CHAPTER 2

REVIEW OF LITERATURE

2.1 INTRODUCTION

The term pesticide encompasses a great range of diverse substances, falling into a number of broad groupings such as herbicide, insecticide and fungicide. The use of pesticides has substantially increased since the introduction of synthetic chemicals in the 1940’s, revolutionizing agricultural production. Specifically, pesticides have been used in: (i) agriculture to increase productivity, quality, quantity of food, fibre and feed; (ii) forestry for pest control; (iii) industrial, commercial, municipal and military sectors for rodent and weed control around plant sites; (iv) medical sector for control of mosquitoes and rodents; (v) urban environment for termite control around structures and pest control in gardens (Carthwaite et al 1996; Centner 1998 and Falconer 1998). Agriculture is the principal market for pesticides (Hirnel et al 1990; Bower et al 1993).

The generic term Persistent Organic Pollutants (POP’s) encompasses hundreds of chemicals which are (i) persistent in the environment, having long half-lives in the soils, sediments, air and biota; (ii) are typically hydrophobic and lipophilic; (iii) have the property to enter the gas phase under environmental temperatures and are subject to long range transport and (iv) are distributed globally and even found in pristine environments such as Artic region. The combination of their resistance to
metabolism and lipophilicity means that they will bioaccumulate and be transported through food chains.

Among the important classes, POP’s are many families of chlorinated organics (eg. DDT, chlordane, dieldrin, aldrin, endosulfan, etc).

2.2 TYPES OF PESTICIDES FOUND ON AGRICULTURAL LAND

There are large number of pesticides currently in use, with a wide range of physico-chemical properties and belonging to a wide variety of chemical classes. Clearly, the physico-chemical properties of a given pesticide will govern its behavior and biological activity in the soil. Molecular size, ionizability, water solubility, lipophilicity, polarizability and volatility are all key properties, but generally one or two properties have a dominating influence (White 1976). Figure 2.1 presents a classification scheme for selected pesticides on the basis of their significant chemical properties.

2.3 ORGANOCHLORINE PESTICIDE POLLUTION IN THE ENVIRONMENT

2.3.1 Residues in soil

Soil acts as a major sink for majority of the pesticides applied to the crop. Kole et al (1998) conducted a field trail by applying endosulfan in soil at the recommended dose (0.051% at 500 L/ha) and 0.10% at 500 L/ha. The maximum residues found on soil after 7 days of application was 0.029 to 0.092 ppm. The terminal residues of endosulfan at harvest stage in different soils varied from 0.007 to 0.0042 ppm. Residues of endosulfan and other selected organochlorine pesticides in farm areas of the lower Fraser Valley,
British, Coloumbia and Canada was studied by Wan et al (2005). A large amount of endosulfan residues were detected in soils (< 0.02 to 5.6 mg/kg dry weight) and ditch sediment (< 0.02 to 3.33 mg/kg dry weight). p,p-DDD and p,p-DDE were found in agricultural soils (< 0.02 to 16.2 mg/kg dry weight) and sediments (< 0.02 to 9.73 mg/kg dry weight). Ferreira et al (1998) reported 97.9% residues of organochlorine such as HCH, DDT, endrin, dieldrin, aldrin and heptachlor epoxide were in soils of Sao Paulo. Highest

![Classification of pesticides](image)

**Figure 2.1 Classification of pesticides**
levels of HCH (up to 0.02 mg/kg) were detected in soils with coffee and soyabean crops, while highest DDT levels (up to 0.43 mg/kg) were encountered in sugarcane soils.

Kathpal et al (1997) showed the presence of HCH residues such as beta and gamma isomers, beta HCH up to 3 ppb and gamma HCH up to 2 ppb in soils of Haryana state after 11 years of application. Aldrin and Chlordane isomers were present in the form of its parent compounds at concentrations up to 2 ppb and 1 ppb. Falconer et al (1997) examined the chiral organochlorine pesticides in soils collected in 1989 from six farms in the Tette area of Canada and showed the presence of pesticide residues of 1-2 orders of magnitude in musk soils with 27-56 % organic matter than in silt loams with 3-7 % organic matter.

Nagami (1997) surveyed organochlorine insecticides in 3 agricultural fields in Japan. Dieldrin was 0.05 µg/g of soil in the 1st field. Trans-chlordane, cis-chlordane and trans-nonachlor were found at higher concentrations in the other 2 fields. Bizik and Kotercova (1995) reported the occurrence of organochlorine pesticides and their residues (Aldrin, dieldrin, endrin, heptachlor, heptachlorepoxide, HCH, p,p-DDT, o,p-DDT, p,p-DDE, p,p-DDD and o,p-DDE) at 20 cm intervals to a depth of 1m in an orthic luvisol soil in Portugal. DDT and its metabolites were highest (0.4 to 4.8 mg/kg) in the upper soil layers. El Marsafy (1999) reported that the soil of Ismailea governorate was contaminated with organochlorine and pyrethroid groups of pesticides.

Cavanagh et al (1999) studied the presence of organochlorine pesticide residues in Australian soils. Organochlorine pesticides were widely used in the Australian sugarcane industry from the early 1950’s until the late
1980’s. Sugarcane soil samples recorded 0.001-45 ng/g of organochlorine pesticide residues.

Aigner et al (1998) reported the concentration of 11 compounds such as o,p-DDT, o,p-DDD, o,p-DDE, p,p-DDT, p,p-DDD, p,p-DDE, cis-chlordane, trans-nonachlor, \( \alpha \)-endosulfan, \( \beta \)-endosulfan, endosulfan sulfate in 80 agricultural soils and two garden soils. Organochlorine concentrations in soils from agricultural areas were greater ranging from 0.006 ppm to 5.41 ppm.

Puchwein et al (1993) reported the occurrence of organochlorine pesticides and PCB cogeners at low concentration in the soils of upper Austria. PAH contents varied from 10 ng/g to 20 \( \mu \)g/g. High level of chlorinated hydrocarbon insecticide and polychlorinated biphenyl residue found in cultivated soils (290 ng/g of soil) of southern provinces of Vietnam. concentrations of organochlorines in cultivated and non cultivated soils were 0.09-2.3 and 0.09-2.1 ng/g of soil (Thao et al 1993).

Kannan et al (2003) reported DDT concentrations from 0.11-to 45 ng/g dry weight and 0.34 to 34 ng/g dry weight from Georgia. p,p-DDT was the predominant metabolite of DDT, accounting for an average 69% of the total DDT concentrations and p,p-DDE accounted for 33% of total DDT concentrations. Concentrations of total HCH’s were less than 1 ng/g in all the soils. Among HCH isomers, beta and gamma HCH isomers were found in some soils. Fu et al (2001) reported the presence of DDT and HCH in soils from Tibet during 1993-95. The concentration of DDT’s ranged from undetected level to 2.83 ng/g soil.

In South Korea soils, the main pesticides found were gamma and delta-HCH, Heptachlor epoxide and dieldrin in the range of 0.17 - 0.94,
0.77 - 2.97, 1.38 - 48 and 0.32 - 0.49 ng/g of soil respectively (Kim et al 2001). In Poland, the soils in the cities of Kranow and Katowise are more polluted by organochlorines (Falandysz et al 2001).

The soil samples from New South Wales, Australia contains DDE concentration in the range of 0 to 2 ppm (Sivaramaiah et al 2002). Concentration of organochlorine pesticide in top soils of a selected farm in Beijing in 1993 and 2003 was studied. The results indicated that DDT concentration was 93.2 % and 81.2 % (Shi et al 2005). The study conducted in agricultural soil of Argentina showed organochlorine contamination. Among the organochlorine pesticides, lindane was the most dominant one (32.6 ng/g dry weight in the upper and 173.9 ng/g dry weight in the lower horizon). DDT was found at low levels in the surface soils (6.8 ng/g dry weight) (Miglioranza et al 2002). HCH contamination in Tianjin, China was studied by Gong et al (2004). They reported that concentrations of delta HCH ranged from 1.3 to 1095 ng/g among which beta HCH accounted for 52%. Stream sediments from Spain contained organochlorine pesticides like HCB, HCH (Alpha and Beta) at 29 and 392 μg/kg indicating that there is a moderate to severe pollution in the area even though they were banned few decades ago (Lopez et al 2005).

Bashour et al (2004) reported the level of residual DDT in labanese soil. A total of 113 surface soil samples were collected for analysis from major agricultural regions. The values of residual DDT in the soil ranged between 10 and 1190 ng/g. The rhizosphere soils and bulk soils were collected from two sites (A and B) in Tianjan, China for the determination of HCH and DDT and their derivatives. The average concentrations of total HCH and DDT in the bulk soils were 3.6 and 80 ng/g for site A and 102 and 235 ng/g for site B respectively. Relative accumulation of HCH and DDT in the rhizosphere soil from site A but not from site B (Tao et al 2005).
2.3.2 Residues in water

The samples from rural area Farrukabad in the vicinity of the Ganger river in Utter Pradesh, Northern India were found to be contaminated with residues of HCH, DDT and aldrin. The values exceed the WHO guideline value (Mohapatra et al 1995 and Nayak et al 1995). Herman et al (2005) reported the contamination of endosulfan, atrazine, aldrin, dieldrin and chlorpyrifos in canal of South Florida with an average concentrations of 16, 11, 9, 2.6 and 6 ng/L respectively. The endosulfan and other pesticide contamination was maximum during high agricultural activity. Fatoki and Awofolu (2004) reported that the organochlorines range from 5.5 ng/L (2,4-D) to 160 ng/g (HCB) in the water samples of South Africa.

2.3.3 Residues in vegetables

The contamination of growing plants occurs by adhesive of volatile substances from the air to the plasmid surface and by the migration of contaminants through xylem in inner ascendant transport. Rajabhaskar et al (2001) reported the presence of endosulfan residues in Bhendi (Lady’s finger) under field conditions revealed that the initial deposit of 3.18 ppm and 4.39 ppm for 700 and 1400 g a.i/ha doses. These residues dissipated to an extent of 0.523 and 0.868 ppm level by 7 and 15 days after third spraying with the corresponding half-life values of 3.20 and 5.07 days and the safe waiting periods of 2.1 and 4.5 days.

Pesticide residues in 127 vegetables like brinjal, bhendi, cucumber, tomato and carrot from Vijayawada of Andhra Pradesh were analysed for BHC, α-BHC and DDT. Out of 54 samples, 42 samples were contaminated with pesticide residues. The concentration of pesticide residues exceeded the tolerance limits in 14 vegetable samples (Sreenivasa Rao and Ramamohana Rao 2000; Gonzalez et al 2003).
Singh and Kulshrestha (2004) reported the presence of endosulfan residues in *Solanum melongena*. The residues in different samples were in the range of 5-22 ppb. The concentrations of total HCH and DDT in vegetable roots were 3.6 to 60 and 4.2 to 73 ng/g for site A, 50 to 152 and 7.1 to 136 ng/g for site B respectively (Tao et al 2005).

Chandrasekar et al (2001) collected and analysed the pesticide residues in farm gate, market samples of vegetables like brinjal, tomato, cauliflower, cabbage, green chilli, peas, etc. Out of 164 vegetable samples monitored for the pesticide contamination, 113 vegetables were contaminated with one or more pesticides.

### 2.3.4 Residues in birds

Colonially nesting aquatic birds can indicate site specific contamination because they are high level predators and depended upon local resources during breeding seasons. DDE were greater in eggs of *F. sparverius* (10.8 mg/kg), American robins (17.3 mg/kg), European starlings (8.8 mg/kg) (Hebert et al 1994). The eggs of black crowned night heron (*Niiclorax niclicorax*) contained PCB, p,p-DDE, oxichlordane, heptachlor epoxide with higher amounts in Newyork harbour (Matz and Parsons 2004; Manosa et al 2003).

### 2.3.5 Residues in milk

Breast-feeding has been responsible for elevated concentration of these organochlorines compounds as well as harmful effects in children latter in life. The serum levels of BHC, HCB, p,p-DDT, p,p-DDE and p,p-DDT in 41 volunteers (14 men and 27 womens) in the rural area of northern Japan were studied by Hanaoka et al (2002). The level of ß-HCH was
0.5 (0.05 to 1.5), HCB 0.2 (0.02 to 0.7) and total DDT was 5.0 (0.9 to 31 ng/mL of serum). The beta HCH levels increased with rice and milk intakes.

Blood samples were taken from each 10 breast-fed and bottle-fed infants at six weeks of age. The serum concentrations of organochlorine compounds in breast fed infants were significantly higher than the bottle fed infants (PCB 0.38 vs 0.1 mg/L), (HCB 0.13 vs 0.04 mg/L), (p,p-DDE 1.05 vs 0.18 mg/L) (Diamond et al 2005; Kunisue et al 2004; Minh et al 2004). Organochlorine pesticides were analysed in human breast milk samples collected in 2003 primipara mothers living in Penang, Malaysia. Organochlorines were detected in all the samples with DDT’s, HCH and PCB as the major contaminants. The residue levels of all the organochlorines are higher than those in general population of other countries (Sundaryanto et al 2005). Human breast milk collected from Hongkong samples contained p,p-DDT (0.39 ng/g), p,p-DDE (2.48 ng/g) and HCH was 0.95 ng/g (Wong et al 2002).

Alawi and Hawadi (2005) reported the organochlorines in sheep milk in Jordan. The total HCH isomers were detected in 59 samples out of 60 (98.33%). The gamma isomer was found in 39 samples (65%) in the range of 0.024-5.29 mg/kg milk fat. The alpha isomer was found in 27 samples at a concentration range of 0.002-1.11 mg/kg milk fat. The beta isomer was found in 56 samples (93.3 %) in the range of 0.009-4.45 mg/kg milk fat.

2.3.6 Residues in food

Honey samples collected from Portugal and Spain during the year 2002 contains organochlorines. Among them γ-HCH was the most frequently detected in 50% of the samples, followed by HCB in 32% of the samples and
other isomers of HCH (α and β) in 28% and 26% of the samples. Residues of DDT and their metabolites were detected in 20% of the samples (Blasco et al 2003 and Manirahiza et al 2002).

2.3.7 Residues in fish

HCH, DDT and PCB’s were analysed in fish flesh collected from fish ponds in the Pearl river delta. The concentrations of these contaminants were < 0.01 to 7.8 ng/g lipid for HCH, 22.3 to 38.1 ng/g lipid for DDT and 6.0 to 48 ng/g lipids for PCB (Zhou and Wong 2004). Fish samples collected from a local market in Zhoushan city, an Island in the East China showed the concentrations of organochlorines and PCB’s from 0.67 to 13 and 0.24 to 1.4 ng/g dry weight respectively. Concentrations of p,p-DDE in fish meat were comparatively high (3.9 ng/g wet weight). The daily fish consumption based on a dietary survey conducted among 160 local healthy residents was determined to be 105 g/person. The relevant cancer bench mark concentrations of HCB, dieldrin, chlordane, DDT’s and PCB’s were 0.36, 0.04, 1.6, 1.7 and 0.29 ng/kg respectively based on the local diet. The hazard ratios (HR’s) based on non cancer end points were all less than 1.0 while the HR’s based on cancer were greater than 1 for certain contaminants based on the 95th centile concentration in fish tissue (Jiang et al 2005).

2.3.8 Residues in fodder

The total HCH isomers were detected in 19 out of 20 fodder samples (95%). The gamma isomer was found in 15 samples in the concentration range of 0.9-31.5 mg/kg. The alpha isomer was found in 12 samples in the concentration range of 0.9-14.0 mg/kg. The beta isomer was found in 11 samples in the concentration range of 0.7-12.6 mg/kg (Alawi and Hawadi 2005).
2.3.9 Residues in animals

Blubber samples from ringed seal (*Phoca hispida*) and polar bear subcutaneous fat (*Ursus maritimus*) were collected near a barrow, Alaska in 1996. The samples contained $\Sigma$PCB’s were 732 ± 282 ng/g in seals and 3395 ± 1442 ng/g in polar bears. The $\Sigma$DDT, $\Sigma$HCH and HCB concentrations in seals and bears were 562 ± 57 ng/g vs 74.8 ± 39 ng/g, 380 ± 213 ng/g vs 515 ± 35 ng/g and 17.4±10.1 ng/g vs 183±153 ng/g respectively (Kucklick et al 2002; Bartuszevige et al 2002; Hoeksra et al 2003).

The bivalves *Anadara senilis*, *Crassostrea tulifpa* and *Perna perna* from Gana, Canada were analyzed for their organochlorine concentrations. The results showed that $\Sigma$PCB was 0.1mg/g dry weight. There was no correlation between PCB concentration and lipid content reflecting the importance of indirect contamination of the bivalves. The most abundant pesticides were the $\Sigma$DDT 73 and $\Sigma$HCH 29ng/g dry weight. DDT/DDE and hept/heptachlor epoxide ratios reflect their recent applications, while the ratio from aldrin and dieldrin suggest that the use of aldrin has been discontinued, at least one of the coast of Gana. Global scenario of organochlorine pesticide pollution in different field samples and countries are presented in Table 2.1.

2.4 SOURCES OF PESTICIDE CONTAMINATION ON AGRICULTURAL FARMS

The main sources of pesticide contamination on agricultural farms are classified into direct and indirect contamination. The direct contamination includes spray application (pesticides are voluntarily spread on soils or sprayed over crops to control pests in agriculture) and accidental spillage or irresponsible disposal.
<table>
<thead>
<tr>
<th>Country</th>
<th>Type of Sample</th>
<th>HCH and its derivatives</th>
<th>DDT and its derivatives</th>
<th>Endosulfan and its derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>Soil</td>
<td>32.6 to 173.9 ng/g</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Australia</td>
<td>Soil</td>
<td>16.2 mg/kg</td>
<td>1.35 to 32 mg/kg</td>
<td>0.02 to 4.3 mg/kg</td>
</tr>
<tr>
<td>China</td>
<td>Soil</td>
<td>2.5 to 55 mg/kg</td>
<td>2.4 to 130 µg/kg</td>
<td>2.5 to 5.8 mg/kg</td>
</tr>
<tr>
<td>Columbia</td>
<td>Soil</td>
<td>---</td>
<td>0.02 to 16.2 mg/kg</td>
<td>0.02 to 5.6 mg/kg</td>
</tr>
<tr>
<td>Columbia</td>
<td>Sediment</td>
<td>---</td>
<td>0.02 to 9.73 mg/kg</td>
<td>0.02 to 3.3 mg/kg</td>
</tr>
<tr>
<td>Georgia</td>
<td>Soil</td>
<td>---</td>
<td>0.11 to 45 ng/kg</td>
<td>---</td>
</tr>
<tr>
<td>India</td>
<td>Soil</td>
<td>5 ppb</td>
<td>130 µg/g</td>
<td>5.8 µg/g</td>
</tr>
<tr>
<td>Japan</td>
<td>Milk</td>
<td>0.5 to 1.5 ng/mL</td>
<td>09 to 31 ng/mL</td>
<td>---</td>
</tr>
<tr>
<td>Lebanon</td>
<td>Soil</td>
<td>10 to 1190 ng/g</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Nigeria</td>
<td>Soil</td>
<td>5.7 to 24.3 ng/g</td>
<td>7.9 to 57.6 ng/g</td>
<td>0.3 to 11.3 ng/g</td>
</tr>
<tr>
<td>Spain</td>
<td>Soil</td>
<td>392 µg/kg</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Portugal</td>
<td>Soil</td>
<td></td>
<td>0.4 to 4.8 mg/kg</td>
<td>---</td>
</tr>
<tr>
<td>USA</td>
<td>Water</td>
<td>3 to 350 µg/L</td>
<td>0.2 to 1.3µg/L</td>
<td>0.12 to 2.3 µg/L</td>
</tr>
<tr>
<td>USA</td>
<td>Soil</td>
<td>0.2 to 16.3 mg/kg</td>
<td>0.1 to 45 mg/kg</td>
<td>0.02 to 5.7 mg/kg</td>
</tr>
<tr>
<td>USA</td>
<td>Fish</td>
<td>0.1 to 13.6 µg/kg</td>
<td>0.5 to 9.3 µg/kg</td>
<td>0.05 to 11.3µg/kg</td>
</tr>
</tbody>
</table>

Source: [www.global scenario/pesticidepollution.com](http://www.global scenario/pesticidepollution.com)
A survey carried out in the USA, for instance, estimated that millions of tonnes of unwanted products have accumulated over the past 60 years in thousands of barns across the country (Jones 1993). Pesticides are unwanted for various reasons such as (i) some are products whose registration has been cancelled (ii) they did not perform as desired or intended (iii) others have been replaced by alternative products or (iv) pests became resistant to the pesticides (Centner and Gunter 1999).

The disposal of an unwanted pesticide is costlier as farmers have to incur the full cost of legal disposal. This leads to its disposal in landfill or dispersal or spillage into the environment, where they may cause pollution and other problems.

The indirect contamination includes spray drift, atmospheric transport and deposition. Whenever pesticides are applied to agricultural fields as spray from either ground based equipment or aircraft, there is an initial irrigation of some of the active ingredients away from the target area. This takes the form of droplet drift at the actual time of spraying. The smaller size fraction is preferentially transferred downward rather than deposited out in the target area. Then, following the application and extending over several hours at least, more pesticide may be carried down wind through volatilization depending on the compound’s volatility (Crover et al 1997).

Global pesticide consumption was estimated to be 2.5 billion kg/y. The maximum consumption led to greater persistence of pesticides. However greater their persistence, greater their potential for transport away from the target area. Agricultural pesticides therefore enter the atmosphere by spray drifts, volatilization and wind erosion of soil. Volatilization can remove a large fraction of the pesticide initially applied to the field.
Pesticides are emitted from non-agricultural usage for example, from lawns, parks, gardens and buildings treated for pest control (Majesvki and Capel 1996). Emissions from soil and water that were contaminated with pesticides in the past may be a significant contributor to contemporary atmospheric burdens especially organochlorine compounds. Pesticides with low Henry’s constants (eg. Dieldrin and HCH) are removed by gas scavenging, while high constants pesticides (chlordane) are removed mainly by washout of water. Once they have been deposited on soil or vegetation, persistent compound can reenter the atmosphere and recycle below the atmosphere or surface, depending on the temperature (Muir et al 1996; Bidleman et al 1999 and Atterby et al 2002)

2.5 FATE OF PESTICIDES IN THE ENVIRONMENT

Once pesticides have entered the soil, they can undergo several different fates in the soil systems. These are summarised in Figure 2.2. Their retention by the soil and the extent to which they volatalize, leach, degrade etc. is controlled fundamentally by their interaction with the soil itself.
2.5.1 Degradation

Pesticide degradation is one of the major processes in the environment and can proceed through biological and non-biological pathways. The rate of degradation of pesticides is a function of the chemical properties (i.e., structure, toxicity, solubility and concentration etc). Biodegradation can be hampered by poor bioavailability of the compound. Mass transfer limitations through the process of sorption, desorption and solubilization can potentially reduce the amount of chemical available for microbial uptake, mineralization (Beck and Jones 1995; Gerson and Zajic 1979 and Zehnder 1990). The rate of degradation is also a function of the prevailing temperature, moisture regime, pH, mineral nutrients, micronutrients, treatment rate, treatment frequency, the content, type of organic matter and clays present (Kookana et al 1998). The way of an
organic molecule to the metabolizing bacterium is mainly described by diffusion. Diffusion, however, is influenced by both medium density and distance (concentration gradient). Both parameters have important features concerning remediation techniques (Plass et al 1993).

The biodegradation of hydrophobic substances was affected by soil moisture, pH, mineral nutrients, micronutrients, organic supplements, treatment rate, treatment frequency and incubation temperature. Biodegradation was optimal at a soil water holding capacity of 30 to 90%, a pH of 7.5 to 7.8, C:N, C:P ratios of 60:1 and 800:1 respectively and a temperature of 20ºC or above. The solubility of DDT increased with increasing concentration of humic acid when the pH of the samples were low (adjusted to about 5.5). Higher soil temperature and less organic matter in the soil showed the disappearance of DDT in a subtropical environment. Addition of micronutrients and organic supplements were not beneficial. Breakdown of the saturated hydrocarbon fraction was the highest at low application rates favoured the biodegradation of the aromatic and asphaltic fractions (Dibble and Bartha 1979; Masters and Inman 2000; Haarstad and Freswig 2000; Liu and Lin 1994).

Kaiser et al (1998) reported that high amounts of Al and Fe oxides are often accompanied by a huge accumulation of soil organic matter (SOM). Intimate association of the organic matter with the soil minerals may also protect hydrophobic substances from further microbial degradation.

Alawi et al (1995) reported that the interacted amount of pesticide was directly proportional to the concentration of soluble humic acid and inversely proportional to pesticide polarity. There was no significant change in the interacted amount of pesticide with respect to change in temperature,
with pH increases, there was a small decrease in the interacted amount of tetradifon, while no change were detected for DDE and DDT.

The dissolved organic carbon enhances the transport of OCP’s in the soil columns. In the OCP-DOC column, the migration of aldrin, DDT and its metabolites are faster than those in the OCP’s column, when DOC is applied for remediation of soil pollution (Ding and Wu 1997; Breedveld and Karlsen 2000).

Kumaran and Suseeladevi (2001) reported that the soil from Arivurite soil series with higher hydraulic conductivity (2 cm$^{-1}$ h$^{-1}$) and light textured nature exhibited more distribution of atrazine and pendimethrin in the soil column. But, the soil from Devanur series with heavy texture and low hydraulic conductivity (0.92 cm$^{-1}$h$^{-1}$) resulted in poor distribution of these herbicides. It was found that duration of leaching reduced the retention of herbicides retained in the top layer of soil column and increased in lower layers.

Soil moisture content and soil temperature are two most influential factors on the degradation rates and patterns of both alpha and beta endosulfan isomers. The degradation of alpha endosulfan is very fast when both soil moisture content and temperature are high with an average half-life of around 7 days. At low moisture content and low temperature, the average half-life increases to around 27 days and in water logged soil to around 50 days. The degradation curve for alpha endosulfan has a bi-exponential characteristics and the sharp slow down in its degradation rate happens at about 20 days after application (Ghadiri and Rose 2001).

Laabs et al (2000) conducted the free-draining lysimeter field study in Brazilian sandy clay soil with the temperature of 23ºC and pH value of 4.3.
They reported that half-life of alpha endosulfan is 25 days. Submerging the soil substantially increased the half-lives of both alpha and beta endosulfan isomers. The half-life of beta endosulfan remained higher than that of alpha isomer in the submerged experiments carried out under different temperature regimes. The degradation of soil bound alpha endosulfan is always faster than beta isomer under submerged condition at high incubation temperatures.

Endosulfan decreased to less than 45% of the original level under non-flooded conditions irrespective of organic addition as compared to decrease of 15-18 % under flooded conditions during the same period (Sethunathan et al 2002). Awasthi et al (2000) identified moisture content as one of the influential factors in endosulfan degradation. They demonstrated that pH, concentration of organochlorines and size of inocula to be the principal factors in endosulfan degradation.

Shakoori et al (1999) reported that the degradation of endosulfan was optimum at pH 8.5 and temperature of 36º C. The half-life of beta endosulfan was 22 days followed by biphasic pattern in 52 days in the municipal solid waste compost treatment and the exploited half-life was about 115 days for the other treatment (Alhassan et al 2004).

2.5.1.1 Biological mechanism

The microbially mediated breakdown of pesticides has been identified to be more important in degradation compared to physical means (Figure 2.3). The transformation process can be mediated by one or several organisms (Skipper and Turch 1995). The microbial influence either proceeds directly through mineralization, co-metabolism, polymerization or conjugation/accumulation or indirectly through secondary effects of microbial activity altering soil pH and redox conditions (Bellag and Liu 1990).
Several intensive studies on the biodegradation of endosulfan in soils or in water environments have been conducted using pure and mixed cultures of microorganisms (Awasthi et al 2003; Siddique et al 2003; Lee et al 2003; Sethunathan et al 2004; Sutherland et al 2002). Generally, both isomers are degraded by attack at the sulfate group by oxidation to form the toxic metabolite, endosulfan sulfate. In the environment, the formation of endosulfan sulfate occurs only through biological transformation, whereas hydrolysis to the diol occurs readily under alkaline conditions (pH 10) (Martens 1976; Junca et al 2003; Schmidt et al 1997), however Awasthi et al (2002 and 2003) have proved that only at pH above 9.5 resulted in endodiol formation. Endosulfan was subjected to degradation by oxidation and hydrolysis conclusively, endosulfan sulfate formation was found to be favoured as an oxidation production and a novel hydrolysis product
tentatively identified as endosulfan monoaldehyde (Sutherland et al 2000). Molecular structure of endosulfan isomers and their metabolites are presented in Figure 2.4.

![Molecular structure of endosulfan isomers and their metabolites](image)

**Figure 2.4** Molecular structure of endosulfan isomers and their metabolites

Degradation of endosulfan and its utilization as sole carbon and energy source by plasmid harboring and cured strains of two different *Micrococcus sp.* was studied by Guha et al (2000). Plasmid cured strains in comparison to parental strain utilized endosulfan more efficiently as evident from increased biomass accumulation in culture broth with concomitant decrease in residual endosulfan. Addition of endosulfan to the culture grown...
in mineral medium (MM) containing glucose resulted in further increase in degradation of endosulfan by both harboring and cured strains where 25 to 38% and 43 to 81% of added endosulfan could be degraded by different strains under two experimental conditions respectively.

Sutherland et al (2002) identified an enzymatic method for endosulfan sulfate detoxification. The culture described in the study degrades approximately 50 mole\(^{-1}\) endosulfate in 5 days. This degradation is mainly by the action of a hydrolase enzyme directly on endosulfan sulfate. Endosulfan sulfate appeared to be a terminal product. The formation of endosulfan monoaldehyde is from beta endosulfan than from alpha endosulfan. The different oxidation state of the sulphur atom of the two compounds, endosulfan, endosulfan sulfate by the hydrolase enzymes, catalyse the hydrolysis of both the compounds.

The role of blue green algae (*Anabaena sp.* in the soil environment to dissipate endosulfan was studied by Lee et al (2003) who reported that *Anabaena sp.* PCC 7120 produced three principal biotransformation compounds namely endosulfan monoaldehyde, endosulfandiol and minor amounts of endosulfan sulfate. Endosulfan was subjected to degradation by oxidation and hydrolysis is conclusively, endosulfan sulfate formation was found to be favoured as oxidative production and a novel hydrolysis product tentatively identified as endosulfan monoaldehyde.

Sutherland et al (2002) isolated a *Mycobacterium* strain (Strain ESD) capable of metabolizing endosulfan by both oxidative and sulfur separation reactions. The endosulfan degrading reactions are a result of the sulfur starvation response of this bacterium. Studies on the fate of endosulfan in low moisture environments such as soils (Kathpal et al 1997) sterile aqueous systems (Peterson and Batley 1993; Guerin 2001; Guerin and
Kennedy 1992) non-sterile aqueous systems indicate that hydrolysis dominates and suggest that formation of endosulfan sulfate is due to biological oxidation. Walse et al (2002) measured the heterogenous and homogeneous rate constants of endosulfan hydrolysis and indicate that \( \beta \)-endosulfan hydrolysed faster than alpha endosulfan.

Siddique et al (2003) reported that the biodegradation kinetics of endosulfan and metabolic pathway utilized by *Fusarium ventricosum* and *Pandoraea sp*. Complete disappearance of both alpha and beta endosulfan was observed during 12 days of incubation of endosulfan sulfate. *F. ventricosum* degraded about 95 and 100 % of alpha and beta endosulfan respectively in 18 days of incubation in flask spiked with 100 mg of endosulfan.

Kwon et al (2002) reported that the endosulfan degrader *Klebsiella pneumonia* could biologically degrade 8.72 \( \mu \)g endosulfan ml\(^{-1}\) without formation of endosulfan sulfate by a non oxidative pathway. The degradation of alpha endosulfan is faster than of beta endosulfan. Endosulfan diol is the major metabolite even when pH decreases (Mitra et al 2001; Jirku et al 2001 and Yagafarcva et al 2001).

The degradation of alpha and beta isomers of endosulfan by two bacterial co-culture *Bacillus* strains was studied by Awasthi et al (2003). The degradation of both isomers was accompanied by the formation of endosulfan diol and endosulfan lactone. Accumulation of the metabolites, endosulfan sulfate was not observed during the reaction. The metabolism of alpha and beta isomers was found to be comparable and after 12 days of incubation, 94% of the alpha endosulfan and 82% of beta endosulfan has been metabolized. Thirdly, the metabolism of pure alpha and beta isomers of
endosulfan if present individually, 92% of the alpha isomers and 86% of the beta isomer was metabolized during 12 days of incubation.

Enriched culture from soil was able to utilize alpha and beta endosulfan as sulphur sources each producing hydrolysis product endosulfan monoaldehyde as the sole chlorine containing metabolite (Sutherland et al 2002). Alpha endosulfan was more readily hydrolyzed than beta endosulfan.


Alpha endosulfan is known to undergo rapid chemical hydrolysis with increased pH of the medium or soil above pH 7.0. The phototrophic organisms by generating oxic conditions through oxygen release during photosynthesis, may be directly responsible for the enhanced oxidation of alpha endosulfan in soil samples. The introduced algae and cyanobacteria by virtue of their photosynthetic capabilities can promote the proliferation of other members of microbial community such as bacteria and fungi that may in turn facilitate the transformation of alpha endosulfan. Algae degraded the alpha endosulfan to endosulfan sulfate. The major metabolite was endosulfan sulfate and minor metabolite was endosulfan ether in a defined liquid medium. When a high density of the algal inoculum was used, both metabolites appeared to undergo further degradation as evident from their accumulation only in small amounts and the appearance of an endosulfan derived aldehyde (Sethunathan et al 2004).

Walse et al (2002) reported the effect of suspended soils on the oxidation and hydrolysis of the insecticide endosulfan (Alpha and Beta
isomers) and its degradation products. Suspensions of sea sand T10, Alpha Fe₂O₃, Alpha-FeO-OH, lapenite and SiO₂ catalyzed the hydrolysis of endosulfan to the less toxic endosulfan diol. Suspended creek sediment (Bread and Butter creek SC, 4% Organochlorine) inhibited endosulfan hydrolysis. Heterogeneous and homogeneous rate constants of endosulfan hydrolysis were measured and indicated that beta endosulfan hydrolyzes faster than alpha endosulfan.

Sutherland et al (2002) isolated a Mycobacterium sp. (Strain ESD) capable of metabolizing endosulfan by both oxidative and sulfur-separation reactions. Strain ESD did not degraded endosulfan when sulfite, sulfate or methionine were present in the medium along with the insecticide. Partial degradation of endosulfan was occurred when the culture was grown in the presence of DMSO (Dimethyl sulfoxide), cysteine or sulphonin and complete degradation occurred in the presence of glutathion.

2.5.1.2 Physical mechanism

2.5.1.2.1 Photolysis

Photolysis is the transformation of pesticides due to their exposure to radiation. Ultraviolet radiation of sunlight can break down pesticide molecules. Earlier, photolysis was not considered as a degradation pathway for pesticides in soil. Recently, however evidence has emerged to suggest that photo-induced transformations, in some instances be significant. Zayed et al (1994) reported that the degradation of DDT to DDE in soil was enhanced by exposure to sunlight. Over a 90 days exposure period, 65% of the initial DDT remained compared to 91% in the control. It is also known that photo-degradation proceeds faster in moist soils than in dry soils (Klehr et al 1983). Light and heat are the physical agents of primary importance for photolysis of residues on the vegetation, on the soil surface or in water.
Direct photoreactions account for only a part of sunlight induced reactions. Other reactions involving photochemically produced reactive transients such as hydroxyl, hydroperoxyl, super oxide, organoperoxyl and other radicals as well as singlet molecular oxygen may influence the fate of organochemicals in the environment. Thermal decomposition can also be induced by solar radiation.

The algal degradation of alpha endosulfan and endosulfate by pure cultures of Chlorococcum sp. and Scenedesmus sp. was examined by Sethunathan et al (2004). They reported that the phototrophic organisms, by generating oxic conditions through oxygen release during photosynthesis may be directly responsible for the enhanced oxidation of alpha endosulfan in soil samples incubated under light induced condition.

2.5.2  Retention mechanisms

2.5.2.1 Sorption-desorption

Sorption encompasses the physico-chemical processes by which a pesticide molecule present in soil solution binds to the soil particle. It is the major process that attenuates the mobility and accessibility of a chemical to target organisms. The extent of sorption depends on the properties of soil and the compound, which include size, shape, configuration, molecular structure, chemical fractions, solubility, polarity, charge distribution of interacting species and the acid/base nature of the pesticide molecules (Pignatello and Xing 1996).

From a toxicological perspective, binding of pesticides in soil constituents notably soil organic matter, leads to i) a decrease of material available to interact with biota (ii) reduction in the toxicity of the compound (iii) immobilization of the compound, there by reducing its leaching and transport properties (Dec and Bollag 1997).
The reversibility of sorption reactions determines whether the soil solid phase provides a temporary or long term home for pesticides when the process is reversible. The pesticide can be released back in solution, in response to decrease in its solution concentration, (Nicholls 1991) sequestration and soil bound residues are also important in pesticide degradation in the soil ecosystem.

2.5.3 Chemical mechanism

2.5.3.1 Hydrolysis

Hydrolytic degradation of pesticides in soil occurs in soil pore water or on the surface of clay minerals. Hydrolysis has not been identified as a primary route of degradation for members of several classes of pesticides (eg. organophosphates) (Racke et al 1996). Temperature is an important factor governing the rate of hydrolysis in soil pore water. The acceleration of hydrolytic reactions can be described by the Arrhenius equation and may be used to predict the behavior of pesticides in soil. Soil pH also an important property influencing hydrolytic reactions of pesticides. The effect of soil pH in the degradation of a given pesticide depend greatly on whether the compound is most susceptible to alkaline or acid catalysed hydrolysis (Kerpraditshul et al 1993).

2.5.3.2 Redox reactions

Pesticides are susceptible to oxidation or reduction reactions, which occur predominantly in aerobic and anaerobic soils respectively. DDT was rapidly converted to DDD through reductive dechlorination in flooded soils, the rate being dependent on the organic matter of the soils. These degradative mechanisms are important in the tropics associated with flooded rice paddy agriculture.
2.5.4 Dissipation and Transport of Pesticides

The overall dissipation of a pesticide from soil results from a combination of loss mechanisms such as microbial degradation, chemical hydrolysis, photolysis, volatility, leaching and run-off. Each of these transport pathways is considered in the following sections.

2.5.4.1 Volatilization

Volatilization of applied pesticides from soil is one of the important pathways for loss. The rate and extent of the emission during application depends primarily on the application technique and the type of formulation used, whereas the emission after application depends primarily on the properties of the pesticide, soil and environmental conditions (Spencer and Cliath 1990).

Volatilization rates of pesticides from adsorbing surfaces are directly proportional to their vapour pressures. The actual rates of loss are almost entirely dependent on external conditions that affect movement away from the evaporating surface, such as surface roughness, wind speed and air turbulence. In agricultural soils, ploughing/tilling will increase volatilization of pesticides from soil (Dubus et al 2000). The high volatalization rate of alpha endosulfan is due to its low water solubility and relatively high vapour pressure or its high Henry’s constant. The ratio of liquid phase vapour pressure and solubility or solid phase vapour pressure and solubility, provides a value for the Henry’s constant. Alpha endosulfan has a Henry’s constant of 0.72 approximately 18 times that of beta’s (H = 0.04) (Ghadiri and Rose 2001).
2.5.4.2 Leaching

Leaching is a fundamental soil process whereby constituents dissolved or suspended in soil solution are lost from the soil profile by the action of percolating liquid water. The factors which appear to have the most significant influence on pesticide mobility in soils are: physical properties of soil, chemical properties of the pesticide, climatic conditions at the time of application, adsorption, pesticide formulation and method of application (Kan and Tomson 1990).

The prerequisites for leaching of pesticides are entry of the compound into solution and sorption of the compound on to soil particles. Additionally, the moisture content of the soil at the time of pesticide application and the evapotranspiration ratio influence the leaching of pesticides into soil. Pore size distribution and the presence of an impervious layer (horizon) influences the rate of water movement and would probably increase the extent of both desorption and pesticide movement (Jarvis 1998).

2.5.4.3 Surface run-off

Run-off is defined as water and any dissolved or suspended matter it contains that leaves a plot, field or small single cover watershed in surface drainage (Leonard 1990). Pesticide run-off includes dissolved, suspended particulate and sediment adsorbed pesticide that is transported by water from a treated land surface (Raupach et al 2001).

Run-off water to the low land site was the most important factor affecting difference in the concentrations and fluxes of the agricultural chemicals between the two sites. Run-off water to the low land sites appears to have played a dual role by diluting chemical concentration in the
unsaturated zones as well as increasing the concentration at the water table compare to the up land sites (Delin and Landon 2002).

Waterborne pathways include run-off, subsurface percolation and deliberate discharge of tail water. Of these three, run-off can transport endosulfan either in dissolved form or attached to suspended sediments.

Kennedy et al (2001) measured the rates of dissipation from foliage and soil, volatalization from the field and transport of endosulfan residues in irrigation and storm run-off waters. Half-lives of total endosulfan toxic residues (alpha, beta and sulfate product) were 1.6 d in foliage and 7.1 d in soil and could be explained by the rapid volatilization of the parent isomers in the first 5 d. Concentration of endosulfan residues in run-off water varied from 4.5 to 12.5 \( \mu g \) depending on the residue level present on field soil at the time of the irrigation.

2.5.4.4 Biomagnification of organochlorine pesticides

Kuttanad, known as the bowl of Kerala in India is a unique wetland ecosystem with intensive agricultural operation. Pesticide pollution has become a very serious problem in this region. Krishnan et al (2001) reported that the HCH residues was distributed in different trophic levels in food chain. Accumulation of HCH in the Kuttanad ecosystem was in the order of sediment > fish > benthos > frog > plankton > clam > earthworm > human blood > cow milk.

Sharma et al (1999) studied the concentration and biomagnification of organochlorine pesticides in the benthic macro invertebrate (biota) samples collected from 12 different stations of river Yamuna from Hathninund to Juhina (U.P) during premonsoon period in 1996. The biomagnification
factors for BHC, aldrin, endosulfan, dieldrin and DDT ranged from 1700-20 lakhs, 4600-46 lakhs, 400-12 lakhs, 1540-13.5 lakhs and 2375-1.7 lakhs respectively.

Biomagnification of organochlorines in Canada was studied by Harris et al (2000). A soil – earthworm – robin food chain was chosen for study. Organochlorines and PCB’s were measured in soil, earthworm, robin egg and robin nesting samples collected from fruit orchards and reference sites. High average DDE was in soil - 5.2 mg/kg, earthworm - 52 mg/kg, robin egg - 484 mg/kg dry weight and that of DDT was soil - 9.2 mg/kg, earthworm - 21 mg/kg, robin egg 73 mg/kg dry weight. This showed that the contamination of pesticides is common in the region.

Bioaccumulation of organochlorines in fresh water fish *Cyprinus carpio* was studied by Satyanarayanan et al (2005). The rate of bioaccumulation was found to be maximum at 4.38 μg/g wet weight in liver tissue and a minimum of 0.0021 μg/g wet weight in gill tissue in 30 days exposure.

The bioaccumulation of organochlorine contaminants in Bowheads from Barrow, Alaska was studied by Hoekstra et al (2002). The concentration of organochlorine in blubbers samoles were toxaphene (455 ng/g) > polychlorinated biphenols (410 ng/g) > DDT and its related compounds (331 ng/g) > HCH isomers (203 ng/g) > chlordane (183 ng/g) > chlorobenzene (106 ng/g). The dominant analyte in blubber and liver was p,p-DDE and HCH respectively.

Senthilkumar et al (2001) reported the accumulation pattern of organochlorines in biota was generally in the order of HCH > DDT > PCB > CHS < HCB. Magnitude of organochlorine concentrations increased in the
order of sediments < green mussel < earthworm < frog < lizard < fish < bird eggs < bats < bird tissue. Accumulation of the DDT in migratory birds during wintering in India may be of concern due to the great biomagnification potential of DDT’s.

Pollution with organochlorine compounds was evaluated in the upper Oka river in Russia in 1990. The order of ability of the organs of 7 species of fish to accumulate polychlorinated biphenyl was gonads > liver > brain > muscles. Comparison of individual fish species showed that the liver, muscles and gonads of sabrefish were most contaminated with organochlorine compounds (Sukheparova et al 1994).

2.6 EFFECT OF ORGANOCHLORINE PESTICIDES ON THE ENVIRONMENT AND HUMAN HEALTH

2.6.1 Effect on crop

Khan et al (2000) indicated that the endosulfan was phytotoxic even at lower concentration. The adsorption of certain nutrient elements such as Ca, Mn, Co and Cu gets facilitated at lower doses (upto 500 mg/kg soil) and there after an inhibitory trend for the nutrients adsorption was noticed. However, the inhibitory trend for other nutrient uptake such as Mg, P, K, Fe and Zn was observed through the entire range of endosulfan amendments. The residue level of paraquat, which was greater than 100 ppm in the volcanic soil produced chlorosis and decreased the yield in affected crops.

Laboratory and field experiments conducted at Mumbai, India showed that DDT is detrimental to the oil seed crops such as groundnut (Arachis hypogoea L.), soyabean (Glycine max) and Indian mustard (Brasica juncea). The inhibitory effect was directly proportional to DDT concentration on total biomass and economic yield in these crops. Soyabean and Indian
mustard plants showed a large degree of chlorosis in DDT contaminated plots, while nodule formation in groundnut was reduced considerably and was proportional to DDT concentration in soil. Yield of soyabean and Indian mustard was reduced significantly at the lower (100 mg a.i) dose (Mitra and Raghu 1999).

Field experiment in Madhya Pradesh, India was conducted to study the effect of soil application of pesticides containing N, P and S (including BHC (HCH)) on the available nutrients for egg plants (Solanum melongena) at Mandsaur. N, P and S contents of pesticides had no effect but their toxicity increased the available N, P and S contents of the soil. Thimet, Furadon and Temin (aldicarb) treatments resulted in more available N, P and S at each stage of sampling (Khandkar et al 1994).

2.6.2 Effect on fresh water system

Effect of pesticides on the fresh water crab Barytelphusa cuniculris was studied by Shanmugam et al (2000) who reported that the lethal concentration of phosphomidon carbaryl and endosulfan for 24, 48, 72 and 96h leads to behavioural changes, which includes hyperexcitedness, uncoordinated movements, poor response to external stimulus leading paralysis and death. The experimental data revealed that Barytelphusa cuniculris found to be more sensitive to endosulfan and least to phosphomidon among the three chemicals tested.

Monocrotophos has induced marked pathological changes in fish gills. The changes include bulging of tips of primary gill filaments. The secondary gill filaments lost their original shape and curing of secondary gill filaments were observed. The pillar cell nucleus showed necrosis and development of vacuoles in the secondary gill epithelium (Yazdandoost and
Katdare 2000). The effect of insecticide endosulfan on cogenic tadpoles on Australian tree frog \( (Litoria freycenti) \) was studied by Broomhall and Shine (2003). The growth of tadpoles were more slow when exposed to 0.3 or 1.3 \( \mu \text{g/L} \) of endosulfan for 96 hours. This concentration also caused 17\% tadpole mortality and the survivors were more vulnerable to invertebrates predation and tested 15 days after transferred to clean water. The short time exposure to endosulfan in natural water bodies may influence tadpole viability either immediately or over an extended period.

### 2.6.3 Effect on humans

Pesticides are responsible for different diseases, the dreaded one being cancer. In general, pesticides are implicated as inducers of colorectal carcinoma. Organochlorine pesticides normally accumulate in adipose tissue and induce leukemia. They also caused neoplastic lesions in gastric mucosa (Sivaswamy 1991). Brandt et al (2001) reported that, endosulfan exposure implicated fatality and the suggested etiology for sudden onset of status epilepticus with resulting severe neurological weakness. He also explained the neurotoxicity as the primary concern in humans following occupational exposure to endosulfan.

### 2.6.4 Effect on microflora

Meghraj et al (2000) studied the long-term effects of contamination of DDT on soil microflora. Viable counts of bacteria and algae declined with increasing DDT while fungal counts, microbial biomass and dehydrogenase activity increased in 27 mg DDT residues/kg contaminated soil. Species composition of algae and cyanobacteria was altered in contaminated soils and sensitive species were eliminated in high level (37 mg DDT/kg) contaminated soils.
The effect of continuous application of aldrin, HCH, 10% carbofuran, 2,4-D, Baseline and Machetic on the *Nitrosomonas* and *Nitrobacter* populations and N turnover was studied by Pandey and Rai (1993) in Bihar, India. They reported that the reduction in the numbers of nitrifying bacteria and in the concentration of NH$_4$-N and NO$_3$-N was only observed when the pesticide application rates exceeded the recommended doses. Lofez et al (2002) reported the effects of aldrin at rates 10 and 50 mg/mL on microbial activity in soil under aerobic condition. The presence of aldrin decreased denitrification activity at a concentration of 50 mg/mL. Nitrifying population were not affected as a consequence of the addition of aldrin, showing that these microorganisms can tolerate the concentrations of aldrin tested.

The effect of eleven pesticides on the population of bacteria, actinomycetes, fungi and protozoa was investigated by treating a garden soil with the recommended rates. Of the eleven pesticides investigated, Phenyl mercuric acetate at 50 µg/g inhibited bacterial density the most, that is from $5 \times 10^5$ to 220 cells/g. PCB’s at 240 mg/g reduced bacterial population from $5 \times 10^5$ to 2100 cells/g. Whereas, Thiram at 100 µg/g suppressed it by 2 log orders of magnitude. PCB’s reduced actinomycetes density from $3 \times 10^4$ to 320 cells/g and completely eliminated all fungal and protozoan propagules from the soil. In general, protozoa and fungi were most susceptible to fungicides than bacteria and actinomycetes (Ekundayo 2003; Digrak and Ozcelik 1998).

Ghadiri et al (1995) reported that the endosulfan applied to the soil has reduced either the population or the activities of soil microorganisms responsible for the degradation of organochlorines in the soil. The adverse effect of added endosulfan on the degradation of aldrin and dieldrin is more
pronounced at a higher soil-water content of 30%. Endosulfan is mutagenic to bacteria, yeast and mammalia (Chaudhuri et al 1999, ASTDR 2000).

2.6.5 Effect on reptiles

Increasing use of pesticides in Sub-Saharan Africa progressively threatens population of amphibians and reptiles. DDT treatment at 180 g/ha and endosulfan at 200 g/ha caused death among snakes, lizards and frogs. Lizards were killed by chlorpyrifos (Lambert 1997).

2.6.6 Effect on milk

The presence of pesticides residue in milk destruct the fat globules (Chubiko et al 1998).

2.6.7 Genotoxicity

Hop plants treated with heptachlor showed 8 DNA abducts among 16 abducts, 9 of which are new detected in declining hopes. It is suggested that the presence of these hyper modified nucleotides perturbs gene expression and contributes to hop decline. In addition, the genotoxicity of heptachlor was also confirmed by induction of DNA abducts in bean cell suspension culture (Laouedj et al 1995).

2.6.8 Effect on birds

Herbicide 2, 4-D and 2,4,5-T reduced egg production in exposed chicken to 8% and 18% respectively, while DDT exposure caused egg breakage and complete reproductive failure in brown pelican (Gupta et al 2004).
2.7 SOLUBILIZATION OF HYDROPHOBIC SUBSTANCES

One major problem faced during bioremediation of endosulfan is its poor solubility in water, which results in low availability of these compounds to microbial cells and restriction of appropriate biocatalyst (Guerin 2001; Sutherland et al 2002; Kullmann and Matsumura 1996). One promising approach in increasing the bioavailability of these organic compound is addition of surfactants.

2.7.1 Synthetic surfactants

Surfactants are surface active agents, amphiphilic with both a polar head and nonpolar tail. Surfactants fall into four categories nonionic, anionic, cationic and zwitterionic. Surfactants are classified according to the nature of the hydrophilic portion of the molecule.

The head group may carry a negative charge (anionic), a positive charge (cationic), both negative and positive charges (zwitterionic) and no charge (nonionic). Examples of four type of surfactants are presented in Table 2.2. A phenomenon unique to surfactants is the self-assembly of molecules into dynamic clusters called micelles. Micelle formation occurs above a critical concentration of surfactant monomers referred to as the Critical Micelle Concentration (CMC). Examples of surfactant micellisation are presented in Figures 2.5 and 2.6. Surfactants are particularly attractive for mobilization of hydrophobic substances as they potentially have low toxicity and favourable organic solvent based systems.
Table 2.2 Examples of the four types of surfactant

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Ionic type</th>
<th>Molecular structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dodecyl Sulfate</td>
<td>Anionic</td>
<td>CH$_3$(CH$<em>2$)$</em>{10}$OSO$_3^-$Na$^+$</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>Nonionic</td>
<td>CH$_3$-CH$_2$-CH$_2$-CH$_2$-(OCH$_2$CH$_2$)$_x$OH</td>
</tr>
<tr>
<td>Tween 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzytrimethyl Ammonium bromide</td>
<td>Cationic</td>
<td>[CH$_3$N-CH$_2$-(C$_6$H$_5$)]Br$^-$</td>
</tr>
<tr>
<td>3-N-alkyl amino Proionic acid</td>
<td>Zwitterionic</td>
<td>$\text{RNH}_2\text{CH}_2\text{CH}_2\text{COO}^-$</td>
</tr>
</tbody>
</table>

Surfactant enhanced soil washing can result from two distinct mechanisms. One occurs below the critical micelle concentration (soil roll up mechanism) and the other occurs above the critical micelle concentration (solubilization). Surfactant monomers are responsible for the soil roll up mechanism which occurs in two steps. At first the surfactant monomers accumulate at the soil contaminant and soil water interfaces and increases the contact angle between the soil and the contaminant (i.e., change the wettability of the system) surfactant molecules adsorbed on the surface of the contaminant cause a repulsion between the head group of the surfactant molecule and the soil particles thereby promoting the separation of the
Figure 2.5  Examples of surfactant micellisation

Figure 2.6  Basic surfactant structures
contaminant from the soil particles. In the second step, convective currents create agitation and abrasion, which provides the energy necessary to create additional surface area of the oil phase and thus displace the oil from the soil (Rosen 1989).

The second mechanism for enhanced soil washing is solubilization. It results from contaminant partitioning into the hydrophobic core of surfactant micelles. As the number of micelles increase solubilization increases. Thus a concentration well above the CMC is necessary for this enhancement (Deshpande et al 1999). Two methods can be used to examine the appropriateness of the surfactant to a specific contaminant namely the hydrophilic and lipophilic balance (HLB) method and Winsor method.

**HLB Method**

HLB scale was designed for matching surfactant structure to an organic chemical be emulsified in water. Each surfactant has an HLB number indication of the types of oils it can emulsify. HLB requirement of an organic chemical is directly related to hydrophobicity.

**Winsor Method**

In Winsor system, basic approach is the observed relationship between interfacial tension (IFT) and the formation of a middle phase microemulsion. The interfacial tension between the oil and the water phase goes to zero. The capillary number goes to a maximum and the oil is mobilized (West and Harwell 1992).

Zhang et al (1999) reported that the concentration of surfactant is of primary interest in biodegradation studies. The addition of Tween 80 has been
shown to stimulate the utilization of hexadecane by several strains of *P. aeruginosa* (Singer and Finnerty 1984). Similarly, sodium dodecyl sulphate also induced mineralisation of decane by *Pseudomonas* C12B (Suchanek et al 2000). Tiehm (1994) reported that the nonionic surfactants and sodium dodecyl sulphate increased the concentration of PAH in the water phase because of solubilization and also reported that the non toxic surfactants enhanced the degradation of fluorene, phenanthrene, anthracene, fluoranthene and pyrene.

Bruell et al (1997) examined the removal of NAPL in xylene from porous media using a biodegradable 5% sodium lauryl sarsinate surfactant flushing solution. Recovery of 90% residual m-xylene from washed sand was obtained with an average of 43.2 pore columns of surfactant solution. The DOWFAX (8390) components increased the solubilization of phenanthrene (Deshpande et al 2000). The nonionic surfactants (Witconol, SN70) on biodegradation of phenanthrene and hexadecane in soil was studied by Macur and Inskeep (1999). Measurements of phenanthrene solubilization and surface tension indicated that phenanthrene was solubilized at supra CMC levels of surfactant. Addition of surfactant at supra CMC may give changes in interfacial chemistry and subsequent mass transfer processes.

The presence of the synthetic nonionic surfactants Triton X-100, Tergitol NK, Brij 35 and Igepal CA-720 on the bioavailability and biodegradation of crystalline, naphthalene and phenanthrene was investigated. Batch growth experiments showed that the rates of biodegradation of naphthalene and phenanthrene in the dissolution limited growth phase were increased by the addition of surfactant. No toxic effect of the surfactants at concentrations up to 10 g/L were observed (Volkering et al 1995). The rate of biodegradation was reduced at a surfactant concentration above but not below the CMC when the test bacterium grow on biphenyl in the presence of
Triton X-100. This decrease correlated with the CMC and was more pronounced at a biphenyl concentration of 0.2 μg/g/mL than at 2 μg/g/mL, the rate of glutamate degradation was reduced by Triton-X 100 (Roch and Alexander 1995).

Surfactant enhanced solubilization was studied by Walters and Aitkin (2001) who reported that approximately 12 mg surfactant/g soil was required before concentrations greater than the CMC were observed in the liquid phase in soil microcosms. At greater doses solubilization of each DDT, DDD and DDE isomer increased linearly with the surfactant dose. The surfactant substantially increased the rate of DDT degradation during first 9 weeks.

Surfactant even at very low concentration shown to enhance the biodegradation of certain xenobiotics in soil (Haigh and Alcock 1996). Aronstein and Alexander 1993 conducted the study to determine whether nonionic surfactant (Novel 1111412-56) added to the surface of lime silt loam soil enhance the biodegradation of phenanthrene and biphenyl. Water containing the surfactant at 10 μg/mL surfactant markedly enhanced the rate and extent of phenanthrene mineralization. The stimulation was less if the water added to the soil surface contained 100 μg/mL surfactant. The effect of non-ionic surfactants on the solubility and biodegradation of polycyclic aromatic hydrocarbons (PAH) in the aqueous phase and in the soil slurry phase, as well as the fate of these surfactants were investigated by Kim et al (2000). The PAH solubility was linearly proportional to the surfactant concentration when above CMC and increased as the hydrophile-lipophile balance (HLB) value decreased. Substantial amounts of the sorbed phenanthrene in the soil particles were desorbed by non-ionic surfactants into the liquid phase when the ratio of soil to water was
1:10 (g/mL). Brij 30 was the most biodegradable surfactant, showed no substrate inhibition upto 1.5 g/L and was definitely used as a source by the bacteria.

The role of some selected non-ionic, anionic and cationic surfactants was investigated in solubilizing and mobilizing PAH’s from soil. The data from the batch experiment showed that Brij 30 started transporting the PAH’s from soil to water at concentrations well below its apparent CMC. At its high concentration, Brij 30 transported more PAH’s to the aqueous phase. The tested anionic and cationic surfactants did not show the solubilization effect until the concentrations reached their above CMC (Sun and Puri 2000).

The presence of SDS in the culture medium with n-decane as main source of carbon and energy accelerated the growth of *Pseudomonas C12 B*. SDS disappeared from the culture medium in early stages of cultivation suggesting preferential degradation by the bacterium, while the consumption of n-decane was accelerated. This may be associated with the capacity of SDS to induce decane mineralization system in *Pseudomonas C12B* and the ability of SDS to stimulate the surface attachment of competent bacteria resulting in close proximity of the cells and thus enhanced break down of the hydrocarbon pollutant (Suchanek et al 2000). Chun et al (2002) investigated the solubilization of naphthalene and phenanthrene into the micelles formed by three different anionic surfactants. The three surfactants were Sodium Dodecyl Benzene Sulfonate (SDDBS), Monoalkylated disulfonated diphenyl oxide (MADS-C12) and Dialkylated disulfonated diphenyl oxide (DADS-C12). The order of increasing solubility enhancement of naphthalene and phenanthrene was SDDBS < MADS - C12 << DADS - C12 which
indicates that hydrophobic chains in micellar core play more important role for the solubilization of PAH’s than the benzene rings in the palisade layer of micelle.

2.7.2 Biosurfactants

Biodegradation of slightly soluble organic compounds is slow because of low availability of these compounds to microbial cells. Microorganisms possess a number of adaptive mechanisms for accumulating and transporting hydrocarbons into the cells for initial enzymatic catabolism. Many bacteria are capable of emulsifying hydrocarbons in solution by producing surface-active agents, such as biosurfactants, thereby increasing the bioavailability of these compounds to microbial cells (Desai and Banat 1997; Rocha and Infante 1997).

2.7.2.1 Structure of biosurfactants

Biosurfactants are a chemically unique class of compounds produced by many bacterial and fungal genera (Miller and Zhang 1997). The molecular structure of biosurfactants comprises of a hydrophilic portion, which may consist of mono-, oligo-, or polysaccharides, amino acids or peptides or carboxylate or phosphate groups and a hydrophobic portion, which is composed of saturated or unsaturated fatty acids or fatty alcohols (Karanth et al 1999).

Biosurfactants can be classified into several broad groups – glycolipids, lipoamino acids and lipopeptides, polymers of lipoproteins, lipopolysaccharides, phospholipids, mono- and diglycerides, fatty acids and fatty acids/neutral lipids (Fiechter 1992).
In addition, there are species level differences in the chemical structure of biosurfactants. For example, glycolipid containing rhamnose are called rhamnolipids and rhamnolipids produced by *Pseudomonas aeruginosa* differ in the number of rhamnose molecules (mono- or dirhamnolipids) (Rendell et al 1990). The different types of biosurfactant produced by microorganisms and structures are presented in Table 2.3 and Figure 2.7.

### 2.7.2.2 Properties of biosurfactants

Biosurfactants are amphipilic molecules, that can modify the properties of a liquid medium at a surface or interface by reducing the surface tension. Biosurfactants reduce surface tension by accumulating at the interface of immiscible fluids and solids, there by increasing the surface area of insoluble compounds, which leads to increased bioavailability and subsequent biodegradation of hydrocarbons (Kosaric 2001). The glycolipid produced by *Pseudomonas fluorescens* and the biosurfactant from *Bacillus licheniformis* have been shown to reduce the surface tension of aqueous solutions to 26-27 mN/m (Lin et al 1998). At low concentration, surfactants are present as individual molecules. However, as the concentration of the surfactant is increased, where no further change in interfacial properties take place.

The amount of biosurfactant needed to reach this concentration is called the “Critical Micelle Concentration” (CMC) (Miller 1995). At the CMC, molecules aggregate to form monolayer (micelle) or bilayer (vesicle and lamella) structures that have the ability to encapsulate hydrocarbon molecules resulting in either solubilisation or emulsification of the hydrocarbons.
Table 2.3  Production of different types of biosurfactant by microorganisms

<table>
<thead>
<tr>
<th>S.No</th>
<th>Microorganism</th>
<th>Type of biosurfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Pseudomonas aeruginosa,</em> <em>P. oleovarans</em></td>
<td>Rhamnolipid</td>
</tr>
<tr>
<td>2.</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>Peptidolipid</td>
</tr>
<tr>
<td>3.</td>
<td><em>Candida lipolytica</em></td>
<td>Polysaccharide-protein-lipid complex</td>
</tr>
<tr>
<td>4.</td>
<td><em>Rhodococcus erythropolis</em></td>
<td>Trehalose lipid</td>
</tr>
<tr>
<td>5.</td>
<td><em>Bacillus licheniformis</em></td>
<td>Lipopeptide</td>
</tr>
<tr>
<td>6.</td>
<td><em>Acinetobacter calcoaceticus</em></td>
<td>Emulsan (anionic heteropolysaccharide)</td>
</tr>
<tr>
<td>7.</td>
<td><em>Torulopsis bombicola</em></td>
<td>Sophorose lipid</td>
</tr>
<tr>
<td>8.</td>
<td><em>Acinetobacter sp.</em></td>
<td>Phospholipid</td>
</tr>
<tr>
<td>9.</td>
<td><em>Bacillus subtilis</em></td>
<td>Surfactin (Lipoprotein)</td>
</tr>
<tr>
<td>10.</td>
<td><em>Arthrobacter paraffineus</em></td>
<td>Sucrose lipid or fructose lipid</td>
</tr>
<tr>
<td>11.</td>
<td><em>Corynebacterium sp.</em></td>
<td>Glycolipid</td>
</tr>
<tr>
<td>12.</td>
<td><em>Streptosporangium amethystogenes</em></td>
<td>Lipopeptide</td>
</tr>
</tbody>
</table>

2.7.2.3 Production and recovery of biosurfactants

Biosurfactants yield and composition are affected by growth conditions including carbon sources, culture medium nutrients (N, P and Fe),
temperature, pH and aeration (Homes and Ratledge 1993; Banat et al 2000; Kosaric 2001). The carbon source is one of the critical factor affecting the structure and yield of biosurfactant. For example, *P. fluorescens* produced a bioemulsifier during growth on different hydrocarbon substrates and maximum yield was obtained with gasoline as substrate (Desai et al 1998). Rahman et al (1999) also reported biosurfactant production by *Pseudomonas sp.* MR-3 on different carbon source with maximum yield (6.46 g/L) on glucose as substrate.

Nutrients like nitrogen, phosphate etc., could also affect biosurfactant production. For example, biosurfactant production was enhanced when *P. aeruginosa* was grown in nitrate and proteose peptone media (Mulligan and Gibbs 1989). Arino et al (1996) reported that rhamnolipid production by *P. aeruginosa* GLI was stimulated under conditions of nitrogen limitation.

### 2.7.2.4 Mechanism of interaction of biosurfactant with microorganisms

Biosurfactants enhance the emulsification and solubilisation of hydrocarbon substrate and thereby facilitate the growth of microorganisms as hydrocarbons (Lin 1996). Biosurfactants produced by microorganisms may be cell bound or extracellular, when it is secreted into the growth medium. For example, the *Rhodococcus sp.* produced cell-surface associated biosurfactant when grown on hydrocarbon substrates which exhibited increased cell surface hydrophobicity. The organism thus assimilates both solid and liquid alkanes by adhering to the alkane phase (Whyte et al 1999). *P. aeruginosa* ATCC 9027 facilitates degradation of hydrocarbons with limited water solubility by producing extracellular rhamnolipids (Zhang and Miller 1992). A mutant of *P. aeruginosa* which lacked extracellular rhamnolipids was isolated by
Koch et al (1991) which was unable to grow on hexadecane but retained growth upon addition of small amounts of rhamnolipids indicating that rhamnolipids play a major role in hexadecane uptake and utilization by *P. aeruginosa*. A protein like activator is also produced by *P. aeruginosa* and the co-operative action between the activator and rhamnolipid stimulates growth of the organism on hexadecane (Hisatsuka et al 1972). The rhamnolipids increased the bioavailability by increasing both aqueous dispersion and cell hydrophobicity (Zhang and Miller 1994). Modes of microbial uptake of hydrocarbons was presented in Figure 2.8.

Microbial adhesion to hydrocarbons proceeds by many methods. In *Acinetobacter calcoaceticus* RAG-1 it occurs via fimbriae (Rosenberg et al 1982). Baldi et al (1999) observed two types of adhesion in *Acinetobacter venetianus* VE-C3. First cell-to-cell interaction proceeds cell adhesion to n-alkane followed by incorporation of nanodroplets of n-alkanes into the hydrophilic capsular polysaccharide to form a more hydrophobic polysaccharide n-alkane matrix surrounding the cell wall. This results in partitioning of the bulk polar phase between the aqueous medium and the outer cell membrane enabling the organism to grow on diesel oil.

There are 2 mechanisms by which biosurfactants enhance the biodegradation of slightly soluble organic compounds. First, biosurfactants can solubilize hydrophobic compounds in micelle structures, effectively increasing the apparent aqueous solubility of the organic compound and its availability for uptake by a cell.
Figure 2.8  Modes of microbial uptake of hydrocarbons
(Adopted from Miller 1995)

A - Uptake of hydrocarbons dissolved in the aqueous phase surrounding degrading cells;
B - Uptake via direct contact of degrading cells at the aqueous hydrocarbon interface of large oil drops in water;
C - Uptake through direct contact of degrading cells with fine or submicron size oil droplets dispersed in the aqueous phase;
D - Enhanced uptake as a result of production of biosurfactants.

Second biosurfactants can cause the cell surface to become more hydrophobic thereby bring the association of the cell with the slightly soluble substrate (Al-Tahhan et al 2000).
2.7.2.5 Applications of biosurfactants

Biosurfactants have several advantages over synthetic surfactants such as biosurfactants present surface active properties differing in some cases from synthetic surfactants, providing new possibilities for industrial applications. Microbial surfactants have been shown to be more effective and specific than many conventional synthetic surfactants in specific applications and they are usually nontoxic and biodegradable. The various application of biosurfactants were presented in Table 2.4.

The application of surfactants from *Bacillus subtilis* 09 on the bio remediation of soils polluted with crude oil was studied by Cubitto et al (2004) reported that *Bacillus subtilis* (09) did not negatively affected the hydrocarbon degrading microbial population and concentrations of *Bacillus subtilis* 19 and 19.5 mg stimulated the growth of the population involved in the crude oil degradation and accelerated the biodegradation of the aliphatic hydrocarbons.

The release of surface active compound promotes an emulsification of the hydrocarbon phases, rendering such lipophilic molecules available to the metabolic pathways of microorganism (Zhang and Miller 1992). The biodegradation of hexadecane by five biosurfactant producing bacterial strains (*Pseudomonas aeruginosa* UG2, *Acinetobacter calcoacticius* RAG 1, *Rhodococcus erythropolis* DSM 43066, *R. erythropolis* ATCC 19538 and strain BCG 112) was determined in the presence and absence of exogeneously added biosurfactants.
Table 2.4 Applications of biosurfactants

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ceramics processing</td>
<td>Horowitz and Currie (1990)</td>
</tr>
<tr>
<td>5.</td>
<td>Therapeutic</td>
<td>Takizawa et al 1995</td>
</tr>
</tbody>
</table>

The degradation of hexadecane by *Pseudomonas aeruginosa* UG2 was stimulated only by the rhamnolipid biosurfactant induced by the same organochlorines (Noordman and Janssen 2002). The literature overview on the effect of surfactants on biodegradation of sorbed hydrophobic organic compounds in contaminated soil was presented in Tables 2.5 and 2.6.
Table 2.5  Literature overview on the effect of surfactants on biodegradation of sorbed hydrophobic organic compounds

<table>
<thead>
<tr>
<th>Authors</th>
<th>Matrix</th>
<th>Contamination</th>
<th>Microorganisms</th>
<th>Setup</th>
<th>Surfactant Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viney and Bewley (1990)</td>
<td>Sand</td>
<td>Polychlorinated biphenyls</td>
<td>Several isolates and pure strains</td>
<td>Microcosm</td>
<td>Alkylphenol ethoxylates</td>
</tr>
<tr>
<td>Aronstein et al (1991); Aronstein et al (1992); Aronstein and Alexander (1993)</td>
<td>Sand (0.4% o.c). Slit loam (7.6% o.c), Muck (32.9% o.c)</td>
<td>Phenanthrene biphenyl</td>
<td>Mixed culture</td>
<td>Shaking soil slurries and column experiments</td>
<td>Alcohol ethoxylates</td>
</tr>
<tr>
<td>Laha and Luthy (1991); Laha and Luthy (1992)</td>
<td>Slit loam (1.5% o.c)</td>
<td>Phenanthrene</td>
<td>Mixed culture</td>
<td>Shaking soil slurries</td>
<td>Several Nonionic</td>
</tr>
<tr>
<td>Dohse and Lion (1995)</td>
<td>Sand (0.05% o.c)</td>
<td>Phenanthrene</td>
<td>Mixed culture</td>
<td>Shaking soil slurries</td>
<td>Microbial Polymers</td>
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<tr>
<td>Providenti et al (1995a,b)</td>
<td>Sandy loam</td>
<td>Phenanthrene creosote</td>
<td>Mixed culture</td>
<td>Shaking soil slurries</td>
<td>Rhamnolipid Biosurfactants</td>
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<tr>
<td>Surfactant Concentration</td>
<td>Effect on Desorption</td>
<td>Effect on Biodegradation</td>
<td>Explanation</td>
<td></td>
<td></td>
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<td>--------------------------</td>
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<td>--------------------------</td>
<td>-------------</td>
<td></td>
<td></td>
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<tr>
<td>5 gL⁻¹</td>
<td>Stimulation</td>
<td>Inhibition</td>
<td>Surfactant Toxicity</td>
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<tr>
<td>Sub-CMC</td>
<td>No effect</td>
<td>Inhibition at high surfactant Concentration</td>
<td>(No Toxicity)</td>
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</tr>
<tr>
<td>Varying</td>
<td>Stimulation of Partitioning</td>
<td>No Effect</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td>30 or 100 mg TOC L⁻¹</td>
<td>Stimulation</td>
<td>Stimulation</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varying</td>
<td>Not determined</td>
<td>Mixed</td>
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<tr>
<td>100-400 mgL⁻¹</td>
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<td></td>
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</tr>
<tr>
<td>Authors</td>
<td>Items</td>
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<td>Contamination</td>
<td>Microorganisms</td>
<td>Setup</td>
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</tr>
<tr>
<td>Bewley et al (1989)</td>
<td></td>
<td>Complex</td>
<td>Coal tar (PAHs)</td>
<td>Mixed Culture</td>
<td>In situ treatment and landfarming</td>
</tr>
<tr>
<td>Ellis et al (1991)</td>
<td></td>
<td>Sandy clay and clay loam</td>
<td>Creosote</td>
<td>Pseudomonas sp.</td>
<td>Microcosms, columns</td>
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<tr>
<td>Marks et al (1992)</td>
<td></td>
<td>Refining sludges and petrochemical sludge</td>
<td>PAHs</td>
<td>Mixed culture</td>
<td>Continuous slurry reactors</td>
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<tr>
<td>Ghosh et al (1995)</td>
<td></td>
<td>MGP soil with 75% o.c.</td>
<td>PAHs</td>
<td>Enriched mixed culture</td>
<td>Batch</td>
</tr>
<tr>
<td>Effect on Desorption</td>
<td>Stimulation</td>
<td>Stimulation of solubilization and leaching</td>
<td>Possible stimulation of partitioning</td>
<td>Not determined</td>
<td>Stimulation of Biodegradation</td>
</tr>
<tr>
<td>----------------------</td>
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<td>------------------------------------------</td>
<td>-------------------------------------</td>
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<td>----------------------------------</td>
</tr>
<tr>
<td></td>
<td>Possible stimulation of biodegradation</td>
<td>Stimulation in microcosms, possible stimulation in full scale treatment</td>
<td>Possible stimulation of biodegradation</td>
<td>Not determined</td>
<td>Possible stimulation of biodegradation</td>
</tr>
<tr>
<td>Explanation</td>
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<td>No blanks, several parameters changed</td>
<td>Two parameters changed</td>
<td>No blanks, several parameters changed</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Surfactant concentration to low</td>
<td></td>
</tr>
</tbody>
</table>