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Review Article

REMEDIATION OF LINDANE FROM ENVIRONMENT – AN OVERVIEW

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ABSTRACT
Lindane has been used historically as a broad spectrum pesticide in agricultural, livestock, forestry, veterinary and human health applications. Several factors have contributed to concern over the production and use of lindane including its properties of persistence, toxicity, bioaccumulation and potential for long-range transport. During the last two decades, extensive attention has been paid on the cleanup of environment polluted with this hazardous material. A number of methods have been developed for the removal of lindane from soil and water such as sorption by activated carbon, remediation by nanoparticles, photocatalysis, biocatalytic dechlorination, phytoremediation, biosorption and microbial degradation. Among all these methods, microbial degradation offers an effective approach to remove such toxicant from the environment. This article provides a selective overview of the achievements and present scenario of remediation studies carried out using bacteria, fungi, algae and actinomycetes as potential agents for removal of lindane and its isomers from contaminated soil and water.

KEYWORDS: HCH, Lindane, Bioremediation, Bacteria, Fungi, Algae.

INTRODUCTION

Lindane is an organochlorine insecticide widely used throughout the world and considered as Persistent Organic Pollutant (POP).Due to the deleterious effect of POP's on environment and human health, the use of many of them has been reduced or even banned in developed countries, but they are still widely used in developing nations. Among them, India is one of the major sources of organochlorine contamination, producing and utilizing chlorinated pesticides in large quantity throughout world (Rajendran *et al*., 1999).

Lindane is also known as gamma –HCH since it is made up of at least 99 % of the gamma isomer of hexachlorocyclohexane (HCH). Almost pure γ-HCH i.e lindane, is known as benzene hexachloride (BHC) (Li, 1999).Technical HCH can include varying proportions of α, β, δ, and γ-HCH isomers which have been shown to have serious short and long term health effects. Being a POP, HCH is nowadays found in air, water and soil samples all over the world. Though all HCH isomers are toxic, carcinogenic, endocrine disrupters and are known to exert damaging effects on the reproductive and nervous systems in mammals (Metcalf, 1955; Smith, 1991; Walker *et al*., 1999),it is ubiquitously used in tropical countries to reduce vector-transmitted diseases and to protect livestock and to increase agricultural yields because of its low production cost and effective pesticide properties.

Scientists all over the world are involved towards the development of remediation technologies including physical, chemical and biological remediation. Each of these methods has its own merits and demerits. The physical and chemical methods like removal of lindane by adsorbents, nanoparticles and chemical dechlorination are effective with only lower concentrations of the pollutant. Disposal of the sorbed lindane is another emerging problem. Bioremediation, which includes the gainful utilization of microorganism for the biodegradation of target pollutants, is a potential technique for the biological treatment of industrial waste and contaminated soils (Crawford and Crawford, 1996; Alexander, 1999). Several soil microorganisms capable of degrading and utilizing HCH as a carbon source have been reported over last two decades. In selected bacterial strains, the genes encoding the enzymes involved in the initial degradation of lindane have been cloned, sequenced, expressed and the gene products were characterized. Most studies report the biodegradation of relatively low (<500 mg/kg) concentrations of HCH in soil. Information on the effect of inorganic nutrients, organic carbon sources or other soil amendments is scattered and inconclusive. More in-depth study is needed to develop the effective bioremediation protocols to treat the soil with high HCH concentrations, (Philips *et al*., 2005). This paper presents an updated information on lindane removal using various remediation agents under different ecosystems.

Mechanism of Lindane biodegradation

Different microorganisms have been found to be capable of using halogenated compounds as their growth substrate. The key reaction during microbial degradation of halogenated compounds is the removal of the halogen atom, i.e., dehalogenation of the organic halogen. During this step, the halogen atoms, which are usually responsible for the toxic and xenobiotic character of the compound is most commonly replaced by hydrogen or a hydroxyl group. Halogen removal reduces the risk of forming toxic intermediates formed during the subsequent metabolic steps of biodegradation. For instance, the oxidative

conversion of several halogenated organic compounds may lead to the production of acylhalides or 2-haloaldehydes .The haloaldehydes are very reactive products due to their electrophilicity and may cause cellular damage (Janssen *et al*., 2001; Camacho-Pérez *et al*., 2011).

It was initially believed that HCH biodegradation is largely an anaerobic process, and variable levels of anaerobic degradation of α, β, γ, and δ-HCH have indeed been observed. Isolates capable of degrading one or more of the other four HCH isomers under anaerobic conditions include *Clostridium rectum* (Ohisa, *et al*., 1978), *Clostridium sphenoides* (MacRae *et al*., 1969 ; Heritage and MacRae.,1977), *Clostridium butyricum*, *Clostridium pasteurianum* (Jagnow *et al*., 1977), *Citrobacter freundii* (Jagnow *et al*., 1977), *Desulfovibrio gigas*, *Desulfovibrio africanus*, *Desulfococcus multivorans* (Boyle *et al*.,1999), and a *Dehalobacter* sp. (van Doesburg *et al*., 2005). The first report of an aerobic bacterial strain *Pseudomonas paucimobilis* SS86 that degraded HCH appeared around 1990, isolated from Japan (Senoo and Wada, 1989). *Sphingomonas paucimobilis* UT26 is a nalidixic acid resistant strain of *Pseudomonas paucimobilis* SS86 which degraded α-, γ- and δ-HCH aerobically (Imai *et al*., 1989). Another HCH degrading *Pseudomonas sp.* was isolated from sugarcane fields in India (Sahu *et al*., 1990).

Nagata *et al*.(1993a,b) proposed the degradation pathway of lindane using *Pseudomonas paucimobil*is. They reported that 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) is transformed to 1,2,4-trichlorobenzene (1,2,4-TCB) by non-enzymatic reactions due to the fact that 1,4-TCDN contains an instable bond whereas the aromatic ring of 1,2,4-trichlorobenzene (1,2,4-TCB) is more stable. The gene LinB codes for the enzymes of the family of haloalkane dehalogenases that are responsible for catalyzing the reaction of β -HCH to 2,3,4,5,6 -pentachlorocyclohexane (PCCH) by hydrolytic dehalogenation. The gene LinC is envolved with dehydrogenases that transforms 2,5-dichloro-2,5-cyclohexadiene-1,4-diol (2,5.DDOL) to 2,5-dichlorohydroquinone (2,5-DCHQ). The latter is further converted to β-ketoadipate by the reductive enzyme dechlorinase LinD (Miyauchi *et al*., 1998), a ring-cleavage dioxyenase LinE (Miyauchi *et al*., 1999), and the maleylacetate reductase, LinF (Endo *et al*., 2005). The degradation pathway of lindane was extensively analyzed in *S. paucimobilis* UT26 (Nagata *et al*., 2007). The γ-HCH is transformed to 2, 5- dichlorohydroquinone via sequential reactions catalyzed by enzymes LinA, LinB and y LinC. 2,5- dichlorohydroquinone, in turn, is metabolized by enzymes LinD, LinE, LinF, LinGH y LinJ to succinyl-CoA and acetyl-CoA, that are further channelled into and metabolized in the tricarboxylic acid cycle.

The aerobic actinomycetes *Streptomyces* M7 strain can utilize γ-HCH as its sole source of carbon and energy. Synthesis of dechlorinase in *Streptomyces* sp. M7 was induced when the microorganism was grown in the presence of lindane (γ-hexachlorocyclohexane) as the only carbon source (Cuozzo *et al*., 2009).

Cyanobacteria, *Anabaena* sp. strain PCC7120 and *Nostoc ellipsosporum* transformed lindane first to γ-pentachlorocyclohexene and then to a mixture of chlorobenzenes. This process was co-metabolic and depended on the presence of nitrate (Kuritz *et al*., 1995).

In case of fungi, not much work has been done on the pathways of HCH degradation. Most of the HCH-degrading fungi known to date are the members of the family of white rot fungi, and a very few non white rot fungi has been noted as degraders of lindane. The biodegradation is accomplished with the action of extracellular oxidative enzymes, produced by the fungus to decompose woody substrates, such as Laccase, Manganese peroxidise, Lignin peroxidase (McErlean *et al*., 2006; Rigas, *et al*., 2005).

Factors affecting biodegradation of Lindane

A number of factors viz. presence of oxygen, bioavailability, initial lindane concentration, temperature, pH, and biomass concentration have been recognized by many workers to affect the biodegradation of lindane (Castro & Yoshida 1974; Sethunathan *et al*., 1983; MacRae *et al*., 1967, 1969, 1984). HCH biodegradation was initially thought to be an anaerobic process. Bachmann *et al*. (1988) studied the degradation of α-HCH under aerobic, denitrifying, sulfate-reducing and methanogenic 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. Factors that affect the volatility of HCHs (temperature, humidity, vapor pressure, soil organic matter and moisture) can influence the rate of biodegradation. Lindane can be volatilized through air pockets of the soil, or escape from the surface affecting their concentrations in the solid and liquid phases of the soil along with their bioavailabililty (El Beit *et al*., 1981, Phillips *et al*., 2005). pH is the most important factor as the reduction in pH may hinder the growth of the degraders during the process of degradation. The effect of pH has been studied in *Pandoraea* sp. The optimum pH for growth and biodegradation of α and γ-isomers in soil slurries was 9.0 (Okeke *et al*., 2002). In another mixed consortium, dechlorination of α HCH was noted at more neutral pH range (6-8) (Manonmani *et al*., 2000). A strain of *Clostridium rectum* was isolated which degraded lindane optimally at pH 7-8 in anaerobic pure culture studies (Ohisa and Yamaguchi, 1978). Zheng *et al*., (2011**)** reported the rapid degradation of lindane by S*phingobium* strains at low temperature (4˚C).

In the present article, the reported works on possible applications of various agents towards lindane removal have been discussed.

Role of bacteria in Lindane remediation

Efforts have been given on studying the lindane removal by bacteria. MacRae *et al*., (1969) reported degradation of technical HCH by anaerobic *Clostridium* sp. Francis *et al*., (1975) reported lindane degradation by *Escherichia coli* isolated from rat faeces. About 10% of the added lindane was metabolized by the bacterium producing single metabolite, PCCH. Sahu *et al*., (1992) reported the dechlorination of lindane by *Pseudomonas aeruginosa.* Nalin *et al*., (1999) isolated a new strain of *Rhodanobacter*

lindanclasticus which degraded technical grade HCH under aerobic condition.

Gupta *et al*., (2000) reported lindane degradation using acclimatized culture of *Bacillus circulans* and *Bacillus brevis* in 5µg/mL lindane and 80% degradation was noted within 8 days. Datta *et al*., (2000) reported the growth characteristics and degradation of the aerobic bacterial strain *Arthrobacter citreus* BI-100 in mineral salts medium with γ-HCH (100 mg/L) as the sole source of carbon. Gupta *et al*., (2001) reported the degradation of γ-HCH by *Alkaligens faecalis,* isolated from agricultural fields.

A gram-positive *Microbacterium* sp. strain ITRC1 was isolated and characterized by Manickam *et al*., (2006). DNA fragments corresponding to the two initial genes involved in lindane degradative pathway, encoding enzymes for gamma-pentachlorocyclohexene hydrolytic dehalogenase (linB) and a 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase (linC) were amplified by PCR and sequenced. For the first time a *Xanthomonas* sp. was isolated from a contaminated soil which utilized γ-HCH as sole carbon and energy source by successive dechlorination (Manickam *et al*., (2007). A root-colonizing HCH-degrading *Sphingomonas* was isolated by two step enrichment process which shed light on the possibilities of rhizoremediation (Boltner *et al*., 2007). Bioconversion and biological growth kinetics of *Pseudomonas aeruginosa* degrading technical HCH was investigated in batch process under aerobic condition by Lodha *et al*., (2007). At lower technical HCH concentrations (1-10mg/l), degradation (above 99%) was observed whereas at higher concentration (20-50 mg/l), the degradation efficiency was reduced. Bioremediation of lindane contaminated soil by *Streptomyces* sp. M7 and its effects on *zea mays* growth was reported by Benimeli *et al*., (2008). A yellow-pigmented, (HCH) degrading bacterial strain, *Sphingobium quisquiliarum*,P25(T) was isolated from an HCH dump site located in the northern part of India (Bala *et al*.,2009). There was a similar report by Dadwal *et al*. (2009) regarding the isolation of another yellow-pigmented bacteria, *Sphingobium chinhatense*. Zheng *et al*., (2011) reported that bacterial strain *Sphingobium indicum* B90A could serve as a good candidate for developing novel bioremediation technique for cold regions to decontaminate γ-HCH from soil / waters. Comparative studies on remediation potential of four rhizospheric bacterial species viz. *Kocuria rhizophilla, Microbacterium resistens, Staphylococcus equorum and Staphylococcus cohnii* on lindane removal was reported by Abhilash *et al*., (2011).

Role of fungi in Lindane remediation

Biomaterials like fungi have been proved to be efficient for the removal of lindane from soil and aqueous solutions. Bumpus and Aust (1987) published the first report on the biodegradation of lindane by white rot fungi *Phanerochaete chrysosporium*. Application of fungal technology for the cleanup of contaminants has shown promise since 1985 when the white rot species *Phanerochaete chrysosporiumwas* was found to be capable of metabolizing a number of important environmental pollutants (Sasek, 2003). Mougin *et al*., (1997) reported the enhanced mineralization in soils supplemented with lindane by *Phanerochaete* sp .and the fungus seemed to modify lindane degradation pathway by increasing the conversion of volatile intermediates to CO_2. The ability of the white-rot fungus *Trametes hirsutus* to degrade lindane in liquid culture was investigated by Singh and Kuhad (1999) which was compared *Phanerochaete chrysosporium*. It was shown that *Trametes hirsutus* degraded lindane faster than *P. chrysosporium* but the mechanism of degradation for both the fungi appeared to be the same. Singh and Kuhad (2000) studied the performance of *Cyathus bulleri* and *Phanerochaete sordida*, two white rot fungi for γ-HCH degradation and reported that *C. bulleri* degraded lindane more efficiently than *P. sordida*.

The degradation of lindane at various concentrations by a sub-tropical white rot fungus DSPM95 was studied in batch and packed bed bioreactor systems. Percentage degradation of 82 ± 6% was achieved in batch cultures and 81% degradation was noted in packed bed reactor (Tekere *et al*., 2002).) Bioremediation process was evaluated by polypore fungus, *Ganoderma australe* in mixtures of a sandy soil and wheat straw doped with lindane (Rigas *et al*., 2007). Fungal growth was found to be a function of temperature and moisture for a proper colonization. Quintero *et al*. (2007) evaluated the degradation of hexachlorocyclohexane (HCH) isomers present in a spiked soil by the white-rot *Bjerkandera adusta* in a slurry system. At optimal conditions, maximal degradations of 94.5%, 78.5% and 66.1% were attained after 30 d for γ-, α- and δ-HCH isomers. Biodegradation of lindane by Phycomyceteous fungus *Conidiobolus* 03-1-56, a non white rot fungus was reported by Nagpal *et al*., (2008). The fungus completely degraded lindane on the 5th day in the culture medium. Two *Fusarium* species (*F. poae* and *F. solani*) isolated from the pesticide contaminated soil showed better degradability of lindane used as a sole carbon source when compared with the growth performance of other fungal isolates from the same contaminated soil (Sagar and Singh 2011).

Role of algae in Lindane remediation

Use of algal biomass as remediation agent may be attractive and economical because algae have low nutrient requirements, being autotrophic they produce a large biomass, and unlike other biomass and microbes, such as bacteria and fungi, they generally do not produce toxic substances. Lindane dechlorination by the cyanobacteria *Anabaena* sp. strain PCC7120 and *Nostoc ellipsosporum*, was reported by Kuritz *et al*., (1995; 1997). It was found that nitrate is essential for biodegradation of lindane. Potential use of environmental cyanobacterial species in bioremediation of lindane – contaminated effluents was reported by El – Bestawy *et al*., (2007). Cyanobacterial species isolated from the two Egyptian Lakes (Qaroun & Mariut) were exposed to 5 and 10 ppm Lindane for 7days and growth inhibition or stimulation percentage of lindane removal efficiency (RE), were calculated. Lindane exhibited different degrees of toxicity or stimulation for the selected cyanobacteria. Stimulation of growth ranged between 0.0- and 13.16-fold higher than controls, while inhibition ranged between 0.0% and 100%.The study also revealed that Mariut species were more resistant to lindane

toxicity than Qaroun species. Resistance to lindane among Qaroun spp. was in the order *Oscillatoria* sp. 12> *Oscillatoria* sp. 13>*Synechococcus* sp. > *Nodularia* sp. > *Nostoc* sp. > *Cyanothece* sp. > *Synechococcus* sp. Among Mariut spp., the order was *Microcystis aeruginosa* MA1 >*Anabaena cylindrica* >*Microcystis aeruginosa* MA15>*A. spiroides* >*A. flos-aquae*. Lindane was removed by all the species, either as individuals or mixtures, at both concentrations. The lindane RE percentage of Qaroun species ranged between 71.6% and 99.6% and that of Mariut species between 45.23–100.0%. Mixed culture RE percentages ranged between 91.6% and 100% at 5 ppm, while at 10 ppm, the RE percentage ranged between 90.4% and 100%. The researchers concluded on the potential of natural resources as efficient agents for pollution control.

Recently, adaptation of microalgae to lindane as a new approach for lindane bioremediation has been reported by Gonzaalez *et al*., (2012). The lindane resistant cells were used to test the potential of microalgae to remove lindane. Three concentrations of lindane (4, 15 and 40 mg/L) were chosen as a model. In these exposures, the lindane resistant cells showed a great capacity to remove lindane until 99 % lindane was eliminated.

Role of Actinomycetes in Lindane remediation

Actinomycete strains isolated from soil samples of Argentina have been reported (Fuentes *et al*., 2010a). The lindane removal ability of isolated actinomycete, individually and as a mixed culture under controlled laboratory conditions was evaluated. Lindane degradation was studied using pure and mixed microbial cultures. Mixed cultures were shown to be more suitable for bioremediation compared with pure cultures because their biodiversity could enhance environmental survival and increased the catabolic pathways available for contaminant biodegradation (Rajashekara and Manonmani, 2007).

Streptomyces sp. M7 (M7), *Streptomyces coelicolor* A3 (ScA3) and four actinomycete isolates (A2, A5, A8 and A11) were cultivated, as pure and mixed cultures, in Minimal Medium with lindane (1.66 mg/L). Microbial cells were used to obtain cell free extracts for dechlorinase activity assays and the supernatants from these cultures were used to determine residual lindane by gas chromatography. The actinomycetes isolates were characterized by 16S rRNA amplifications, sequenced and were mostly identified as members of *Streptomyces* genus. These native *Streptomycetes* showed the ability to grow as microbial consortium and removed lindane. Therefore they could be used as potential agents for the biological removal of lindane (Fuentes *et al*., 2010b).

CONCLUSION

This article provides the updated information on remediation of lindane and its isomers from contaminated soil and water using various agents like bacteria, fungi, algae and actinomycetes. There is information describing individual environmental parameters and how they affect HCH degradation in soil microcosms as well in aqueous environment. However, this information is insufficient and sometimes not applicable for field level studies where other parameters can not be controlled or are less predictable. Reports are scanty on remediation technology applicable for actual field-scale treatment of soil and water contaminated with HCHs. In addition, the biodegradation pathways of micro-organisms have been elucidated at the molecular level with a limited number of species. More research is needed to understand the basic mechanism of interactions of HCH-degrading microorganisms with the soil environment which regulate the lindane remediation.

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