ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 2, Issue 4, pp: (105-128), Month: October - December 2014, Available at: www.researchpublish.com

# A Comparative Study on the Endosulfan Degradation Efficacy of Bacillus Sp. and Pseudomonas Sp., Isolated From Pesticide Polluted Soil

Shilpa Kuttikrishnan

## 1. INTRODUCTION

For centuries, humans believed that atmospheric, terrestrial and aquatic systems were sufficient to absorb and breakdown wastes from population centres, industries and farming. Today there are two problems related to wastes. First regarding the disposal of large quantities of xenobiotic wastes. Second, regarding the removal of toxic waste accumulating at dump sites, in the soil and in water systems.

Xenobiotic being beneficial it is being widely used by modern man without considering about its harmful effects.The term "xenobiotics"refers to artificial compounds that are foreign to biological systems and contain structures and bonds that are do not occur in biological systems.Xenobiotics may be polymers,gases,polychlorinated or polybrominated compounds or pesticides**.** A very common example of xenobiotic is pesticide.The chemicals used to control all kinds of pests are known as pesticides.The word pesticide is a general term that includes insecticide, herbicide and algicides.

From the historical perspective different natural substances have been used as pesticides. Initially salts of metal, sulphur, natural oils and tobacco products were utilized.During the last 50years or so, the chemical synthesis of pesticides has increased considerably. One of the main contribution of pesticides is to control the spread of diseases by arthropods and other vectors. Outbreaks of malaria, lowseborne typhus, plaque and urban yellow fever are controlled by an organochlorine DDT.

When new pests as well as increased usage of them were introduced in the late 1970's, a fundamental shift in agricultural practice was also introduced .Insecticide application are often needed to kill the insect that are formerly easily controlled through rotation.In addition,the farmers must now depend on synthetic fertilizer rather than rotation to maintain soil nutrients.

Other problem resulting from pesticide use is the phenomenon of pest resistance.In 1938,scientist knew that mites that are resistant to pesticide.But by 1984,this number escalated to a shocking 447 species of insects and mites.As problem with broad scale use of pesticide as the sole method of control increased ,a new ecologically oriented concept was introduced to lessen these problems.This concept known as integrated pest management(IPM),relies on natural enemies of pests to eliminate pest problems.

Pesticides may be classified according to either target organisms like insecicides,fungicides and herbicides and if they are gaseous nature,they are called fumigants.Chlorinated hydrocarbon compound discovered by Paul Hermann Muller and was the earliest as "wonder chemical" in the year 1938 provided greatest benefits to humans in the protection of agricultural production and harvest and ongoing programmes of public health also received a tremendous boost to combat vector borne epidemics. DDT and many other pesticide of this group such as aldrin, lindane, endosulfan etc have been introduced which are essentially stomach poison for the insects and pests.The remarkable biological persistence combined with great lipophility leads to accumulation of residue of these insecticides in food chains human adipose fat **(**Burger et al.,1995**).**

In India, the annual pesticide consumption has increased by 20-25 folds during the past over three decades and 8% increase is expected every year. Currently 400 members of three groups of pesticides are being used in India.

Since most pesticides are toxic in nature, their continuous ingestion by man, animals, avian etc even though in very minute quantities can lead to accumulation in body tissue causing adverse effects such as metabolic aberration, carcinogenesis, autogenesis, immunosuppression etc. several factors are known to influence the pesticide in soil. They include chemical decomposition, phytochemical decomposition, volatilization, plant uptake, absorption and microbial decomposition.

A problem which arises particularly in the case of chlorinated hydrocarbon is their susceptibility to biomagnification.it is also known as bioamplification or biological magnification. This is an increasing concentration of insecticide in organisms at higher trophic levels, as these toxins accumulate in the body tissues and cannot be denatured or metabolised. This results in the increasing concentration of the insecticide at each step in the food chain until the top predators suffer very high doses. The mechanism by which chlorinated hydrocarbons exert their toxic effects on organism is not known with certainity. They dissolve in the fatty membrane surrounding nerve fibre and interfere with the transport of ions in and out of the fibre. This leads to nerve impulse transmission which in turn results in tremors, convulsions and death(A.K.DE,1987).

Biodegradation refers to the process in bioremediation by which xenobiotics are transferred to a less toxic state (Shelly Sinha, 2009). Mineralization usually means decomposition of a xenobiotic to inorganic ions and carbondioxide.This is the most desirable situation because the end products are usually non-toxic.

There are several types of insecticides. For example DDT, Lindane, Aldrin, Dieldrin Heptachlor, Endosulfan, Chlordane, Parathion and Diazinon (J.M.Vargas,1975).

Endosulfan (6,7,8,9,10,10 -hexachloro -1, 5, 5 a, 6, 9a-hexahydro -6, 9- methano -2, 3, 4- benzodioxathiepin-3-oxide) is a cyclodiene insecticide,recalcitrant in nature under the name thionex,thidan and benzoepin.Endosulfan is a mixture of two isomers, alpha and beta isomers.It is sometimes called a ‘chlorinated hydrocarbon of the cyclodiene group’(Rainer Martens,1976).Due to its persistant nature,its entry in ecosystem via food chain and produce harmful effects to plants, animals and even human beings.

Endosulfan is usually sprayed in the agricultural setting to protect crops and can also be used as a wood preservative. Endosulfan does not dissolve easily in water but sticks to particles or soil.It breaks down slowly and may accumulate in organism that are exposed to it.It is toxic to workers when improperly handled and can disrupt wildlife. It is neuro toxic to rats in the laboratory settings and considered to be an endocrine disrupter, an xenoestrogen. Endosulfan has been detected in the atmosphere, soils, sediments, surface waters, rain water and food stuff (Seth W.Kullman, 1995).

Stimulation of the central nervous system is the major characteristics of endosulfan poisoning.Symptoms in actualy exposed individuals include incoordination, imbalance, and difficulty in breathing, gagging, vomiting, diarrhoea, convulsion, and loss of consciousness. Organs like kidneys, liver, blood and parathyroid gland are also affected.

Endosulfan is degraded by soil microorganisms Pseudomonas sp, Arthrobacter sp and Bacillus sp**.** These organisms detoxify/degrade endosulfan to different intermediates and thus reduce its toxicity in the environment(Osama EI Gialani Elsaid et al.,2010).Earlier reports have confirmed that some strains of Pseudomonas sp.and mixed consortium of microbes are able to transfer to its non toxic metabolities.But the actual interconversion and the rate of reaction could not be detected as the interconversion if once started, is very fast. Although it is not yet confirmed that these organism can metabolise endosulfan as sole source of carbon and energy source but the interconversion of endosulfan has been reported. The major metabolites are endosulfan sulphate and endosulfan diol (R.Jayashree, 2007).

In 2001, in Kerala, India, endosulfan spraying became suspect when linked to a series of abnormalities noted in local children.Endosulfan is a highly toxic pesticide in EPA toxicity class 1.It is a Restricted Use Pesticide (RUP).Labels for products must bear the signal words DANGER-POISON, depending on formulation. In 2010, in Kerala, India, the complete use of endosulfan in agricultural fields has been banned due to its toxic effects in children.

This project involves a preliminary experiment, how endosulfan is degraded by the pesticide resistant bacteria-Pseudomonas sp. and Bacillus sp.

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 2, Issue 4, pp: (105-128), Month: October - December 2014, Available at: www.researchpublish.com

## 2. OBJECTIVES

1) To isolate and identify the pesticide resistant bacteria from endosulfan polluted soil.

2) To determine the pesticide degradation capacity of bacteria from the soil.

3) To compare the pesticide degradation capacity of *Bacillus* sp and *Pseudomonas* sp.

## 3. REVIEW OF LITERATURE

***XENOBIOTICS:***

Xenobiotics are those compounds that are alien to a living individual and have a propensity accumulate in the environment. Principal xenobiotics include pesticides, fuels, solvents, alkanes, polycyclic hydrocarbons (PAHs), antibiotics, synthetic azo dyes, pollutants (dioxins and polychlorinated biphenyls), polyaromatic, chlorinated and nitro-aromatic compounds. The main concern with xenobiotic compounds is the toxicity threat they pose to public health. It is quite shocking that some xenobiotic compounds (phenols, biphenyl compounds, phthalates, etc.) act as endocrine disruptors (Nagao, 1998; Borgeest *et al*., 2002).

***PESTICIDES:***

Pesticides are good example of xenobiotics.They are substances or a mixture of substances,of chemicals or biological origin,used by human society to mitigate or repel pests such as bacteria, nematodes, insects, mites, mollusks,birds, rodents, and other organism that affect food production or human health.They usually act by disrupting some component of the pest's life processes to kill or inactive it. In a legal context, pesticides also include substances such as insect attractants, herbicides, plant defoliants and plant growth regulators.

Some of the pesticides include endosulfan, DDT, lindane,aldrin etc.Pesticides have vastly increased in agricultural production but their residual accumulations in soil and transport to other components of the environment have stimulated research on their biodegrability.The liberal use of pesticides had a negative impact on the local terrestrial and aquatic environment.(Reynold Murry *et al*.,1996)

Pesticides,decreasing agents,organic solvents etc contain large amount of short chain halogenated aliphatic hydrocarbons and this caused numerous cases of environmental pollution due to improper disposal of wastes, accidental spillage or deliberate release and cause contamination of soil, underground water and surface water.

The contamination of food materials with pesticide is one of the major problem for man.The consumer runs the greater risk of exposure to pesticides through the contaminated food.The intake of pesticide through food and other sources results in the accumulation in the body tissues of human beings.It has been shown that these chemicals can be transferred from mother to foetus so that babies may be born with insecticides in their tissues.(Singh and Dhaliwal G.S., 1992).

Pesticides which are readily degraded in the environment are called non-persistent in comparison with those which are not readily degraded, which are called persistent.

**List of persistent and non-persistent pesticides**

| | PERSISTANT | NON-PERSISTANT |
|---|---|---|
| Insecticides | DDT | Methoxychlor |
| | Aldrin | Sevin(carbaryl) |
| | Dieldrin | Malathion |
| | Chlordane | Lindane |
| Fungicides | PMAS | Benlate |
| | Calo-Clor | Mancozeb |
| | Cadmium | Zineb |
| | | Captan |

| | | |
|---|---|---|
| Herbicide | Simazine | Paraquot |
| | Turbacil | Dalapon |
| | Tordon | Dacthal |
| | | Treflan |

***BIOMAGNIFICATION:***

Biomagnification, also known as bioamplification or biological magnification, is the increase in concentration of a substance, such as the pesticide DDT, that occurs in a food chain as a consequence of:

- Persistence (can't be broken down by environmental processes)
- Food chain energetics.
- Low (or nonexistent) rate of internal degradation/excretion of the substance (often due to water-insolubility).

Biological magnification often refers to the process whereby certain substances such as pesticides or heavy metals move up the food chain, work their way into rivers or lakes, and are eaten by aquatic organisms such as fish, which in turn are eaten by large birds, animals or humans. The substances become concentrated in tissues or internal organs as they move up the chain. Bioaccumulants are substances that increase in concentration in living organisms as they take in contaminated air, water, or food because the substances are very slowly metabolized or excreted.

Although sometimes used interchangeably with 'bioaccumulation,' an important distinction is drawn between the two, and with bioconcentration, it is also important to distinct between sustainable development and over exploitation in biomagnification.

- Bioaccumulation occurs within a trophic level, and is the increase in concentration of a substance in certain tissues of organisms' bodies due to absorption from food and the environment.
- Bioconcentration is defined as occurring when uptake from the water is greater than excretion .Thus bioconcentration and bioaccumulation occur within an organism, and biomagnification occurs across trophic (food chain) levels.

Biodilution is also a process that occurs to all trophic levels in an aquatic environment; it is the opposite of biomagnification, thus a pollutant gets smaller in concentration as it progresses up a food web.

***BIODEGRADATION:***

Biodegradation is one of the natural processes that help to remove xenobiotic chemicals from the environment by microorganisms. This is primarily a strategy for the survival of the microorganisms (Singh, 2008). It is one of the most cost-effective methods amongst remedial approaches. Although most organisms have detoxifying abilities (that is, mineralization, transformation and or immobilization of pollutants), microorganisms, particularly bacteria, play a crucial role in biogeochemical cycles for sustainable development of the biosphere (Tropel and Van der Meer, 2004). Horizontal gene transfer, high growth rates and metabolic versatility make them to evolve quickly and to adapt themselves to changing conditions of environment, even at extreme environment that does not permit the proliferation of other living organisms (Timmis and Pieper, 1999; Diaz and Prieto,2000; Kim and Crowley, 2007). A large number of microbial communities have been characterized with their responses to pollutants, in order to identify the potential bacterial degrader that can adapt to use these chemicals as their novel growth and energy substrates (Janssen *et al*., 1985).

Microbial degradation of petroleum and other hydrocarbons is incredibly an intricate course of action that mainly depends on the composition of community and its adaptive response to the presence of these compounds. Such degradation of petroleum in marine sites is restricted principally by the availability of phosphorus and nitrogen. Apart from this pH, moisture, oxygen and temperature are prime factors influencing the degradation rate (Leahy and Colwell, 1990). The biological treatment of xenobiotic wastes in the conventional activated sludge process varies under different conditions (Widada *et al*.,2002). Relevance of current progress in molecular techniques has to be identified for isolation of plasmid DNA, construction of DNA probes in recent perspective to explore the efficient genes implicated in catabolism of xenobiotics and to study the genetic diversity of environmentally significant microbes.

Co-metabolism is another exclusive phenomenon; it appears to occur widely in microbial metabolism. Here, the microorganisms transform the desired xenobiotic compound even though the compound itself cannot serve as the primary energy source for those organisms. To degrade the contaminant, the microbes require the presence of other compounds (primary substrates) that can support their growth. The enzymes or coenzymes produced to degrade the primary substrate may display some activity for other substrate which is significantly known as cosubstrate. The co-substrate is not the physiologically intended substrate but is just 'accidentally' transformed or in other words, the process occurs in a much randomized manner (Horvath, 1972).

The pesticides are subjected to degradation processes in soil which, depending on the physico-chemical behavior of the active ingredient and the prevailing soil and climatic conditions, lead to different mineralization and fixation rates in the soil. The degradation process determines the persistence of a pesticide in the soil environment and consequently its performance and also its impacts on the environment (Pennington *et al.*, 2004).

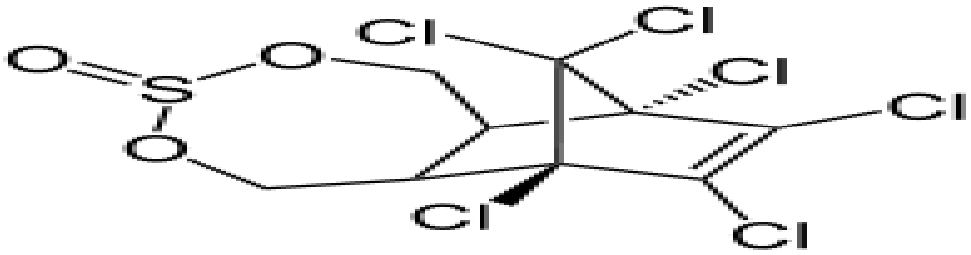
The breakdown of pesticides in soil through the activity of micro organisms require all conditions necessary for good microbial growth in soil. Some pesticides are not complete microbial foods, this means that the other nutrients necessary for microbial growth must be present in sufficient amounts as well as adequate moisture. The presence of other organic compounds in soil often promotes microbial action on pesticides by providing additional source of food for microbial growth. It should be emphazied that only a restricted range of microbial species are able to break down a given pesticide. When a pesticide first used in a given soil, the proper inoculum probably is ambient, so that initial biodegradation is very slow, but gradually, a population adapted to the compound build up in the soil so that biodegradation subsequently proceeds much more rapidly.

Fensulfothion, an organophosphate pesticide extensively used for the control of golden nematode of potato for several years in the Nilgris , India at a very high doses at high elevations was found very persistant in soil, but disappears within a few days to months due to microbial degradation.

***ENDOSULFAN:***

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzo(e)dioxathiepin-3-oxide) is a chlorinated cyclodiene insecticide classified under the category of persistent organic pollutant (POP). Technical-grade endosulfan is a mixture of two stereoisomers, alpha endosulfan and beta endosulfan in a ratio of 7:3. The solubility of endosulfan in water is low, but it persists in soil and water environment for 3 to 6 months or more (Rao and Murty, 1980; Kathpal *et al.*, 1997). Endosulfan is ubiquitous and environmentally persistent, as a result the presence of endosulfan residues were traced in surface water, groundwater, atmosphere, and water bodies by many researchers (Sujatha *et al.*,1999; Bhattacharya *et al.*, 2003; Berrakat *et al.*, 2002; Cerejeira *et al.*, 2003; Golfinopoulos *et al.*, 2003). Many countries imposed ban on endosulfan production and/or usage, whereas in India it is still one of the priority pollutant for pest control.

***CHEMICAL STRUCTURE:***




***MOLECULAR FORMULA:***

C9 H6 Cl6 O3 S

***IUPAC NAME:***

6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-

methano-2,4,3-benzodioxathiepin-3-oxide

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 2, Issue 4, pp: (105-128), Month: October - December 2014, Available at: www.researchpublish.com

***OTHER NAMES:***

Benzoepin, Endocel, Parrysulfan, Phaser, Thiodan, Thionex

***USES OF ENDOSULFAN:***

Endosulfan is a contact and stomach poison that has been used to control insects such as the Colorado potato beetle, flea beetle, cabbage worm, peach tree borer and tarnished plant bug as well as several species of aphid and leaf hopper. It is used in countries throughout the world to control pests on fruit, vegetables, tea, and on non-food crops such as tobacco and cotton. (WHO,1968).

***TOXIC EFFECTS:***

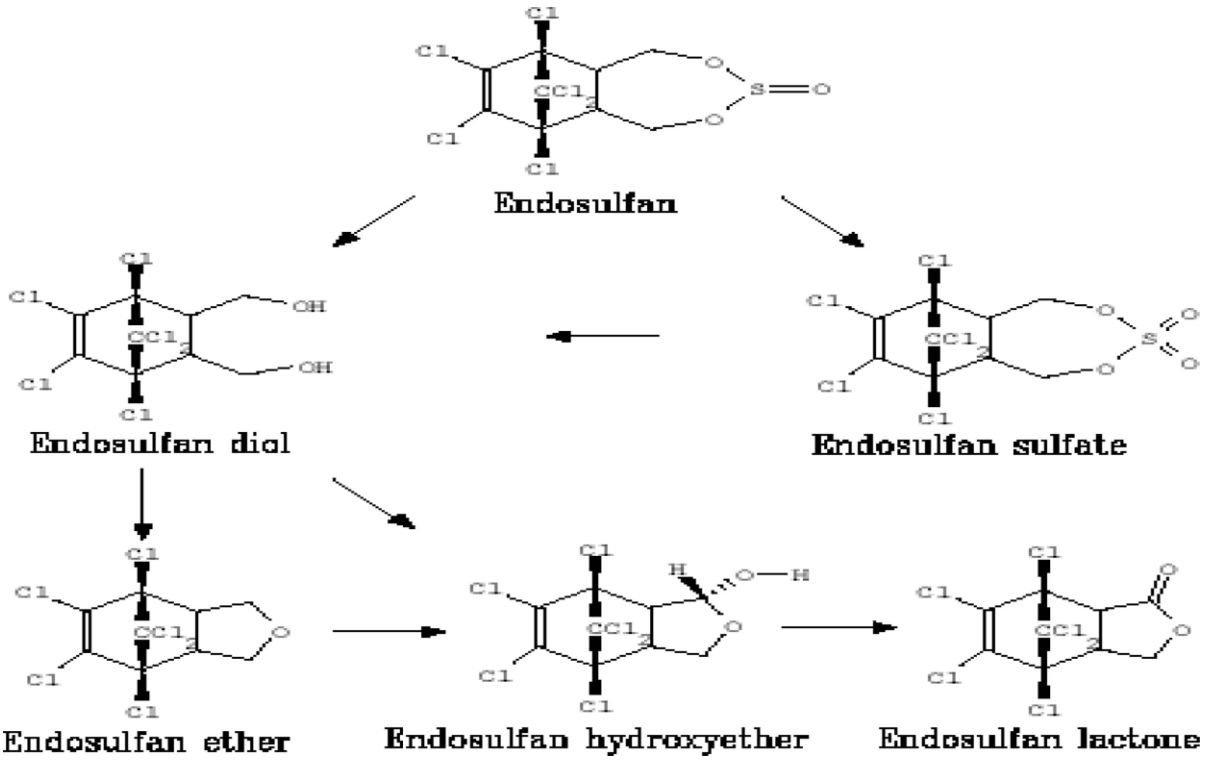
Endosulfan is acutely neurotoxic to both insects and mammals, including humans.Endosulfan is also a xenoestrogen—a synthetic substance that imitates or enhances the effect of estrogens—and it can act as an endocrine disruptor, causing reproductive and developmental damage in both animals and humans. It is extremely toxic to auatic fauna(Sunderam *et al*.,1992)while provoking chronic symptoms like testicular and prostrate cancer(Saiyed *et al*.,2003)breast cancer and sexual abnormality(Arnold *et al*.,1996) genotoxicity(Chaudhari *et al*.,1999) and neurotoxicity (Paul and Balasubramaniam.,1997) in numerous mammalian species.

***SYMPTOMS:***

Symptoms of acute poisoning include hyperactivity, tremors, convulsions, lack of coordination, staggering, difficulty breathing, nausea and vomiting, diarrhea, and in severe cases, unconsciousness.

***MICROBIAL DEGRADATION:***

Many bacteria and fungi including *Cornybacterium sp., Nocardia sp., Mycobacterium sp., Pseudomonas fluorescens, Penicillium sp., Aspergillussp., Phanerochaete chrysosporium* have been reported to be endosulfan degraders. (Kullmann and Matsumura, 1996*)*. Endosulfan could be degraded by attack on the sulfite group by oxidation and or hydrolysis to form the toxic endosulfan sulfate and the nontoxic endosulfan diol respectively(Baar and Aust, 1987).




*Schematic pathway of endosulfan degradation.*

Endosulfan can be utilized as a sole sourceof carbon and/or sulfur during biodegradation (Guerin,1999; Sutherland *et al*., 2000). The biodegradation of endosulfan can be done under aerobic and anaerobic conditions with bacterial and fungal cultures (Katayama and Matsumura,1993;Mukherjee and Gopal,1994; Awasthi *et al*., 1997; Shetty *et al.*, 2000;Siddique *et al*., 2003**).**

Bioremediation of endosulfan is more beneficial under anaerobic condition because of the faster rates of hydrolysis and dehalogenation reactions.It is reported that endosulfan detoxification in anaerobic condition occurred through two different pathways: (i)endosulfan hydrolyzed to endosulfan diol by enzymatic/chemical hydrolysis; (ii) formation of endosulfan monoaldehyde by bacterial metabolism.

Endosulfan can be degraded in about two weeks to nontoxic metabolite under anaerobic conditions (Guerin & Kennedy, 1999).Endodiol is the major degradation product in an undefined mixture of microorganisms obtained from soil suspension (Shivaramaiah and Kennedy., 2006). Degradation of endosulfan occurred in contaminant with bacterial growth when endosulfan was used as only source of sulfur in the culture, while no growth occurred in the absence of endosulfan. (Tariq *et al.,2000).*

Degradation of endosulfan can occur through both abiotic and biotic processes. The two primary reactions though which degradation occurs include oxidation by microorganisms to endosulfan sulfate, which is as toxic or more toxic than the parent compounds, and byhydrolysis (abiotic and biotic) to endosulfan diol, which is much less toxic. Hydrolysis is the dominant pathway for the degradation of endosulfan in water and is greatly dependent on the pH and temperature. Both isomers of endosulfan are susceptible to alkaline hydrolysis (Goebel *et al*.,1982). The β-isomer hydrolyzes faster, as compared to the α-isomer. This has been attributed to less steric hindrance from the S=O bond of the β-isomer structure, leading to more susceptibility for nucleophilic (OH-) to attack at the S atom. Endosulfan is reported to degrade faster in the aqueous phase than in the solid phase. Under strongly alkaline conditions the half-life of the endosulfan in the aqueous phase is approximately 1 day.

Endosulfan does not undergo direct photolysis but is transformed by chemical hydrolysis under alkaline conditions such as in seawater (Armburst,K.L.,1992). In soil, endosulfan has been shown to be degraded by a wide variety of microorganisms (Martens,M.,1976). However, degradation rates are usually low and metabolism often results in the formation of endosulfan sulfate, anoxidative metabolite shown to be equally as toxic and persistent as the parent compound, endosulfan. Because of its persistence and toxicity, endosulfan contamination poses a significant environmental concern.

Soil pH also play a vital role in the degradation process. Some of the bacteria favour a pH near neutral and some are highly tolerant to alkaline conditions. The environmental fate of endosulfan in soils is influenced by the pH, texture, moisture content and also by the presence of organic matter and co-pollutants. The rate of degradation is a function of the prevailing temperature,moisture regime, the content, type of organic matter and clays present.

Fish are very susceptible to endosulfan toxicity at a level of 1-20ng/L. Endodiol is a nontoxic metabolite to fish and other organisms; thus, hydrolysis producing endodiol may been important detoxification pathway of endosulfan. However,endosulfan sulfate has a similar toxicity compared to the parent compound endosulfan. In addition, endosulfan sulfate has amuch longer tolerance in the soil environment in comparison to endosulfan (Kennedy *et al*.,2001). Therefore, the production of endosulfan sulfate seems to cause long persistence of endosulfan in soil.Endosulfan sulfate is produced by several microorganisms including *Mucor thermohyalospora* MTCC 1384 , and *Trichoderma harzianum* , *Phanerochaete chrysosporium*.

Earthworms are usually exposed to many pesticides and other organic pollutants in the soil and their guts are known to have efficient detoxification capability with a large number of aerobic and anaerobic bacteria(Karsten and Drake, 1995), but their use in bioremediation of pesticide-contaminated soil has so far not been studied. A *Rhodococcus* strain was isolated from the gut microflora of earthworms, exposed to endosulfan-contaminated soil and degraded the endosulfan .*Rhodococcus* species are soil organisms but couldbe gastrointestinal commensals as they are obligate aerobes with optimum temperature requirements of 28–30℃ and are capable of utilizing organic compounds as sources of carbon and nitrogen (Barton and Hughes, 1982**).**

The intergeneric transfer of conjugative and metabolizable plasmids harbored *by Echerichia coli* in the gut of the soil microarthropod *Folsomia candida* (Collembla) and found that the plasmid transfer took place in the gut.The live

earthworm gut could also be an ideal culture condition for ‘‘training’’ bacteria which may involve plasmid transfer and can be used as a viable environmental technology for bioremediation of pesticide contaminated and water (Hoffmann et al.,1998).

Cyanobacteria are free living, photoautotrophic microorganisms that have shown their capabilities to degrade both naturally occurring compounds and synthetic chemicals, especially pesticides(Yan *et al.*,1998; Megharaj *et al.*,1994*; Megharaj et al.*,1987). Therefore, cyanobacteria have been considered to be potent alternative organisms for chemical and physicaltreatments to transform environmentally persistent, toxic materials.For example, three blue-green algae, *Synechococcus elongates*,*Nostoc linckia*, and *Phormidium tenue*, strongly participate in the degradation of monocrotophos and quinalphos in soil.The biotransformation of endosulfan can be done by two bluegreen algal species namely, *Anabaena* sp. PCC 7120 and *Anabaena flosaquae*.

## 4. MATERIALS AND METHODS

### SAMPLE COLLECTION:

Soil samples were collected aseptically in pre sterilized conical flasks from agricultural fields of Kasargod and chavadi and brought to the laboratory and analysed immediately.

### *ISOLATION OF BACTERIAL POPULATION:*

Serial dilution technique was employed to isolate the organism. The isolates were stored in nutrient agar for identification and the cultures were streaked on to selective media like Eosin Methylene Blue,Mac Conkey agar,Mannitol Salt Agar,Pseudomonas Agar and SS Agar (Salmonella Shigella Agar).

**Nutrient agar:**

| | | |
|---|---|---|
| Peptone | - | 5g |
| Beef extract | - | 3g |
| Yeast extract | - | 3g |
| Sodium chloride | - | 5g |
| Agar | - | 15g |
| Distilled water | - | 1000ml |
| pH | - | 7 |

**Eosin Methylene Blue Agar:**

| | | |
|---|---|---|
| Peptone | - | 10 g |
| Lactose | - | 5g |
| Agar | - | 13.50g |
| Dipotassium phosphate | - | 2g |
| Eosin dye | - | 0.400g |
| Methylene blue | - | 0.065g |
| Distilled water | - | 1000ml |
| pH | - | 7.2 |

**Mac Conkey Agar**

| | | |
|---|---|---|
| Bacto peptone | - | 17 g |
| Proteose peptone | - | 3g |

Lactose - 10g
Bile salt mixture - 1.5 g
Sodium chloride - 5g
Agar - 13.5g
Neutral red - 0.03g
Crystal violet - 0.001g
Trypticase - 10g
Glucose - 5g
Phenol red - 0.018g
Distilled - 1000ml
pH - 7.1

**SS Agar(Salmonella Shigella Agar):**

Peptic digest of animal tissue - 5g
Beef extract - 5g
Lactose - 10g
Bile salt mixture - 8.5g
Sodium citrate - 10g
Sodium thiosulphate - 8.5g
Ferric citrate - 1g
Brilliant green - 0.00033g
Neutral red - 0.025g
Agar - 15g
Distilled water - 1000ml

**Pseudomonas Agar:**

Casein enzymichydrolysate - 10g
Proteose peptone - 10g
Dipotassium phosphate - 1.5g
Magnesium sulphate - 1.5g
Agar - 15g
Distilled water - 1000ml
pH - 7.2

**Identification of the organism:**

The colonies formed on the solid media were isolated and identified by performing gram staining, motility test, sugar fermentataion and various biochemical tests.

**Gram staining:**

It was done to differentiate bacteria in to two groups such as gram positive and gram negative.24 hours old culture was taken and stained with crystal violet and gram iodine respectively for 1 minute, decolourised with alcohol for 15 seconds and counter stained with safranin for 1-2 minutes and observed under oil immersion. Those bacteria that appeared purple were reffered to as gram positive and those that appeared pink were reffered to as gram negative.

**GRAM STAINING REAGENT:**

**Crystal violet:**

**Solution A**

Crystal violet (90% dye content) - 2.0g

Ethanol (95%) - 20 ml

**Solution B**

Ammonium oxalate - 0.8g

Distilled water - 80 ml

Solution A and solution B were mixed.

**Gram's Iodine**

Iodine - 1g

Potassium iodide - 2g

Distilled water - 300ml

**Ethyl alcohol (95%)**

Ethanol (90%) - 95ml

Distilled water - 5ml

**Safranin :**

Safranin 'o' - 0.25ml

Ethyl alcohol (95%) - 10ml

Distilled water - 100ml

**Motility test:**

**Hanging drop method:**

A clean cavity slide was taken and Vaseline was kept on its edges.A loopful culture was placed on the centre of the sterilized coverslip.The cavity slide was inverted over the coverslip allowing glass slide to adhere to Vaseline.Then the slide was inverted so that the coverslip was uppermost and the drop would be hanging from thecoverslip to the centre of the cavity.Edges of the drop was focused under low power objective for observing motility.

**Spore staining:**

The smear was prepared on a clean glass slide,heat fixed,stained with malachite green for 15 minutes,washed and counterstained with safranin and observed under oil immersion.The spores were stained green in color and the negative cultures were fully stained red.

**Malachite green:**

Malachite green - 5g

Distiiled water - 100ml

**Safranin:**

| | | |
|---|---|---|
| Safranin | - | 0.25ml |
| Ethyl acohol(95%) | - | 10ml |
| Distilled water | - | 100ml |

**Biochemical reactions:**

The peptone broth was prepared and pH was adjusted to 7.2. 5ml of the broth was then dispensed into each test tube and sterilized.The tubes wer then inoculated with the test organism and incubated at 37℃ for 24 hours.following incubation 2-4 drops of Kovac's reagent was added.The cherry ring red indicates a positive test.

**Tryptone broth:**

| | |
|---|---|
| Tryptone | -10.0gm |
| Sodium chloride | -5.0gm |
| Distilled water | -1000ml |
| pH | - 7.4 |

**Kovac's reagent (Di-methyl amino benzaldehyde):**

| | |
|---|---|
| Amyl (butyl) alcohol | -150ml |
| Para di methyl amiobezaldehyde | - 10gm |
| Concentrated hydrochloric acid | - 50ml |

**Methyl Red Test:**

The organism was inoculated into MR-VP broth, incubated at $37^0$C for 24-48 hours. Following incubation, 5-6 drops of methyl red was added and the development of bright red colour was observed as a positive result.

**MRVP broth (pH -7.6):**

| | |
|---|---|
| Buffered peptone | -7.5gm |
| Dextrose | -5.0gm |
| Di potassium hydrogen phosphate | - 5.0gm |
| Distilled water | -1000ml |

**Methyl red reagent:**

| | |
|---|---|
| Methyl red | -0.2gm |
| Ethyl alcohol (95%) | -600ml |
| Distilled water | -400ml |

**Voges-Proskauer Test:**

The organism was inoculated in to MR-VP broth, incubated at $37^0$C for 24-48 hours. Following incubation Barrit's reagent was added added and the development of deep rose colour was observed as a positive result.

**Barrit's reagent:**

**Solution A**

| | |
|---|---|
| Alpha-naphthol | -5.0gm |
| Ethanol absolute | -95ml |

**Solution B**

| | |
|---|---|
| Potassium hydroxide | -40.0gm |
| Creatine | -0.3gm |
| Distilled water | -100ml |

**Citrate Utilization Test:**

The culture was inoculated in to simmon's citrate agar slants containing bromothymol blue and incubated at $37^0$C for 24-48 hours. Following incubation citrate positive cultures showed change in colour from green Prussian blue.

**Simmon's citrate agar:**

| | |
|---|---|
| Ammonium dihydrogen phosphate | -1.0gm |
| Di- potassium phosphate | -1.0gm |
| Sodium chloride | -5.0gm |
| Sodium citrate | -2.0gm |
| Magnesium sulfate | -0.2gm |
| Bromothymol blue | -0.08gm |
| Agar | -15.0gm |
| Distilled water | -1000ml |
| pH | - 7.2 |

**Oxidise Test:**

Using a tooth prick the isolated colonies were picked and rubbed on the oxidize disc which is moistened with a drop of distilled water in a petridish and stand for few seconds. Observe for colour change to purple which was a positive result.

**Catalase test:**

A loop full of test culture was placed at the centre of a glass slide and added hydrogen peroxide. Catalase positive reaction was shown by the formation of effervescence.

**Nitrate Reduction Test:**

The organism was inoculated in to nitrate broth and incubated at $37^0$C for 24-48 hours. Following incubation 5 drops of solution A and solution B was added and the development of red colour was observed as a positive result.

**Nitrate broth:**

| | |
|---|---|
| Peptone | -0.5gm |
| Beef extract | -0.3gm |
| Potassium nitrate | -0.5gm |
| Distilled water | -100ml |
| pH | -7.2 |

**Solution A**

| | |
|---|---|
| Sulphanilic acid | -8.0gm |
| Acetic acid (5N)-1 part of glacial | |
| Acetic acid to 2.5 parts distilled water | -1000ml |

**Solution B**

| | |
|---|---|
| Alpha naphthylamine | -5.0gm |
| Acetic acid | -100ml |

**Carbohydrate Fermentation test:**

The test organism inoculated into nutrient broth supplemented with sterilized sugars. The tubes were incubated at $37^0$C for 24-48 hours. Change in colour of dye and displacement of the medium in Durham's tube were examined.

**Lactose broth with phenol red**

| | |
|---|---|
| Peptone | - 10 g |
| Yeast extract | - 1g |
| Beef extract | - 1g |
| Sodium chloride | - 5g |
| Lactose | - 5g |
| Phenol red | - 0.018g |
| Distilled water | - 1000ml |
| pH | - 7.4 |

**Dextrose broth with phenol red:**

| | |
|---|---|
| Peptone | - 10 g |
| Yeast extract | - 1g |
| Beef extract | - 1g |
| Sodium chloride | - 5g |
| Dextrose | - 5g |
| Phenol red | - 0.018g |
| Distilled water | - 1000ml |
| pH | - 7.4 |

**Sucrose broth with phenol red:**

| | |
|---|---|
| Peptone | - 10 g |
| Yeast extract | - 1g |
| Beef extract | - 1g |
| Sodium chloride | - 5g |
| Sucrose | - 5g |
| Phenol red | - 0.018g |
| Distilled water | - 1000ml |
| pH | - 7.4 |

**Mannitol fermentation:**

| | | |
|---|---|---|
| Mannitol | - | 1g |
| Peptone | - | 10g |
| Sodium chloride | - | 5g |
| Distilled water | - | 100ml |
| pH | - | 7.4 |

**Coagulase test:**

Emulsify a portion of a single colony from a test isolates in to 0.5 ml of sterile plasma in tube. The tubes were incubated at $37^0$C for 1-4 hours. Following incubation the positive result was shown by the formation of clot by gently lifting and shifting the tubes.

**Hydrogen Sulfide Test:**

The test culture was stabbed in to SIM agar deep tubes. The tubes were incubated at $37^0$C for 24-48 hours. Following incubation positive result was indicated by the formation of black precipitate along the lie of stabbing.

**SIM agar:**

| | | |
|---|---|---|
| Peptone | - | 10.0gm |
| Dextrose | - | 40.0gm |
| Agar | - | 15.0gm |
| Distilled water | - | 1000ml |
| pH | - | 5.6 |

**Urease Test:**

The culture was inoculated in to Christenson's agar slants containing phenol red. The tubes were incubated at $37^0$C for 24-48 hours. Following incubation urease positive, culture showed change in colour from red to pink.

**Christensen's Media :**

| | | |
|---|---|---|
| Peptone | - | 1.0gm |
| Sodium chloride | - | 5.0gm |
| Di potassium hydrogen phosphate | - | 2.0gm |
| Phenol red | - | 0.012gm |
| Agar | - | 20.0gm |
| Sterile (10% glucose solution) | - | 10ml |
| Sterile (20% urea solution) | - | 100ml |
| Distilled water | - | 1000ml |
| pH | - | 6.8-6.9 |

**Maintenance of pesticide resistant organism:**

The isolates were cultivated in a special medium known as liquid mineral medium.

**Liquid mineral medium:**

| | | |
|---|---|---|
| Ammonium chloride | - | 1.0g |

| | |
|---|---|
| Potassium dihydrogen orthophosphate | - 2.0g |
| Dipotassium hydrogen orthophosphate | - 7.5g |
| Magnesium sulphate | - 0.2g |
| Sodium chloride | - 0.5g |
| Calcium carbonate | - 0.2g |
| Glucose | - 1.0g |
| Distilled water | - 1.0L |
| pH | - 7.8 |

**Propagation of endosulfan degrading bacteria:**

The liquid mineral medium for the propagation of endosulfan degrading bacteria was supplemented with endosulfan at a concentration of 0.5ml.Then the isolates were inoculated in to the medium.Then it is plated in to nutrient agar plates in half hour gap.

**Degradation of endosulfan by the resistant bacteria:**

The 100ml of liquid mineral medium without glucose was taken which was supplemented with endosulfan, as a sole source of carbon, in different concentration from 0.1ml to 0.5ml, in conical flasks along with control.This was inoculated with the test organisms and incubated in shaking incubator at 37˚c for 24hours.

**Liquid mineral medium (without glucose):**

| | |
|---|---|
| Ammonium chloride | - 1.0g |
| Potassium dihydrogen orthophosphate | - 2.0g |
| Dipotassium hydrogen orthophosphate | - 7.5g |
| Magnesium sulphate | - 0.2g |
| Sodium chloride | - 0.5g |
| Calcium carbonate | - 0.2g |
| Distilled water | - 1.0 L |
| pH | - 7.8 |

**Measurement of absorbance:**

The growth of bacteria in broths was measured by turbidity of the solution.A small volume of liquid mineral medium with endosulfan was taken in a 1.0 cm glass cuvette and its absorbance was read at 600nm in a uv-vis spectrophotometer.

## 5. RESULTS

The organism isolated from the kasargod soil is *Bacillus cereus* and the organism isolated from chavadi soil is *Pseudomonas aeroginosa,*based on biochemical tests. The results were tabulated on table no:1 and 2.

The number of pesticide resistant organism was found to be increased when plated on nutrient agar.The results were tabulated on table no:3 and 4.

The degradation capacity of endosulfan is more for a *Bacillus cereus* than *Pseudomonas aeruginosa.*The results were tabulated on table no: 5 and 6 and graph no. 5.1 and 6.1.

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 2, Issue 4, pp: (105-128), Month: October - December 2014, Available at: www.researchpublish.com

**TABLE: 1**

| IDENTIFICATION OF MICROORGANISM | | |
|---|---|---|
| **TESTS** | **RESULTS** | **ORGANISM** |
| Gram staining | Gram negative rods | *Pseudomonas aeruginosa* |
| citrate | Positive | |
| Oxidase | Positive | |
| Catalase | Positive | |
| Urease | Positive | |
| Glucose fermentation | A/G | |
| Lactose fermentation | A | |

A=ACID

A/G=ACID AND GAS

**TABLE: 2**

| IDENTIFICATION OF MICROORGANISM | | |
|---|---|---|
| **TESTS** | **RESULTS** | **ORGANISM** |
| Gram staining | Gram positive rods | *Bacillus cereus* |
| Voges proskauer | Negative | |
| Citrate | Positive | |
| Catalase | Positive | |
| Glucose fermentation | A | |
| Oxidase test | Negative | |
| Nitrate reduction | Positive | |
| Starch hydrolysis | Positive | |
| Casein hydrolysis | Positive | |
| Gelatin hydrolysis | Positive | |

A=ACID

**TABLE: 3 Total numbers of pesticide resistant bacteria from kasargod soil**

| Sampling area | Sample | Concentration of endosulfan in liquid mineral medium | organism | Time interval | No. of pesticide resistant organisms |
|---|---|---|---|---|---|
| kasargod | Soil | 0.5ml | *Bacillus cereus* | $0^{th}$ hour | 3 |
| kasargod | Soil | 0.5ml | *Bacillus cereus* | ½ hour | 8 |
| kasargod | Soil | 0.5ml | *Bacillus cereus* | 1 hour | 21 |
| kasargod | Soil | 0.5ml | *Bacillus cereus* | 1 ½ hour | 37 |
| kasargod | Soil | 0.5ml | *Bacillus cereus* | 2 hour | 50 |
| kasargod | Soil | 0.5ml | *Bacillus cereus* | 2 ½ hour | 75 |
| kasargod | Soil | 0.5ml | *Bacillus cereus* | 3 hour | 86 |

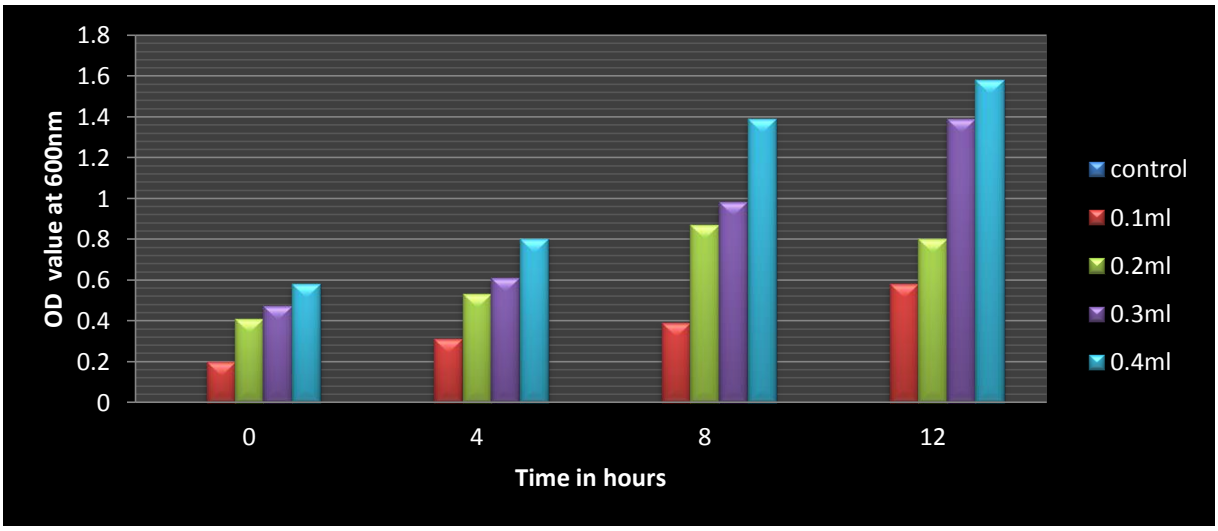
ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 2, Issue 4, pp: (105-128), Month: October - December 2014, Available at: www.researchpublish.com

**TABLE: 4 Total number of pesticide resistant bacteria from Chavadi soil**

| Sampling area | Sample | Concentration of endosulfan in liquid mineral medium | Organism | Time interval | No. of pesticide resistant organisms |
|---|---|---|---|---|---|
| Chavadi | Soil | 0.5ml | *Pseudomonas aeruginosa* | $0^{th}$ hour | 2 |
| Chavadi | Soil | 0.5ml | *Pseudomonas aeruginosa* | ½ hour | 6 |
| Chavadi | Soil | 0.5ml | *Pseudomonas aeruginosa* | 1 hour | 30 |
| Chavadi | Soil | 0.5ml | *Pseudomonas aeruginosa* | 1 ½ hour | 42 |
| Chavadi | Soil | 0.5ml | *Pseudomonas aeruginosa* | 2 hour | 49 |
| Chavadi | Soil | 0.5ml | *Pseudomonas aeruginosa* | 2 ½ hour | 69 |
| Chavadi | Soil | 0.5ml | *Pseudomonas aeruginosa* | 3 hour | 88 |

**TABLE: 5 Growth of endosulfan degrading bacteria-*Bacillus* in mineral medium having endosulfan as a sole carbon source.**

| **$0^{th}$ hour time** | | **$4^{th}$ hour time** | | **$8^{th}$ hour time** | | **$12^{th}$ hour time** | |
|---|---|---|---|---|---|---|---|
| **Conc. Of endosulfan in ml** | **OD at 600nm** | **Conc. Of endosulfan in ml** | **OD at 600nm** | **Conc. Of endosulfan in ml** | **OD at 600nm** | **Conc. Of endosulfan in ml** | **OD at 600nm** |
| control | 0.00 | Control | 0.00 | control | 0.00 | Control | 0.00 |
| 0.1 | 0.20 | 0.1 | 0.31 | 0.1 | 0.39 | 0.1 | 0.48 |
| 0.2 | 0.41 | 0.2 | 0.53 | 0.2 | 0.87 | 0.2 | 1.00 |
| 0.3 | 0.47 | 0.3 | 0.61 | 0.3 | 0.98 | 0.3 | 1.37 |
| 0.4 | 0.58 | 0.4 | 0.80 | 0.4 | 1.39 | 0.4 | 1.58 |

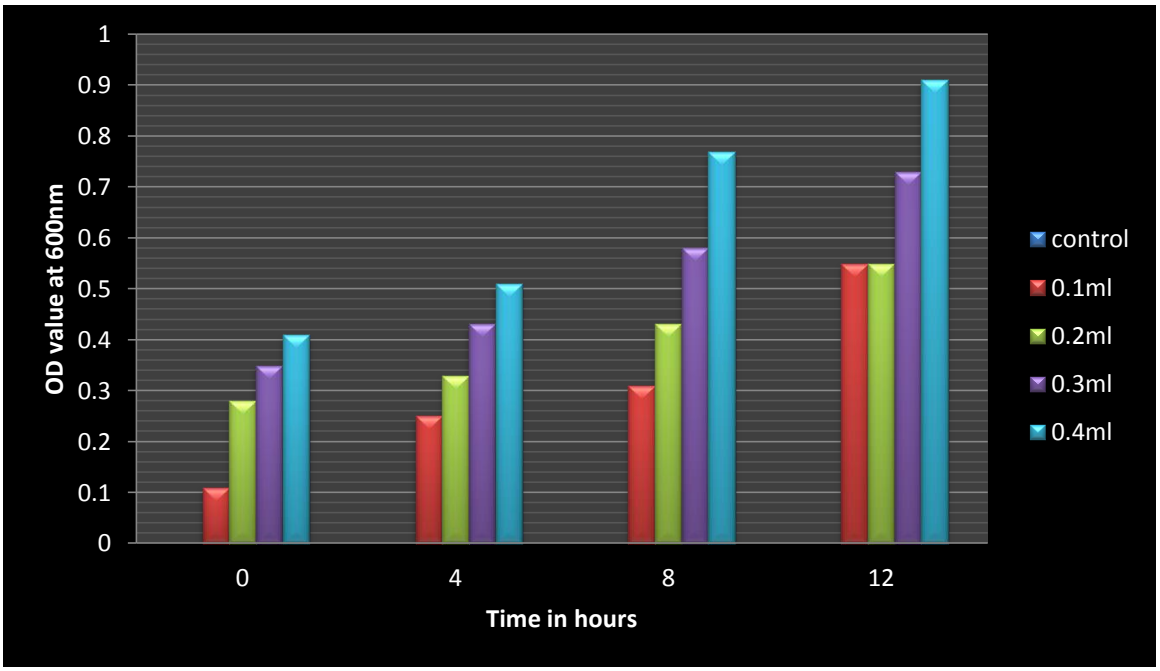



**GRAPH: 5.1- Growth of endosulfan degrading bacteria-*Bacillus* in mineral medium having endosulfan as a sole carbon source.**

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 2, Issue 4, pp: (105-128), Month: October - December 2014, Available at: www.researchpublish.com

**TABLE: 6 Growth of endosulfan degrading bacteria-*Pseudomonas* in mineral medium having endosulfan as a sole carbon source.**

| 0th hour time | | 4th hour time | | 8th hour time | | 12th hour time | |
|---|---|---|---|---|---|---|---|
| Conc. Of endosulfan in ml | OD at 600nm | Conc. Of endosulfan in ml | OD at 600nm | Conc. Of endosulfan in ml | OD at 600nm | Conc. Of endosulfan in ml | OD at 600nm |
| control | 0.00 | Control | 0.00 | control | 0.00 | control | 0.00 |
| 0.1 | 0.11 | 0.1 | 0.25 | 0.1 | 0.31 | 0.1 | 0.39 |
| 0.2 | 0.28 | 0.2 | 0.33 | 0.2 | 0.43 | 0.2 | 0.55 |
| 0.3 | 0.35 | 0.3 | 0.43 | 0.3 | 0.58 | 0.3 | 0.73 |
| 0.4 | 0.41 | 0.4 | 0.51 | 0.4 | 0.77 | 0.4 | 0.91 |




**GRAPH: 6.1- Growth of endosulfan degrading bacteria-*Pseudomonas* in mineral medium having endosulfan as a sole carbon source**

## 6. DISCUSSION

Indiscriminate use of pesticide in agriculture created undesirable effects both the biological community including soil microbial flora and to the other ecosystem.The microbial flora in soil include Autochthonous, Allochthonus and Zymogenous organisms.

The bacteria commonly found in soil generally resistant to pesticide include Pseudomonas,Bacillus,Proteus,Micrococcus, Flavobacterium, Agrobacterium, Corynebacterium, Arthrobacterium etc (Gowri Sanker et al.,1978).

Microbial degradation of organo phosphate and organo chlorine pesticide is of particular interest because of the high mammalian toxicity of such compounds and their widespread and extensive use.For organophosphates such as parathion,

the degrading bacterial isolates were reported as Flavobacterium sp. and Pseudomonas fluorescence can hydrolase endosulfan as sole source of carbon.(Allan Walker et al.,2000).

In our work we isolated *Bacillus cereus* from the endosulfan polluted soil and .*Pseudomonas aeruginosa* from chavadi soil.

The pesticide utilization capacity of these bacteria was studied by inoculating the organism into a liquid mineral medium containing endosulfan.

Degradation of endosulfan studied by checking the OD value in uv-vis spectrophotometer at 600nm.It was was observed that *Bacillus cereus* can degrade endosulfan at a higher rate than *Pseudomonas aeruginosa*.

## 7. SUMMARY

Soil samples were collected from Kasargod and chavadi soil and pesticide resistant organism were isolated and identified.

Degradation capacity of these organism were also studied by growing in a medium containing endosulfan.

By comparative study we found that degradation of endosulfan by *Bacillus cereus* is more than degradation by *Pseudomonas aeruginosa.*

## REFERENCES

[1] Allan Walker.,2000,Microbes enhance pesticide breakdown,The royal society of chemistry,vol.3:44.

[2] Armburst, K. L.,1992, Fate of endosulfan, chlorthal-dimethyl and dichloran in the marine environment, Ph.D thesis. University of California, Davis.

[3] Arnold, S.F.,D.M. Klotz,B.M. Collins,P.M. Vonier,L.J. Guillette Jr and J.A.

[4] McLachlan.,1996,Synergistic activation of estrogen receptors with combination of environmental chemicals. Science,vol. 272:1489–1492

[5] Awasthi, N., N. Manickam, and A. Kumar.,1997, Biodegradation of endosulfan by a bacterial co-culture. Bull. Environ. Contam. Toxicol,vol.59:928–934

[6] Baar, D.P.S.A. Aust., 1987, Mineralization of recalcitrant environmental pollutants by white rot fungus. In: proceedings of the National conference on Hazardous materials. 146-151.

[7] Barton, M.D.,K.L. Hughes.,1982, Is *Rhodoccocus* equi a soil organism? J. Reprod. Fertil. Suppl.vol. 32: 481–489.

[8] Berrakat, A. O., M. Kim, Y. Qian, and T. L. Wade.,2002, Organochlorine pesticides and PCB residues in sediments of Alexandria harbour, Egypt., Mar. Pollut. Bull.vol. 44:1421–1434

[9] Bhattacharya, B., S. Sarkar, and N. Mukherjee.,2003, Organochlorine pesticide residues in sediments of a tropical mangrove estuary. India.Implications for monitoring. Environ. Int.vol. 29:587–592

[10] Borgeest, C.,C. Greenfeld ,D. Tomic, J.A. Flaws., 2002, The effects of endocrine disrupting chemicals on the ovary. Front. Biosc , vol.7: 1941-1948.

[11] Burger,s., B.LAfkham and A.E. Karakaya., 1995,Organochlorine pesticide contaminants in human adipose tissue collected in Tebriz.Bull.Environ contam Toxicol,vol.4:546-553.

[12] Cerejeira, M. J., P. Viana, S. Batista, T. Pereira, E. Silva, M. J. Valerio, Silva, M. Ferreira, and A. M. Silva-Fernandos., 2003, Pesticides in Portuguese surface and ground waters. Water Res, Vol. 37:1055– 1063.

[13] Chaudhuri, K,S. Selvaraj ,A.K. Pal.,1999, Studies on the genotoxicity of endosulfan in bacterial systems. Mutat Res,vol.439:63–67

[14] De,A.K.Environmental chemistry.ed 5:108-109.

[15] Diaz, E.,M.A Prieto.,2000, Bacterial promoters triggering biodegradation of aromatic pollutants. Curr. Opin. Biotechnol.vol. 11: 467-475

[16] Goebel, H,S., Gorbanch , W. Khauf .,1982, Properties, Effects, Residues, and Analytics of the Insecicide Endosulfan.vol. 83: 1-100.

[17] Golfinopoulos, S. K. A. D. Nikolaou, M. N. Kostopoulou, N. K. Xilourgidis, M. C. Vagi, and D. T. Lekkas.,2003,Organochlorine pesticides in the surface waters of Northern Greece. Chemosphere. Vol.50:507–516

[18] Gowri Sanker,R.,Palanippan and S.Chandran.,1978,Isolation and characterisation of pesticide (Dimethoate)metabolising bacterial population from rice soil proceedings of 6th national symposium on environment,vol.6:296-299.

[19] Guerin, T. F.,1999, The anaerobic degradation of endosulfan by indigenous microorganisms from low-oxygen soils and sediments. Environ.Pollut.vol. 106:13–21

[20] Horvath, R.S.,1972, Microbial co-metabolism and the degradation of organic compounds in nature. Bacteriol. Rev. pp. 146-155

[21] Janssen, D.B., A.Scheper, L.Dijkhuizen, and B.Witholt. ,1985, Degradation of halogenated aliphatic compounds by *Xanthobacter autotrophicus* GJ10.Appl. Environ. Microbiol,vol.49: 673-677.

[22] Jayashree ,N., N. Vasudevan.,2007, Effect of tween 80 added to the soil on the degradation of endosulfan by *Pseudomonas aeruginosa*,vol.4:203-210.

[23] Karsten, G.R., H.L. Drake.,1995, Comparative assessment of the aerobic and anaerobic microfloras of Earthworm Guts and Forest soils. Appl. Environ. Microbiol,vol. 61:1039–1044

[24] Katayama, A., and F. Matsumura.,1993, Degradation of organochlorine pesticides, particularly endosulfan by *Trichoderma harzianum*. Environ. Toxicol. Chem,vol.12:1059–1065.

[25] Kathpal, T. S., A. Singh, J. S. Dhankhar, and G. Singh.,1997,Fate of endosulfan in cotton soil under sub-tropical conditions of northern India. Pestic. Sci,vol. 50:21–27.

[26] Kennedy, I. R.,F. Sanchez-Bayo, S.W.Kimber,L. Hugo,N.Ahmad., 2001, Off-site movement of endosulfan from irrigated cotton in New South Wales. J. EnViron. Qual,vol. 30, 683-696.

[27] Kim, J.S., D.E. Crowley.,2007, Microbial Diversity in Natural Asphalts of the Rancho La Brea Tar Pits. Appl. Environ. Microbiol,vol. 73: 4579-4591.

[28] Kullmann, S.W., F. Matsumura.,1996, Metabolic pathway utilized *Phanerochaete Chrysosporium* for degradation of the cyclodine pesticide endosulfan. Appl. Environ. Microbio,lvol.62, 593-600.

[29] Leahy, J.G.,R.R Colwell.,1990, Microbial degradation of hydrocarbons in the environment. Microbiol. Rev. vol.54: 305-315.

[30] Martens, M., 1976., Degradation of [8,9-14C]endosulfan by soil microorganisms.Appl. Environ. Microbiol.vol. 6:853–858.

[31] Megharaj, M..D.R. Madhavi, D. R,C. Sreenivasulu,A. Umamaheswari, K. Venkateswarlu.,1994, Biodegradation of methyl parathion by soil isolates of microalgae and cyanobacteria. Bull. EnViron.Contam. Toxicol,vol. 53, 292-297

[32] Megharaj, M.,K. Venkateswarlu, A.S. Rao., 1987,Metabolism of monocrotophos and quinalphos by algae isolated from soil. Bull. EnViron. Contam. Toxicol,vol. 39, 251-256

[33] Mukherjee, I., and M. Gopal.,1994, Degradation of beta-endosulfan by *Aspergillus niger*. Toxicol. Environ. Chem.vol. 46:217–221.

[34] Nagao T.,1998, Health effects of endocrine disruptors. Japanese J.Toxicology Environ. Health,vol. 44: 151-167.

[35] Osama El Gialani Elsaid, Azhari Omer Abdelbagi, Elsiddig Ahmed Elmustafa Elsheikhc.,2010, Microbial Degradation of Endosulfan in Carbon Free Media and Selective Mediavol,vol.6:257-562

[36] Pau,l V,E,. Balasubramaniam .,1997,Effects of single and repeated administration of endosulfan on behaviour and its interaction with centrally acting drugs in experimental animals: a mini review. Environ Toxicol Pharmacol,vol. 3:151–157.

[37] Rainer Martens.,1976,Degradation of [8,9-14C]Endosulfan by Soil Microorganismsvol.31:853-858.

[38] Rao, D. M. R and A. S. Murty., 1980, Persistence of endosulfan in soils. J. Agric. Food Che,.vol. 28:1099–1101.

[39] Reynold Murry, Peter Philips and Judith Bender.,1996,Degradation of pesticides applied to banana farm soil,comparison of indigenous bacteria and a microbial mat;journal of soil research.vol.33:925-942.

[40] Saiyed, H.,A. Dewan ,V. Bhatnaga ,U. Shenoy ,R. Shenoy and H.Rajmohan .,2003,Effect of endosulfan on male reproductive development. Environ Health Perspect,vol. 111:1958–1962.

[41] Seth, W. Kullmanand Fumio Matsmura.,1995, Metabolic Pathways Utilized by *Phanerochaete chrysosporium* for Degradation of the Cyclodiene Pesticide endosulfan,vol.62:593-600.

[42] Shelly Sinha, Pritam Chattopadhya, Ieshita Pan, Sandipan Chatterjee, Pompee Chanda,Debashis BandyopadhyayKamala Das and Sukanta K. Sen.,2009,.Microbial transformation of xenobiotics forenvironmental bioremediation,vol.8:6016-6027.

[43] Shetty, P. K., J. Mitra, N. B. K., Murthy, K. K., Namitha, K. N., Savitha, and K. Raghu., 2000, Biodegradation of cyclodiene insecticide endosulfan by *mucor-thermo-hyalospora* MTCC 1384. Curr. Sci.vol. 79:1381–1383

[44] Siddique, T., B. C. Okeke, A. Arshad, and W. T. Frankenberger. Jr.,2003,Enrichment and Isolation of endosulfan-degrading microorganisms J. Environ. Qual.vol. 32:47–54.

[45] Singh,B and G.S.Dhaliwal,G.S.,1992,Envt impact of pesticides.In :G.,S., Dhaliwal,B.,S., Hansra and N.,Jerath.Changing scenario of our envt.Punjab Agri Univ.Ludhiana.165-174.

[46] Singh, D.K .,2008,Biodegradation and bioremediation of pesticide in soil:concept, method and recent developments. Ind. J. Microbiol.vol. 48: 35-40.

[47] Sujatha, C. H., S. M., Nair, and J. Chacko. Determination and distribution of endosulfan and Malathion in an Indian estuary. Wate.r Re,vol. 33:109–114.

[48] Sunderam ,R.I.M.,D.M.H. Cheng , G.B.Thompson. ,1992, Toxicity of endosulfan to native and introduced fish in Australia.Environ Toxicol Chem,vol. 11:1469–1476.

[49] Sutherland, T., I. Horne, M. Lacey, R. Harcourt, R. Russell, and J. Oakeshott.,2000, Enrichment of an endosulfan-degrading mixed bacterial culture. Appl. Environ. Microbiol. Vol.66:2822–2828.

[50] Timmis, K.N.,D.H Pieper. ,1999, Bacteria designed for bioremediation. Trends Biotechnol.vol. 17: 200-204.

[51] Tropel, D., Van der Meer JR.,2004, Bacterial Transcriptional Regulators for Degradation Pathways of Aromatic Compounds. Microbiol. Mol.Biol. Rev. vol.68: 474-500.

[52] Vargas,J.M, jr.,1975,Pesticide Degradation.232-233.

[53] Widada, J,H. Nojiri,T. Omori., 2002, Recent developments in molecular techniques for identification and monitoring of Xenobiotic-degrading bacteria and their catabolic genes in bioremediation. Appl. Microbiol. Biotechnol.vol. 60: 45-59.

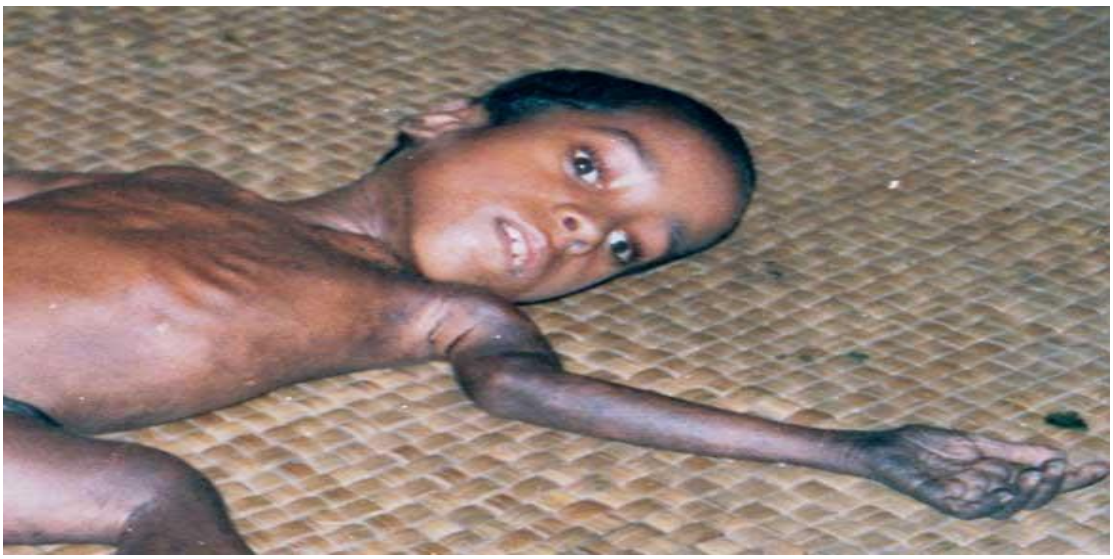
[54] WHO.,1968. Environmental health criteria for endosulfan.world health organisation,vol.40.1-47.

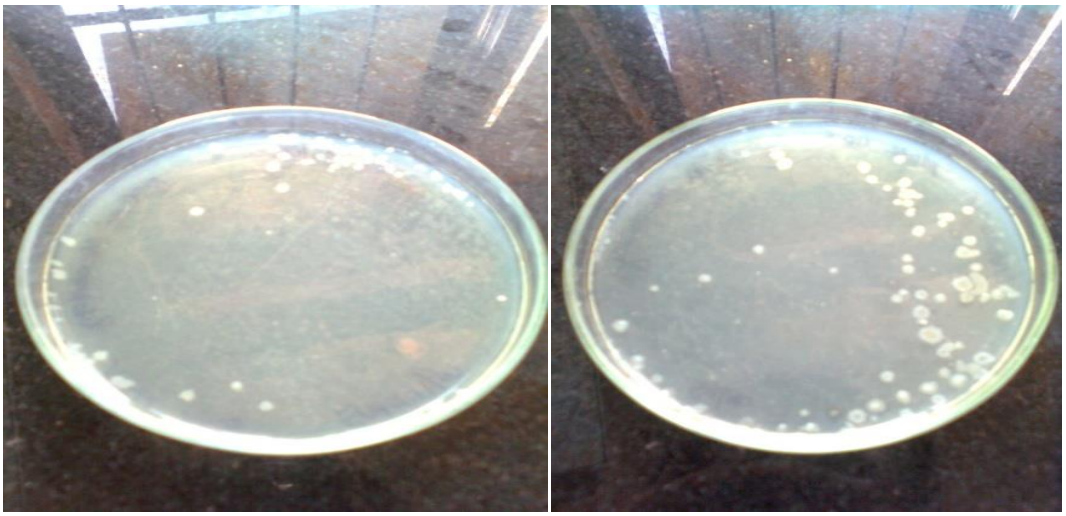
[55] Yan, G. A.,J.W. Jiang, G. Wu,X. Yan.,1998, Disappearance of linear alkylbenzene sulfinate from different cultures with *Anabaena sp*. HB 1017. Bull. EnViron. Contam. Toxicol,vol. 60, 329-334.

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 2, Issue 4, pp: (105-128), Month: October - December 2014, Available at: www.researchpublish.com

## APPENDIX - A

### FATE OF ENDOSULFAN

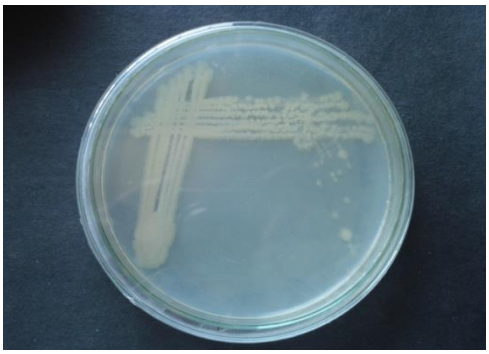




**Organism isolated from Chavadi soil.** **Organism isolated fromKasargod soil**

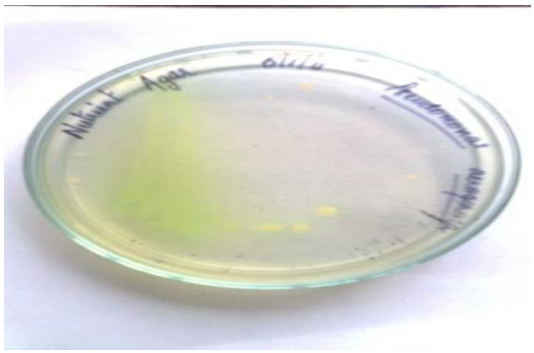
ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 2, Issue 4, pp: (105-128), Month: October - December 2014, Available at: www.researchpublish.com


**ENDOSULFAN**



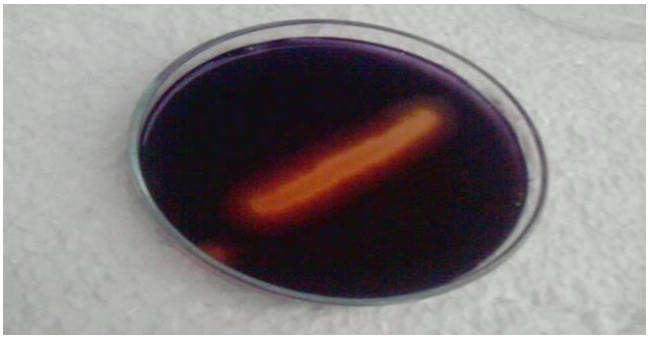
PESTICIDE RESISTANT

*Bacillus cereus*

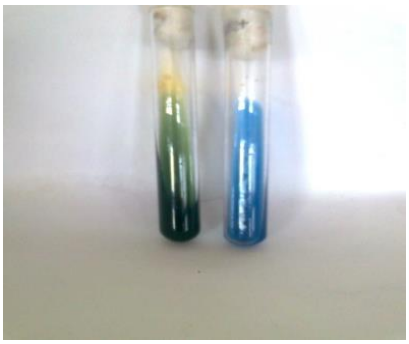


PESTICIDE RESISTANT

*Pseudomonas aeruginosa*



**Starch hydrolysis positive result**



**citrate positive result**

**Organisms isolated from 0.5ml conc. of endosulfan**