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Review Article

LINDANE AND ITS DEGRADATION FROM ENVIRONMENT USING BIOTECHNOLOGICAL APPROACH: A REVIEW

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ABSTRACT

This review describes the different aspects of microbial degradation of Hexachlorocyclohexane from different source in the last decade and it is envised in terms of its different routes through food chain which will lead to bio magnifications (effect on living system), characterization of genes and enzymes in microbial system responsible for degradation, and last is the exploration of novel potential strains of degrading bacteria from different environmental sources/sites. Technology based approach for the identification, isolation of potent genes and enzymes from the unculturable sources or transfer of degrading genes to diverse host system will provide us platform for future research. Since toxicity of lindane is well known now, it is imperative to develop methods to remove it from the environment. Bioremediation technologies, which use microorganisms and/or plants to degrade toxic contaminants, have become focus of interest. Microorganisms play a significant role in the transformation and degradation of xenobiotic compounds. Many gram negative bacteria have been reported to have metabolic abilities to attack Hexachlorocyclohexane. Although the use of this insecticide has been banned or restricted in most of countries due to their toxicity and long persistence in upland soil but it continues to be a global issue. There are innumerable scopes in the degradation of persistent organic pollutants from environment but they need to be decoded.

INTRODUCTION

Toxic organic compounds like polycyclic aromatic hydrocarbons, poly chlorine biphenyls, chlorophenols and dyes released into the environment due to man-made activity and rapid industrializations. The micro organisms have the ability to use these compounds for their metabolic activities and leads to physical and chemical change or mediate complete degradation (Raymond *et al.*, 2001; Wiren-lehr *et al.*, 2002). The pesticide degradation mediated by a variety of microorganisms such as bacteria, fungi and actinomycetes. Fungi mediate the minor change in pesticide / xenobiotic compounds and their bio transformed compound released into soil where it is further accessed by bacteria for degradation (Gianfreda and Rao, 2004). The intoxication and removal of POPs and HOCs from contaminated site is major problem today and need for attention at global level particularly in developing countries (Bezama *et al.*, 2008). POPs like PCBs, dibenzo-p-dioxins, dibenzofurans and organo chlorine pesticides, they resist breakdown in the environment by physical, biological, chemical and photolysis, therefore they continue to persist in the environment for the long time. They accumulate in food chain due to their lipophilicity, bio magnifications and bio magnifications property. The effect of most common OCPs (DDT, HCH and Endosulfan) reviewed recently. (Mrema *et al.*, 2013)

Research in this aspect needs to give attention as during the last decade it has been increased. Persistent use leads to contamination of soil, sediment, ground waters and surface waters (Mertens, 2006). Lindane (1, 2, 3, 4, 5, 6-Hexachlorocyclohexane, γ-HCH) is a broad spectrum chlorinated pesticide and its production started in 1940s since than it was used in agriculture to prevent the damage caused by vector borne diseases. The HCH formulation consists of γ-(10–12%), α- (60–70%), β- (5–12%) and δ- (6–10%) isomers and out of these only γ-HCH possesses insecticidal activity (Li *et al.*, 2003). Therefore γ-HCH is generally purified with 99% purity; the remaining four isomers are discarded and released as HCH muck (Nagata *et al.*, 2007). The use of γ-HCH has been banned in most countries in agriculture due to their toxicity and long persistence in soil but the pharmaceutical use is permitted till 2015. Large amounts of HCH still remain at the production sites even the units have been closed (Iwata *et al.*, 1993). It is classified as a persistent organic pollutant (POP), potent carcinogen and hazardous as identified POP by

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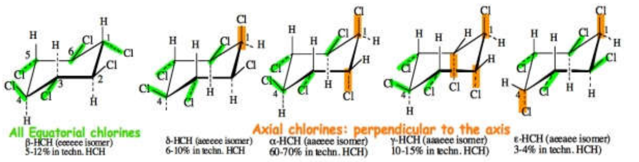
Stockholm Convention 2009. The residues of lindane have been detected in drinking water sources, beverages and in foods (Anon, 2003).

The component of hexachlorocclohexane persists in soil for years and its traces reach to different living organisms through food chain. On percolation in soil it leads to disturbance of the natural micro flora of soil. There is need for great attention for the identification of locally adapted micro organisms from the environment for the development of environment management program by *in situ* and *ex situ* remediation. In recent years a diverse kind of micro organisms being characterized from the habitats *viz*. soil, industrial effluent, polluted sites and other sites. Thereby new strains are being constantly explored and sequenced by the workers from different parts of world. The degrading efficiency of the isolated strains can be increased by the application of gene cloning, mix culturing approach. By the development of new technique like metagenomics and nano biotechnology may prove boon to explore new organisms and potent strains isolated should be utilized in bioremediation program. The developments in microbiology, genomics, gene cloning and sequencing technologies will help in fast identification and characterization of useful genes from the degrading micro organisms. The analysis of the environment at the contaminated sites and design of optimization experiments using mathematical algorithms will help us to achieve the goal in this direction. Therefore, complete analysis of restoration technique employed for a variety of contaminated site cleanup programs (Khan *et al*., 2004). This review is step forward in this direction and reviews the research done in this area. This further gives insight for the characterization of new potent degrading strains with respect to increased efficiency of the γ-HCH degradation.



Aerobic Hexachlorocyclohexane Degradation

In Gram Negative Bacteria

Most of the HCH degrading aerobes known to date are members of family sphingomonadaceae (Lal *et al.,* 2006, 2008). Most important strains named sphingobium jaonicum UT 26, *Sphingobium indicum* B90A, and *Sphingobium francense* Sp^{+} (Boltner *et al*., 2005 Nagata *et al*., 2007). Genes encoding the γ-HCH degradation enzymes have been cloned, sequenced and characterized (Manickam *et al*., 2007). These genes (called lin genes) were initially identified and characterized for *Sphingobium japonicum* UT26 (Nagata *et al;* 1999). During microbial degradation of halogenated compounds the removal of halogen atoms is the key reaction. It is most commonly replaced by hydrogen or a hydroxyl group which results in the reduction of both recalcitrance to biodegradation and risk of forming toxic intermediates.

Degradation pathway of γ-HCH in S. japonicum UT26

The degradation pathway of γ-HCH in UT 26 is shown here in the form of flowchart. This pathway is basically divided into two forms one is upstream and other is downstream. Both the forms are regulated by enzymatic activity so as by their respective genes (*Lin* genes). γ-HCH is converted to 1,2,4-trichlorobenzene (1,2,4-TCB), 2,5- dichlorophenol (2,5-DCP), and 2,5-dichlorohydroquinone(2,5-DCHQ) by enzymatic activities of dehydrochlorinase (LinA; Imai *et al*., 1991), halidohydrolase (LinB; Nagata *et al*., 1993a), and dehydrogenase (LinC; Nagata *et al*., 1994); these steps are referred to as the upstream pathway. Among these three metabolites, 1, 2, 4-TCB and 2, 5-DCP are so-called dead-end products that are not degraded by UT26. Only 2, 5-DCHQ is further metabolized by steps referred to as the downstream pathway. 2, 5-DCHQ is converted to β-ketoadipate by reductive dechlorinase (LinD; Miyauchi *et al*., 1998), ring-cleavage dioxygenase (LinE; Miyauchi *et al*., 1999), and maleylacetate reductase (LinF; Endo *et al*., 2005). Succinyl-CoA: 3-oxoadipate CoA transferase (LinGH) and β-ketoadipyl CoA thiolase (LinJ) were used for the conversion of β-ketoadipate to succinyl–CoA and acetyl–CoA (Endo *et al*., unpublished data). As both compounds are metabolized in the citrate/tricarboxylic acid (TCA) cycle.

γ-HCH
↓
2 pentachlorocyclohexane
↓
1, 3, 4, 6-tetrachloro-1,4-cyclohexadiene
↓
1, 2, 4-trichlorobenzene
↓
2, 4, 5-trichloro-2, 5-cyclohexadiene-1-ol
↓
2, 5-dichlorophenol
↓
2, 5-dichloro-2, 5-cyclohexadiene-1,4-diol
↓
2,5-dichlorohydroquinone
↓
Chlorohydroquinone
↓
Hydroquinone
↓
Acylchloride
↓
γ-hydroxymuconic semialdehyde
↓
Maleylacetate
↓
β-ketoadipate
↓
3-oxoadipyl-CoA
↓
succinyl-CoA
↓
acetyl-CoA
↓
2,6-dichlorohydroquinone
↓
2-chloromaleylacetate
↓
TCA, citrate/tricarboxylic acid cycle; GSH, glutathione (reduced form); GS-SG, glutathione (oxidized form)

In Gram Positive Bacteria

While there have been many reports regarding aerobic degradation of HCH by Gram-negative bacteria, little information is available on the ability of biodegradation of HCH by Gram-positive microorganisms (Lal *et al*., 2010). Regarding this approach, the metabolic pathway for pesticide degradation by actinobacteria (Gram-positive bacteria with high G+C content of DNA) has not been studied extensively; but these can produce extracellular enzymes that degrade a wide range of complex organic compounds. A common feature of the aerobic actinobacterias is the presence of many types of monooxygenases and dioxygenases (Larkin *et al*., 2005). Manickam *et al*., (2011) isolated the actinobacteria *Microbacterium* sp. strain ITRC1 which has the ability to degrade all four major isomers of HCH.

Anaerobic Hexachlorocyclohexane Degradation

The first HCH-degrading microorganism to be isolated was *Clostridium sphenoides* UQM780, which anaerobically reduced the concentration of Lindane in minimal salts media (MacRae *et al*., 1969). Further studies with this microorganism identified the degradation intermediates from α- and γ-HCH as δ- and γ-3, 4, 5, 6-TCCH, respectively (Heritage & MacRae 1977). The primary intermediate of anaerobic Lindane degradation by C. sphenoides was supposed to be the reductive dechlorination product pentachlorocyclohexane (PCCH), but it was too unstable.

α-HCH, β-HCH, γ-HCH

$+2e^{-}\downarrow -2Cl^{-}$

γ-TCCH and δ-TCCH

$+2e^{-}\downarrow -2Cl^{-}$

1, 3 – DCDN (5, 6-dichloro-1, 3-cyclohexadiene)

$+2e^{-}\downarrow -2Cl^{-}$

Benzene

And

$+e^{-}\downarrow -Cl^{-}$

MCB (Monochlorobenzene)

Microorganisms have the ability to withstand the sub lethal concentration of toxic compounds and they utilize these compounds as soul carbon source. Several microorganisms had been reported that degrade γ-HCH are fungi, cyanobacteria, and bacteria (that under both aerobic and anaerobic conditions). Yet, only a few of them have been phylogenetically identified (Nagata *et al*., 2007) and they belongs to the genera *Sphingomonas*, *Rhodanobacter* and *Pandoraea* (Mohn *et al*., 2006). The key reaction during microbial degradation of halogenated compounds is the removal of the halogen atom, i.e., dehalogenation of the organic halogen.

Table 1 Summary of microorganisms with demonstrated ability to degrade HCHs

Microorganism	HCH degradation characteristics	Reference
Bacteria	**Aerobic degradation**	
Bacillus sp.	Dechlorinationa (γ-HCH)	Yule *et al*., (1967)
Cyanobacteria: *Anabaena* sp. strain PCC 7120, and *Nostoc ellipsosporum*	Dechlorination (γ-HCH)	Kuritz & Wolk (1995)
Escherichia coli	Dechlorination (γ-HCH)	Francis *et al*., (1975)
Pandoraea sp.	Utilized as sole carbon source (γ-HCH); Dechlorination (α-HCH)	Okeke *et al*., (2002)
Pseudomonas sp.	Dechlorination (γ-HCH)	Tu (1976)
Pseudomonas sp.	Mineralization (α-, β- and γ-HCH) Dechlorination (δ-HCH)	Sahu *et al*., (1992, 1995)
Pseudomonas sp.	Dechlorination (γ-HCH)	Nawab *et al*. (2003)
Pseudomonas putida	Dechlorination (γ-HCH); Isomerization of γ-HCH to α-HCH	Benezet & Matsumura (1973)
Pseudomonas vesicularis P59	Mineralization (α-HCH)	Huntjens *et al*. (1988)
Rhodanobacter lindaniclasticus	Dechlorination (α- and γ-HCH)	Thomas *et al*., (1996)
Sphingomonas paucimobilis B90	Utilized as sole carbon source (α-, β-, δ- and γ-HCH) implies Mineralization.	Adhya *et al*., (1996)
Sphingomonas paucimobilis UT26	Mineralization (γ-HCH); Dechlorination (α- and δ-HCH)	Nagasawa *et al*., (1993) and Senoo & Wada (1989)
	Anaerobic degradation	
Citrobacter freundii	Dechlorination (γ-HCH)	Jagnow *et al*., (1977)
Clostridium rectum S-17	Dechlorination (γ-HCH)	Ohisa & Yamaguchi (1978)
Clostridium sphenoides UQM780	Utilized as sole carbon source (α-, β-, δ- and γ-HCH)	MacRae *et al*., (1969)
Desulfovibrio sp.	Dechlorination (γ-HCH)	Boyle *et al*., (1999)
Fungi		
Cyathus bulleri	Aerobic dechlorination (c-HCH)	Singh & Kuhad (2000)
DSPM95	Aerobic dechlorination (c-HCH)	Tekere (2002)
Phanerochaete chrysosporium BKM-F-1767	Aerobic mineralization (c-HCH)	Bumpus *et al*., (1985)
Phanerochaete sordida	Aerobic dechlorination (c-HCH)	Singh & Kuhad (2000)
Pleurotus eryngii	Aerobic dechlorination (c-HCH)	Arisoy (1998)
Pleurotus florida	Aerobic dechlorination (c-HCH)	Arisoy (1998)
Pleurotus sajor-caju	Aerobic dechlorination (c-HCH)	Arisoy (1998)
Trametes hirsutus	Aerobic dechlorination (c-HCH)	Singh & Kuhad (1999)

*Dechlorination means degradation of HCH has been measured but mineralization was either not tested or not detected.
*Mineralization means complete degradation to CO2 as detected using radiolabelled tracers.

During this step, the halogen atoms, which is usually responsible for the toxic and xenobiotic character of the compound is most commonly replaced by hydrogen or a hydroxyl group that reduces both recalcitrance to biodegradation and the risk of forming toxic intermediates. Such as in the case of aerobic bacterium *Sphingomonas paucimobilis* UT26 which can utilize γ-HCH as its sole source of carbon and energy (Imai *et al.*, 1989). Under aerobic conditions, the enzyme g-hexachlorocyclohexane dechlorinase (LinA) from *Sphingomonas paucimobilis* UT26 catalyzes the elimination of chlorine atoms from the molecule of γ-HCH (Mencía *et al.*, 2006). But in the case of anaerobic degradation few reports were published and reported in liquid and slurry cultures (Quintero *et al.*, 2005).

Table 2 Data from Nagata *et al* (1999)., Kumari *et al*. (2002), Dogra *et al*. (2004), Boltner *et al*. (2005), Cérémonie *et al*. (2006), Lal *et al*. (2006), Ito *et al*. (2007), Nagata *et al*. (2007), Wu *et al*. (2007), and Yamamoto *et al*. (2009). [b]linX is apparently not required for the γ-HCH degradation pathway of *S. japonicum* UT26 Nagata *et al* (1994).

S. No	Bacteria	Genes reported/Acc. No.
linA1/linA/linA2	UT26 B90/B90A DS2 DS2-2 γ1-7 δ12-7 γ16-1 α1-2 α4-2 DS3-1 BHC-A	(D90355), (AY331258,AY150580), Sp+ (AY903217, AY690622) (AJ871378), (AJ871379), (AJ871380), (AJ871381), (AJ871382), (AJ871383), (AJ871384), (AJ871385) (DQ372106)
linB	UT26 B90A BHC-A MI1205 SS04-1 SS04-2 SS04-3 SS04-4 SS04-5	(D14594), (AY331259), Sp+ (AY903216), (DQ246619), (AB278602), (AB304077), (AB304078), (AB304079), (AB304080), (AB304081)
linC	UT26 B90A BHC-A	(D14595), (AY331258) Sp+ (DQ111068) (DQ462464)
linD	UT26 B90A BHC-A	(D89733) (AY334273) (DQ480725)
linE	UT26 B90A BHC-A	(AB021867) (AY334273) (DQ399709)
linEb	UT26	(AB177985)
linF	UT26 BHC-A	(AB177985), (DQ399710)
linG		
linH		
linI		
linJ		
link	UT26	(AB267475)
linL	UT26	(AB267475)
linM	UT26	(AB267475)
linN	UT26	(AB267475)
linR	UT26 B90A BHC-A	(AB021863) (AY334273) (DQ399711)
linXb	UT26 B90/B90A BHC-A	(D23722) (AY331258, AY150580) (DQ486136)

Role of a desorption-aid silicone oil n performance of slurry bioreactors treating a heavy soil polluted with lindane and the sequential methanogenic-sulfate-reducing slurry bioreactors without silicone oil showed the highest lindane removal efficiency of 98% evaluated by (Camacho-Pérez 2010a,c).the several types of micro organisms characterized which encodes genes and enzymes of HCH degrading pathways.

The γ-HCH in the atmosphere leads to health risks of the population in different country- china and India evidenced by the transport, multimedia expose and risk assessment model indicating the cancer risk(Xu *et al.*, 2013) lake concentration of γ-HCH and DDT in western and southern Tibetan plateau (Tao *et al.*, 2013), sediment contamination by heavy metal, OC pesticides and PAH in southwest france (Arienzo *et al.*, 2013), in Azerbaijan POP contamination (Aliyeva *et al.*, 2013), α-HCH water gases exchange in Canadian Archipalago (Wong *et al.*, 2012), in OC contamination in Pakistan (Ali-Musstjab-Akbar Shah Eqani *et al.*, 2012).

Integrated biological approach

Lindane in the environment

The production and agricultural use of lindane had been banned in more than 50 countries and restricted in 33 countries (Humphreys *et al.*, 2008). The use of γ-HCH for control of agricultural pests has been discontinued, run-offs from the already contaminated agricultural soils or from the dumping sites of adjoining regions can result in high levels of contamination (Prakash *et al.*, 2004). The use of lindane in agriculture leads to 12-30% volatilizes into atmosphere and comes back in the form by rain. It is difficult to predict the fate of POPs in the environment due to rigidity of their compounds by physical, chemical, photolysis and biological degradation. Therefore their fate can be determined using mathematical modelling and system dynamics approach to stimulate fate in soil using Verisim^{R} stimulation software (Chaves *et al.*, 2013), time series different model (Venier *et al.*, 2012), transgene uptake based on lindane system degradation model. Lindane particles get adsorbed very strongly to the organic matter present in soil and therefore become relatively immobile.

Naturally the plants growing in such soil will accumulate HCH in their tissues in the soil; lindane adsorbs strongly to organic matter and is therefore relatively immobile in the soil (Girish and Kuhni, 2013). The most likely mechanisms of HCH accumulation in plants were sorption of soil HCH on roots and sorption of volatilized HCH on aerial plant tissues (Abhilash *et al.*, 2008; Pereira *et al.*, 2008). Soil microflora and aquatic microflora are adversely affected by γ-HCH (Babu *et al.*, 2001). Lindane significantly reduced population of nitrifying bacteria (Martinez-Toledo *et al.*, 1993). The growth and activity of denitrifying bacteria was adversely affected by lindane (Sáez *et al.*, 2006). A reduction of 50% in bacterial cell concentration was observed in lindane-amended soil microcosms (Rodríguez and Toranzos, 2003). HCH also reduces plant seed germination and seedling viguor (Bidlan *et al.*, 2004).

Factors affecting lindane degradation

The degradation of hexachlorocyclohexane occurs by stepwise removal of chlorine atom known as dechlorination and it is influenced by a number of factors. *viz.* initial lindane concentration, temperature, pH, presence of oxygen, bioavailability and biomass concentration, recognized by many workers (Castro & Yoshida 1974; Sethunathan *et al*., 1983). Lindane-bioremediation is subject to the prevailing temperature, moisture and soil conditions (Kearney and Wauchope, 1998). The soil pH, water availability, nutritional status and oxygen levels vary and may not always are optimal growth of white rot fungi (Zervakis *et al*., 2001 Philippoussis 2002) or extracellular enzyme production for pollutant transformation (Gadd, 2001). Thus, the kinetics of pesticides degradation in the soil is commonly biphasic with a very rapid degradation rate in the beginning followed by a very slow prolonged dissipation. The remaining residues are often quite resistant to degradation (Alexander, 1994). When it is adsorbed onto the soil, the degradation rate is much slower due to mass transfer limitations (Rijnaarts *et al*., 1990). The ability of microbes to degrade HCH can be optimized by the process of induction and acclimatization of these microbes, when the enzyme systems of the biodegradation pathway(s) get induced, facilitating effective removal of the pollutant (Girish *et al*., 2000; Elcey and Kunhi, 2010). It was initially thought to be an anaerobic process. Bachmann *et al*., (1988) studied the degradation of α-HCH under aerobic, methanogenic, denitrifying and sulfate-reducing conditions in soil slurries. Degradation was most rapid under aerobic conditions. Sorption to the surface of soil particles reduced the mobility but increased the proximity of contaminants to surface-bound microorganisms. The environmental parameters such as the availability of water in soil may also be a very important factor affecting the rate of degradation, since water availability affects oxygen supply and thus microbial growth and enzyme production (Philippoussis, 2001, Marin *et al*., 1998). Besides affecting microbial growth, water availability affects pesticide binding and distribution in the soil. The behavior of organic compounds in water plays a very signi cant role in their availability for microbial utilization in the environment (Atagana, 2003). Other factors that can contribute to pesticide degradation in soils include chemical nature, amount of soil organic matter, concentration of the pesticide, soil type and microbial community structure and activity of microbes (Schoen, 1987).

Metagenomics

The unculturable microorganism poses a problem for the identification of useful strains and we know that approx. 1% of total micro organisms on this universe are culturable. Therefore total soil DNA isolated and further screened through of DNA library (phagemid vector) construction and clone(s) can be screened for more potent genes. A few reports published in metagenomic analysis of microbial community of HCH degrader microorganisms.

Dadhwal *et al*., 2009 proposed the bio stimulation study for the strain isolated from HCH contaminated site soil in India and analyse the diversity of culturable microorganisms. Similarly Sangwan *et al*., in 2012 performed the soil metagenomic study of three HCH contaminated site in India through 16s RNA amplification. This study found evidence for the horizontal transfer of HCH catabolic genes. The chlorpyriphos, the organophosphate containing insecticide produces a major metabolite 3, 5, 6-trichloro-2-pyridinol (TCP) on degradation recombinant E.coli The metagenomic library was characterized was isolated from cow rumen named as which harbours the tcp3A which codes for its degradation and this was the major breakthrough in the field of metagenomics (Math *et al*, 2010). The herbicides phenoxyalkanoic acid (PAA) its degradation is done by novel and diverse tfdA-like genes, which are dioxygenases dependent reported in *Bradyrhizobium spp., Sphingomonas spp*., and uncultured soil bacteria (Zaprasis *et al*., 2010). In case of polychlorinated biphenyls major cause of contamination of river sediments DNA-stable isotope probing technique was used integrated with metgenomics which reveals the presence of biphenyl dioxygenase genes encodes for its degradation was isolated from genera *Achromobacter* and *Pseudomonas* (Sul *et al*., 2009). A novel 2,4-dichlorophenol hydroxylase gene (tfdB-JLU) was also identified from Escherichia coli and metagenomic library constructed for functional screening of useful gene and enzymes. (Lu *et al*., 2011). Microbial communities from chlorinated pesticides contaminated site also involved in biodegradation such as in case of γ-HCH the presence of biodegradative genes like linA reflects the ability to biodegrade this POP and was reported in *Sphingomonas sp* (Manickam *et al*., 2010). Similar reports were published in case of cellulases and their metagenomic libraries were constructed by a high throughput screen from buffalo rumen and the termite hind-gut (Mewis *et al*., 2011). Same metagenomic approach was applied for the identification and characterization of novel thermostable pyrethroid hydrolytic enzymes against pyrethroid pesticides from Escherichia coli BL21 (DE3) strain (Fan *et al*., 2011).

Degradation of Lindane using Nanoparticles

The chemical degradation and physical adsorption were used for the removal of this chlorinated pesticide (Paknikar *et al*., 2005) and commonly mediated through microwave irradiation (Salvador *et al*., 2002), NaOH-modified sepolite (Ahlborg, 1980) and hydrogen peroxide (Salvador *et al*., 2002). As microbial cultures possess efficient potential for degradation of HCH, so integrated Nano-biotechnological approach for the treatment of pesticides is also becoming popular as a novel and effective technology. This process includes use of zero valent granular iron or nanoparticles of iron for catalytic reductive dehalogenation of various organochlorine pesticides (Zhang *et al*., 2003, Schrick *et al*., 2004). Paknikar *et al*., (2005) used an integrated nano-biotechnological approach for treatment of drinking water to make it free from pesticide residues. They synthesized FeS nanoparticles by wet chemical method and stabilized them by using fungal polymer. These FeS nanoparticles carried out the dechlorination of lindane and the product was further mineralizes by a bacterial culture which also degraded the fungal polymer. Similarly, electrochemical reduction of tricholoroethane using palldised iron oxide (Roh *et al*., 2001), reduction of carbon tetrachloride by Fe^{2+}, S^{2-} and FeS (Assab-Anid and Kun Yu 2002), use of bimetallic particles for ground water treatment (Elliott amd Zhang, 2001), dechlorination of lindane in multiphase catalytic reduction system with Pd/C, Pt/C and Renay/ Ni (Zenovyov *et al*., 2004) has been reported as effective systems for treatment of ground water to make it free from pesticide residues. Although the biotechnological system for biodegradation of organochlorine pesticides is highly promising and effective but it has some limitations of precipitation of metal hydroxides on iron decreasing the reactivity and increase in toxicity of the released products (Zhao, 2008).

Table 3 Pesticide persistence in Soils (Source)

Sr. No.	Low Persistence (half-life 30 days)	Moderate Persistence (half-life 30-100 days)	High Persistence (half-life >100 days)
1	Aldicarb	Aldrin	TCA
2	Captan	Atrazine	Picloram
3	Dalapon	Carbaryl	Bromacil
4	Dicamba	Carbofuran	Trifluralin
5	Malathion	Diazinon	Chlordane
6	Methyl Parathion	Endrin	Paraquat
7	Oxamyl	Fonofos	Lindane
8	2,4-D	Glyphosate	
9	2,4,5-T	Heptachlor	

Phytoremediation

Plants in association with microorganisms degrade, detoxify and ultimately remove pollutants from the environment. The metabolic fate of pesticide is dependent on abiotic factors (temperature, soil, moisture, pH etc), microbial/ plant community, pesticide characteristics. Abiotic degradation is due to chemical and physical transformation of the pesticides by process such as hydrolysis, photolysis, oxidation, reduction and rearrangements. The biotic degradation of pesticides includes the enzymatic transformation which happens mainly through the routes of phytoremediation and microbial detoxification reactions (Laura *et al*., 2003). Plants may carryout degradation of pesticides using anyone of the following mechanisms:-

1. Oxidative transformation e.g. metabolism of fungicide feripromorph by oxidative enzymes in cytochrome P450 (Mougin *et al*., 2001).
2. Hydrolytic transformation reaction (Hoagland and Zablotowicz 2001) ester hydrolysis of thifen-sulfuron-methyl using the plant estrases having Gly-X-Ser-Gly motif (Brown and Kearney, 1991).
3. Aromatic nitroreductive process e.g. metabolism of pentachloronitrobenzene in peanut (*Arachis hypogaea*) via. Aryl nitroreduction and glutathione-S-tranferase mediated dechlorination or nitrite release (Lamoureux and Rusness, 1980).
4. Carbon-Phosphorous bond cleavage (not fully known mechanism in plants).
5. Pesticide conjugation reaction i.e. (a) carbohydrate and amino-acid conjugation e.g. biotransformation of glyphosate via. C-P lyase and glyphosate oxireductase enzymatic reactions (b) Plant glutathione conjugation reaction e.g. increased concentration of glutathione or GSH (γ-L-glutamyl-L-cysteinyl glycine) protected wheat from phenoxaprop injury (Romano *et al*., 1993).
6. Pesticide metabolism in the rhizosphere e.g. lignolytic fungus P. chrysosporium oxidizes the insecticide lindane by putative cytochrome P450 enzyme (Mougin *et al*., 1996, 1997)

Remediation by Rhizospheric Microorganisms

In the rhizosphere plants may enhance co-metabolism of agricultural contaminants by anyone of the following mechinisms: (i) the rhizosphere may allow selective enrichment of degrader organism that have low densities to significantly degrade xenobiotics in root free soil (Nichols *et al*., 1997) (ii) the rhizosphere may enhance growth linked metabolism or stimulate microbial growth by providing a natural substrate when the concentration of xenobiotics is low or unavailable (Alexander, 1999; Haby and Crowley, 1996) (iii) the rhizosphere is rich in natural compounds that may induce co-metabolism of xenobiotics in certain micro-organisms that carry degaradative genes or plasmids (Crowly *et al*., 1997). Research in phytoremediation has focused on densely rooted, fast growing grasses and plants like *Brassica* sp. with fine root system. Mulberry (*Morus alba* L) and Poplar (*Populus deltoides*) trees have been used successfully in the chlorophenols and chlorinated solvents (Stomp *et al*., 1993) which carry out the metabolism of their xenobiotics via. reductive dehalogenation (RDE). In RDE enzymes have broad specificities and there are two mechanisms for this; the first process is co-metabolic RDE which yields no energy for organism. In the second mechanism is halo respiration where organochlorines act as terminal electron acceptors and adenosine triphosphate is generated (Griffith *et al*., 1992). In first case organochlorines are not used as terminal electron acceptors along with allabore phenomenon discovered, further research is needed to fully characterize the role of plants or rhizosphere in biotransformation of organochlorine pesticides.

CONCLUSION AND FUTURE DIRECTIONS

Scientific interest in HCH degradation was initially motivated by environmental and health concerns due to HCH residues, its aerobic degradation is now proving an excellent model for investigating fundamental issues in microbial and molecular evolution.The use of microbes to clean up polluted environments, bioremediation is rapidly changing and expanding the area of environmental biotechnology. Although much work is being done to remediate the polluted environment, but our limited understanding of the biological contribution and their impact on the ecosystem has been an obstacle to make the technology more reliable and safer. In previous studies a defined microbial consortium was able to degrade HCH (technical grade containing all four major isomers) up to 25 ppm level in soil at ambient temperature and neutral pH. The consortium was able to degrade HCH in artificial plots and also in open fields. The inhibition of degradation by the presence of other isomers and native microflora was marginal. With the translation of lab trials to large scale trials coupled with process molecular microbiological techniques can make the bioremediation process more reliable and safer technology.

Although hexachlorocyclohexane removal has been observed under both oxic and anoxic bioremediation treatments, treatments under oxic condition have resulted in the almost complete removal of HCH via mineralization. These observations are on par with the results where in under oxic conditions good degradation of HCH-isomers of technical mixture has been observed. The enzymes should be amenable to various modern research technologies for obtaining improved variants either by the isolation of new variants from uncultured environmental samples/bacteria or by the improvement of existing variants by techniques of *in vitro* evolution. We believe that there are good prospects for developing economically viable HCH bioremediation technologies based on the sphingomonad/Lin systems: soil bioremediation through various biostimulation/ bioaugmentation approaches. Significant work on enzyme characterization, particularly for LinA, and strain and enzyme improvement is still needed. However, the promise of the system evident thus far and the potential of modern microbial and enzyme research technologies to make radical improvements give us confidence that the development of successful technologies for a most pernicious pollutant is quite achievable.

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