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Soil and Pesticides

The deleterious effects of pesticides on soil microbes have attracted considerable attention as the soil microorganisms play a major role in the utilization of both organic and inorganic constituents of the soil by plants (Edward, 1972). Pesticides are toxic chemicals, which have been extensively used to increase crop productivity (Akhtar, 1985). Studies have shown that besides combating the pests, pesticides affect the number, diversity and activity of beneficial microbial communities (Singh & Prasad, 1991). Soil microorganisms show an early indication of soil disturbances by foreign chemicals than any other parameters (Tu, 1995).

Endosulfan is in use world-wide since its introduction in the 1950’s. It is widely considered to be Persistent Organic Pollutants (POP), but was not included in the initial list targeted for phase out under the Stockholm convention. Endosulfan was considered for world-wide elimination in 1994 at the convention in Vancouver, Canada being jointly organized by government of Canada and Philippines, but was later removed from the list according to Quijano, (2000).

Pesticides are bioaccumulative and biomagnifying. Organisms at the top of the food chain are most adversely affected as these pollutants tend to accumulate in maximum quantity in their body through a process of ‘Biomagnification’. They are found in water, air and soil at dangerous magnitude. They are known to be detrimental for the survival of many birds and animals both at terrestrial and aquatic habitats. They also harm many beneficial organisms in farming. According to Anon (2002), endosulfan is recognized as a Persistent Toxic Substance (PTS) by the UNEP, on account of its magnitude of use, environmental levels and ecological effects. It also reveals that India is one of the major producers of endosulfan, and it produces an average of 8206 MT/Yr totaling to 41033 MT between 1995-2000.

Pesticides are also reported to possess mutagenic, carcinogenic and teratogenic activity (Gilden et al., 2010).
Endosulfan and its residues

Soil types affect the persistence and retention of organochlorine pesticide residues (Edwards, 1966) and that DT 50 (degradation time) rates of organochlorine pesticides vary from 4 to 30 years, depending on climatic and other environmental conditions.

Endosulfan residue has been recognized in a variety of environmental media like air, surface water, ground water, soil and sediment and its metabolites have been reported in human and as well as in domestic animal’s milk (Nag & Raikwar, 2008), fruit and vegetable (Mitchell, 1976; Pokharkar & Dethe, 1981).

Rao and Murthy (1980) showed that the endosulfan residues persisted for about 120 days in wet soils and 100 days in dry soil, with the same initial dose (at > 0.05ppm). Further they observed a difference in the persistence of the residues in 3 dry soils that received low, normal and high initial doses and the residues persisted for 60, 100, and 160 days, respectively.

The EPA of the USA considers endosulfan as having a high bioaccumulation potential in fish (Naqvi & Vaishnavi, 1993), but not much evidence is available on its bioaccumulation in higher trophic levels.

The temperature and pH influence the degradation of endosulfan. With increase in temperature, endosulfan sulfate production increases as shown by Sang and Petrovic (1999) and endosulfan persists longer under acidic condition (Anon, 1984, 1996).

Sethunathan et al. (2002) reported that α-endosulfan decreased more rapidly under non-flooded conditions (cotton soil) than under flooded conditions (wet land rice soil) in both unamended and wheatstraw amended soil sample during 120 day incubation. Endosulfan sulfate was detected under both water regimes, but in distinctly greater amounts in non-flooded soil, even when amended with straw. Evidently, endosulfan sulfate persisted under both moisture regimes, even in amended soil.

Micheal et al. (2005) showed varying amounts of endosulfan residues in soil (<0.02-5.60 mgkg⁻¹ dry wt.) and ditch sediments (<0.02-3.33 mgkg⁻¹ dry wt.) in about 3 to 5 farm area samples. The half-life of α-endosulfan is about 1-3 months whereas those of β-endosulfan and
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endosulfan sulfate, it is about 2 - 6 years depending on the environmental conditions (Wan et al., 2005).

Effects of endosulfan

Pandey et al. (1990) studied the genotoxicity of endosulfan on mammalian germs cells and found that it affects the reproductive system by affecting semen quality, sperm count, spermatogonial cells and sperm morphology.

Ingestion or breathing high level of endosulfan may lead to convulsion and death. Endosulfan affects the central nervous system, kidney, and show teratogenic and mutagenic effects (Paul & Subramaniam, 1997; ATSDR, 2000). Symptoms of endosulfan poisoning include hyper activity, excitement, dyspnea (breathing difficulty), apnea (stoppage of breathing), salivation, loss of consciousness, diarrhoea, anemia, nausea, vomiting, insomnia, blurred vision, cyanosis (blue discoloration of skin due to lack of oxygen), foaming at the mouth etc. (US Department of Health and Human Services, 1990).

Naqvi and Vaishnavi (1993) studied the bioaccumulative potential and toxicity of endosulfan on non target animals and found that endosulfan have adverse affect on central nervous system by inhibiting acetyl cholinesterase activity in the brain.

Ghadiri et al. (1995) reported that the endosulfan applied to the soil reduced either the population or the activities of soil microorganisms that are responsible for the degradation of organochlorine compounds. It is shown to produce mutagenic effects in bacteria and yeast (Chaudhuri et al., 1999).

Kamble and Muley (2000) performed static bioassay tests using chloroprophos 20EC and endosulfan 35EC on the fresh water fish, Sarcotherodon mossambicus and showed marked alterations like erratic movement, fast opercular movement, imbalance and darkening and trembling of body after 96 hrs after exposure to LC_{50} concentrations of both the pesticides.

Vengateshwarlu et al. (2000) studied the endosulfan poisoning and reported that endosulfan ingestion affects the kidney and liver.
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Saravanan et al. (2001) reported that endosulfan even at a sublethal dose, reduced the contents of total sugars, free amino acid, cholesterol and protein in blood and liver of an exotic fish, Sarcotherodon mossambicus. According to Dey and Gupta (2002), several behavioral as well as morphological changes were observed under acute toxicity of endosulfan in tadpoles of 03 Anuran sp.

Congenital birth defects, reproductive health problems, cancer, loss of immunity, neurological and mental diseases were reported among the people in 15 villages, especially Padre, Perla in Kasargod in Kerala, on continuous exposure to endosulfan that was aerially sprayed on the crops three times every year for 24 years (Anon, 2002).

Broomhall and Shine (2003) showed that tadpoles of the Australia tree frog, Litoria freycineti, when exposed to 1.3µgL⁻¹ endosulfan grew slowly with high mortality rate. Studies carried out by Saiyed et al. (2003) concluded that defects of the male reproductive system including cryptorchidism were more prevalent in the study group and suggested that endosulfan exposure in male children may delay sexual maturity, interfere with sex hormone synthesis, decreases the quality of semen and damages the testes.

Borell and Aguilar (2005) observed that the organochlorine compound showed more toxicity during pregnancy than in any other stage of the life cycle in Delphinus delphis. Kumar et al. (2005) showed the toxicity of endosulfan to fish by exposing to different concentrations of endosulfan and recording an increased mortality rate.

Endosulfan has also been shown to cause mutations in mammals. High exposure to endosulfan has been shown to result in the incidence of breast cancer (Hoyer et al., 2006).

Rau et al. (2012) showed the presence of endosulfan in the bone marrow of children with acute hematological malignancies and hailing from endosulfan contaminated areas.

Biodegradation of endosulfan

The principal degradation products of endosulfan such as α-isomer disappears readily, but the β-isomer and endosulfan sulfate persist in soil for over two years (Maier, 1968).
Sethunathan (1973) and Siddaramappa et al. (1973) have described the first bacteria with the capability of degrading organophosphorus compounds and since then a number of bacteria with different enzymes involved in pesticide degradation have been studied.

The principal biochemical processes associated with microbial metabolism of pesticides are alkylation, dealkylation, amide or ester hydrolysis, dehalogenation, dehydrogenation, oxidation, reduction, ring cleavage and conjugate formation (Kaufman, 1974; Alexander, 1977).

Martens (1976) studied the degradation of endosulfan by soil microorganisms. Over 15 soil bacteria, 3 actinomycetes and 16 soil fungi were found capable of metabolizing more than 30% of the applied endosulfan. The majority of active fungi formed endosulfan sulfate, while the bacteria formed the endodiol. The study reported a marked increase in the amount of endosulfan diol produced from endosulfan when the pH of the medium was increased from 6 to 8.

Martens (1977) studied the degradation of \(^{14}\)C endosulfan in 7 soil samples. Under aerobic conditions, endosulfan sulfate was detected as the major metabolite in all soils samples. The sulfate was also the major products in soils incubated with N\(_2\)/CO\(_2\) but to a lesser extent. Smaller amounts of the diol and lactones were also produced. Under flooded condition, diol was produced in greater amounts compared to sulfate and hydroxyether.

A mixed culture of soil microorganisms from sandy loam soil was isolated and used for the degradation of endosulfan and its metabolites by Miles et al. (1979) who showed the conversion of both \(\alpha\)- and \(\beta\)-endosulfan to endosulfan sulfate.

The role of microbes in biodegradation of endosulfan has been thoroughly investigated in the concept of enriched culture that utilizes endosulfan as a source of nutrient (Alexander, 1981).

Siddaratha et al. (1990) studied the degradation of \(\alpha\)-, \(\beta\)-, and \(\gamma\)-hexachlorocyclohexane by a Pseudomonas sp. from sugarcane field that readily metabolized \(\beta\)-isomer along with other isomers under aerobic conditions.

Daubaras and Chakraborty (1992) have shown that microbes take active part in the biodegradation and removal of toxic and nontoxic chemicals and have studied the environmental influences on the biodegradability of synthetic compounds by modifying the microorganisms for
new degradation genes. Microbes transform the pesticides in various ways, such as use of the pesticides either as a substrate, or form conjugate, or accumulate or alter it by co-metabolism.

Endosulfan is one of the highly toxic organochlorine insecticides (Katayama & Matsumara, 1993). The residual toxicity of endosulfan byproducts was analyzed by Anon (1993), who showed endosulfan sulfate was highly toxic followed by endosulfan diol, endosulfan lactone, endosulfan ether etc.

Pesticidal degradative genes in microbes have been found to be located on plasmids, transposons, and or on chromosomes. Recombinant DNA studies have made it possible to develop DNA probes that are being used to identify microbes from diverse environmental communities with a unique ability to degrade pesticides (Kumar et al., 1996).

Sutherland et al. (2000) showed that when an endosulfan degrading mixed bacterial culture was isolated from soil with a history of endosulfan exposure, the endosulfan was detoxified by enzymatic oxidation and hydrolytic reactions. The oxidation reaction produced endosulfan sulfate. Hydrolysis involved a novel intermediate, identified as endosulfan monoaldehyde, suggesting that this mixed culture is a source of endosulfan hydrolyzing enzymes that can be exploited in the bioremediation of endosulfan residues.

Shetty et al. (2000) have shown that the fungal strain M-Thermo-hyalospora MTCC 1384 was found to be efficient in transforming endosulfan in carbon deficient medium, through oxidative and hydrolytic pathways as detected by TLC and NMR studies.

Sutherland et al. (2002) reported that a flavin reductase gene in Mycobacterium smegmatis was capable of resulting in the disappearance of β-endosulfan in favour of endosulfan monoaldehyde and endosulfan hydroxyether suggesting that β-endosulfan is metabolized enzymatically to endosulfan monoaldehyde and endosulfan hydroxyether.

Kwon et al. (2002) isolated bacteria from different soil samples that was able to degrade endosulfan without formation of endosulfan sulfate using endosulfan as sole source of carbon and energy. Strain KE-1, identified as Klebsiella pneumoniae, showed highest endosulfan degradation activity within 10 days among the forty isolated bacteria.
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Awasti et al. (2003) have shown the detoxification of endosulfan isomers by using a consortium of different strains of *Bacillus* and observed the accumulation of endosulfan diol and endosulfan lactone. Accumulation of endosulfan sulfate was however not observed with either of the isomer. The microbial degradation of endosulfan isomer was also accompanied by a decrease in its toxicity to the test organism, *Tubifex muller*.

Siddique et al. (2003a) conducted a study on the identification of enriched microorganisms capable of degrading endosulfan. Enrichment was achieved by using the insecticide as either the sole source of carbon or sulfur in parallel studies. Two strains each of fungi and bacteria were selected and they significantly decreased the pH of the nutrients culture media while growing on endosulfan and could degrade the insecticide very efficiently.

Shivaramaiah and Kennedy (2006) isolated soil bacteria capable of metabolizing 50% of the endosulfan into endosulfan sulfate within 3 days of incubation and no other metabolites were documented. Weir et al. (2006) were able to isolate a bacterium from soil that brought about the degradation of α-, β-endosulfan and endosulfan sulfate and also showed that a gene ‘ese’ encoding an enzyme was responsible for the said activity. Prabakaran and Allenpeterson (2006) showed that *Pseudomonas aeruginosa* degraded endosulfan by using it as carbon source even at a high concentration of 500 ppm.

Lee et al. (2006) utilized endosulfan and endosulfan sulfate as the sole carbon source and isolated a bacterium (*Pseudomonas* sp.) capable of degrading both the metabolites around 52% and 71% respectively through enrichment technique.

Kumar and Philip (2006b) studied degradation of endosulfan in both aerobic and anaerobic conditions by mixed bacterial culture which consisted of *Staphylocococcus* sp. and *Bacillus circulans*-I and -II. After 3 weeks of incubation, they were able to degrade 71.5% and 75.88 of endosulfan in aerobic and facultative anaerobic conditions, respectively. Addition of dextrose favoured endosulfan degradation, while pH had a significant effect on endosulfan degradation.

Shanti et al. (2007) characterized the pesticide resistant bacteria from the agricultural fields of Kothanellore in Tamilnadu in South India and found pesticides resistant genera were dominated by *Bacillus* sp. along with other genera like *Corynebacterium*, *Listeria*,...
*Pseudomonas* and enterobacteriaceae members which were able to resist the pesticides upto 0.1%.

Tejomyee *et al.* (2007) conducted biodegradation studies of endosulfan by a soil fungus, *Aspergillus niger* which could tolerate 400 mg/ml of technical grade endosulfan. Complete disappearance of endosulfan was seen within 12 days of incubation with evolution of CO$_2$ and change in pH of medium to acidic range indicated microbial transformations of endosulfan.

Hussain *et al.* (2007) were able to isolate 29 endosulfan degrading bacterial strains from soil using endosulfan as sole sulfur source. Endosulfan diol and endosulfan ether were among the products of endosulfan metabolism by these bacterial strains without the production of endosulfan sulfate. *Pseudomonas cepacia* was the most efficient degrader of both α- and β-endosulfan as it consumed more than 90% of the spiked amount (100 mgL$^{-1}$) in the broth within 14 days of incubation at pH 8.0 and 30°C.

Kumar *et al.* (2007) demonstrated the degradation of endosulfan (between 73 & 81%) by a mixed culture of namely *Stenotrophomonas maltophilia* and *Rhodococcus erythropolis* isolated from a pesticide contaminated soil.

Optimization of environmental parameters for biodegradation of α- and β-endosulfan by *Pseudomonas aeruginosa* was carried out by Arshad *et al.* (2008). The results showed that endosulfan degradation was upto 85% after 16 days at an inoculum size of 600µl, incubation temperature of 30°C, in aerated slurries at pH 8, in loam soil.

Goswami and Singh (2009) carried out studies on microbial degradation of endosulfan by *Bordetella* sp. B9 and showed that it can degrade 80% of α-endosulfan and 86% β-endosulfan in 18 days. The main metabolites detected were endosulfan ether and endosulfan lactone in the broth culture while in soil microcosm endosulfan sulfate was also found along with endosulfan ether and endosulfan lactone.

Bajaj *et al.* (2010) isolated *Pseudomonas* sp. (Strain IITR01) capable of degrading α-endosulfan and endosulfan sulfate but not the β-endosulfan, through enrichment culture technique. GC-MS analysis showed the formation of products such as endosulfan diol, ether and lactone.
The use of immobilized enzymes has also shown promising potential for pesticide detoxification (Karigar & Shwetha, 2011).

Sunitha et al. (2012) have isolated strains of *Pseudomonas putida* using endosulfan and endosulfan sulfate as sole source of sulfur which could bring about more than 70% of endosulfan and 90% of endosulfan sulfate degradation in the broth culture. Metabolites of endosulfan degradation like endosulfan diol, endosulfan lactone and endosulfan sulfate were detected in the cultures.

Yu et al. (2012) have isolated *Stenotrophomonas* sp. *LD-6*, which could degrade 100 mg/L endosulfan completely within 10 days. The major metabolites detected were recorded as endosulfan diol and endosulfan ether with slight decrease in culture pH. They also documented that the biodegradation of both the isomers of endosulfan was relatively better at a temperature range of 25–35°C, with a maximum at 30°C.