Excel**”

## **Major Achievements and Mile Stones:**

- Government College for Women (Autonomous) has been recognized by the UGC in April 2016 as “College with Potential for Excellence (CPE)”
- At the National Level this college has been maintaining higher standards and Re-Accredited with “A” Grade by NAAC in 2011 with CGP 3.03.
- NAAC Accreditation was given in grade B++ in the year 2004.
- From 2014 academic year, this college has become Autonomous.
- Excellence Award in Academics in 2013-14 at State Level.
- Excellence Award in Academics in 2011-12 at A.P., State Level
- Excellence Award in Cultural Events in 2012-13 at A.P., State Level.
- State Level Best Student Study Projects in the last 3 years.

This college is in process of establishing herself as one of the best and as pioneer in Innovation and Creativity Centres to unfold the core potential of Girls of Guntur District and empower them to stand unique in the intensely competitive world. This College pursuing the path of Excellence is organizing National Level Workshops and Seminars on the topics of Academic and Social relevance.

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