Bull. Environ. Contam. Toxicol. (2003) 70:775–782

DOI: 10.1007/s00128-003-0050-0

Bulletin of Environmental Contamination and Toxicology

Enhanced Degradation of Hexachlorocyclohexane Isomers in Rhizosphere Soil of *Kochia* sp.

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Received: 31 May 2002/Accepted: 8 December 2002

Plants are emerging as cost effective tool for remediation of contaminated soil and water. Phytoremediation is a potential green technology for overcoming the inherent limitations of the biological clean-up approaches like inadequate microbial populations and high microbial inactivity. Plants encourage the microbial activity in the root vicinity by exuding organic substances like sugars, alcohol and acids as food for micro-organisms and the loosening of the soil to bring oxygen and water to microbial population, thus providing a microhabitat conducive to microbial proliferation. The overall effect of the plant-microbe interactions is the increase in the microbial population in the vegetative soil compared to nonvegetative soil (Curl and Truelove 1986; Knaebel and Vestel 1994; Cunningham et al. 1996). Another important effect of the plant rhizosphere might be enhancement of the pesticide degrader population in the rhizosphere of the plants exposed to that particular pesticide and reports have shown that rhizosphere soil or micro-organisms isolated from rhizosphere soil often exhibit accelerated rate of xenobiotic degradation (Anderson et al. 1994; Perkovich et al. 1996; Fang et al. 2001; Miya and Firestone 2001). Therefore, biological cleanup of pesticides using plants have become attractive and advantageous for *in-situ* remediation.

Hexachlorocyclohexane (HCH), the most widely used insecticide in developing countries including India, is no longer used for plant protection. Only γ-HCH (lindane) is used as insecticide. This has resulted in accumulation of huge stocks of HCH containing inactive isomers. Few earlier reports have shown that the degradation of HCH isomers is fast in the vegetative soil compared to nonvegetative soil, therefore plants can be exploited as an effective means to degrade/decontaminate the HCH isomers. Probably Wade et al. (1989) the first to report accelerated biodegradation of γ-HCH in HCH-pretreated rice fields under upland condition. Sahu et al. (1990) showed that sugarcane plants grown from the HCH-treated sugarcane setts promoted a very rapid degradation of HCH isomers under aerobic condition. Studies have proved that rhizosphere soil from *Kochia scoparia*, a commonly growing annual at pesticide dealership sites, have successfully degraded several herbicides like atrazine, metolachlor and trifluralin etc. (Anderson et al. 1994; Anderson and Coats 1995; Perkovich et al. 1996) and have very high potential for its probable use in phytodetoxification of pesticides.

Therefore, this study focuses on the degradation of α–, β– and γ-HCH in the rhizosphere soils of *Kochia sp.* Degradation rates of HCH isomers were compared in HCH-treated and untreated rhizosphere soils of *Kochia* and the role of micro-organisms was ascertained in the rhizosphere mediated degradation of HCH isomers.

MATERIALS AND METHODS

Kochia plants were grown in the green house. 10 kg of dried alluvial soil (pH - 7.9, organic carbon - 1.1%) was filled in earthenware pots (12 cm x 10 cm) and 20 days old seedlings of *Kochia* were transplanted. One month after transplanting first application of technical mixture of HCH was made around the root zone of *Kochia* plant. HCH was applied at a rate of 2 μg g^{-1} soil basis. This was followed by two more applications of HCH at 15 days intervals. Another set of HCH-untreated *Kochia* plants were also maintained simultaneously and they served as control. After 15 days of last application *Kochia* plants (both HCH-treated and untreated) were uprooted and the soil held in the roots was collected by tapping the roots. During the course of the study the soil was stored at 4°C.

In order to determine the HCH degrading potential of rhizosphere soil from HCH-treated and untreated plants, both soils were further treated with individual isomers of HCH. 10-g of rhizosphere soil (HCH-treated and untreated) in sterilized 250-ml Erlenmeyer flasks was spiked with 50 μg g^{-1} of α–, β- or γ-HCH. Soils were supplemented with sufficient amount of sterile distilled water to maintain the soil at 60 % water holding capacity. Samples were incubated at room temperature (27±2°C). At regular intervals of 5, 10, 15 and 20 days duplicate samples were removed from both treatments for extraction and analysis of HCH isomers.

Rhizosphere soil from HCH-treated *Kochia* was tested for its ability to degrade HCH isomers in mineral salts medium. To 50-ml sterilized Erlenmeyer flasks α-(100 μg), β- (50 μg) or γ-HCH (100 μg) was added in 1 ml of acetone. Acetone was allowed to evaporate overnight and flasks were supplemented with 9 ml of sterile mineral salts medium [$(NH_4)_2HPO_4$, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; $FeSO_4.7H_2O$, 0.001 ; K_2HPO_4, 0.1 g; $Ca(NO_3)_2$, 0.01 g; distilled water, 1000 ml; pH, 7.0]. Then flasks were equilibrated for 24 h on a rotary shaker to solublise HCH isomers. Flasks were inoculated with 1 ml rhizosphere soil suspension from HCH-treated *Kochia*. Uninoculated mineral salt medium containing respective HCH isomer served as control. Flasks were incubated at room temperature. At regular interval 1 ml portions of medium were removed aseptically from both uninoculated and inoculated flasks, extracted and were analyzed for HCH isomers.

Degradation of γ-HCH was compared in sterilized and nonsterilized rhizosphere soil from HCH-treated Kochia plant. 10-g of rhizosphere soil in 250 ml Erlenmeyer flasks was sterilized for three consecutive days at 15 atmospheric pressure for 1 h each. One set of nonsterilized rhizosphere soil was maintained for comparison. Both sets of soils were treated with γ-HCH (50 μg g^{-1} soil) and were

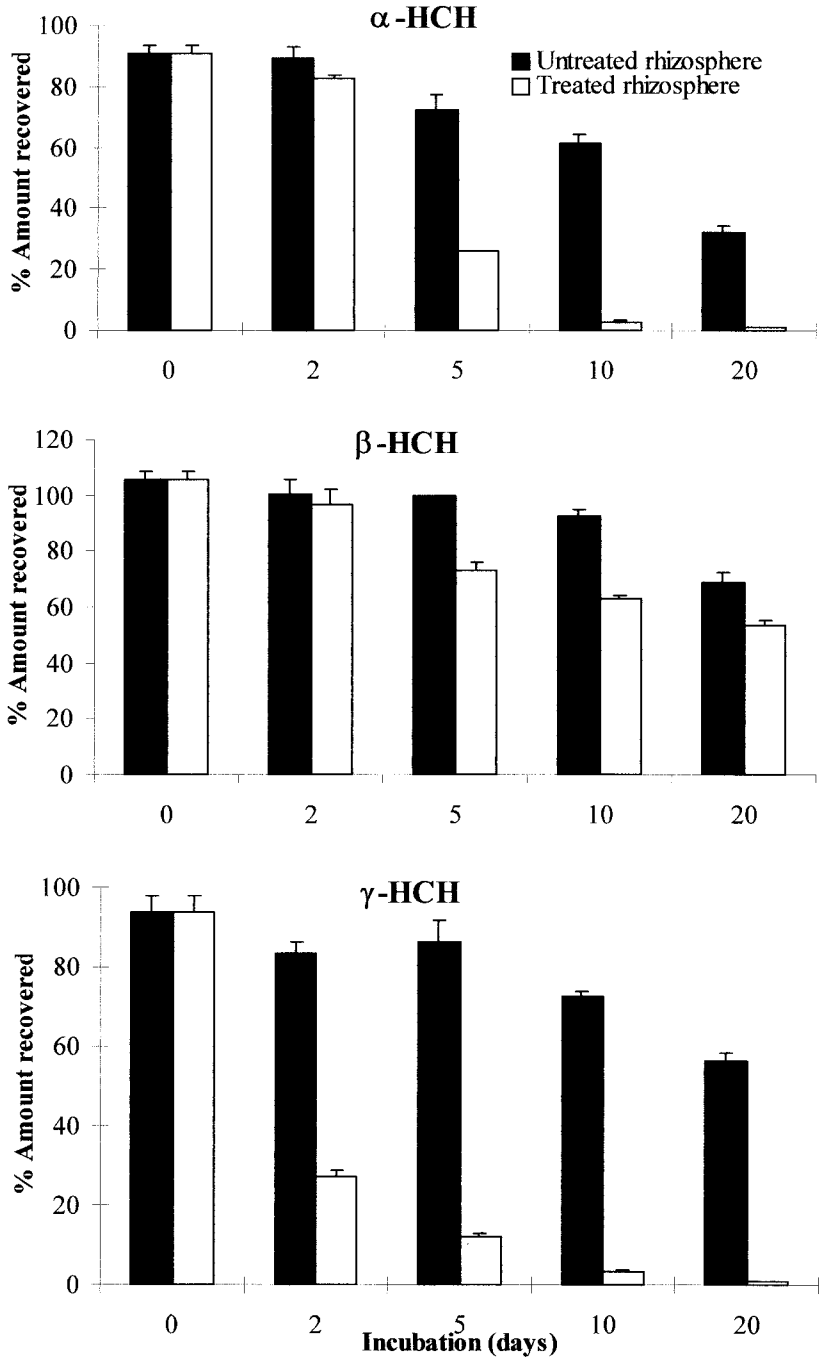

Figure 1. Degradation of HCH isomers in the rhizosphere soil of HCH-treated and untreated *Kochia.*

moistened to 60 % water holding capacity. Samples were incubated at room temperature. At regular intervals both sterilized and nonsterilized samples were removed in duplicate for extraction and analysis.

HCH residues from soil were extracted as follows: 10-g of soil sample was equilibrated with 100 ml hexane in 250 ml Erlenmeyer flasks on a rotary shaker for 2 h. After shaking is over 10-g of anhydrous sodium sulphate was added to each flask to remove traces of moisture from hexane. To quantify HCH-isomers hexane fraction after appropriate dilution was injected in the gas liquid chromatograph (glc).

HCH residues from mineral salt medium were extracted by vigorously shaking 1 ml sample with 2 ml hexane for 2 min. 1 g of anhydrous sodium sulphate was added to each tube and hexane fraction was analyzed for HCH isomers by glc. HCH isomers in hexane (both extracted from soil and mineral salts medium) were analyzed in a Hewlett Packard gas chromatograph equipped with Ni^{63} detector and HP-1 megabore column (10 m x 0.50 mm id x 2.53 mμ film thickness). The operating conditions were: column - 160°C, injector - 300°C, detector - 300°C, carrier gas (nitrogen) flow rate - 45 $ml.min^{-1}$. Under these conditions the retention times for α–, β– and γ-HCH were 1.10, 1.23 and 1.67 min, respectively. The recovery of HCH isomers was more than 90%.

RESULTS AND DISCUSSION

Figure 1 represents the degradation of α–, β- and γ- isomers of HCH in rhizosphere soils from HCH-treated and untreated *Kochia*. Results indicate that HCH isomers were degraded both in HCH-treated and untreated rhizosphere soil. However, rate of degradation was very fast in the rhizosphere soil from HCH-treated plants. α- and γ- isomers of HCH dissipated at faster rate compared to β-isomer. After 10-day incubation nearly 98% of the initially applied α- and γ-isomers of HCH were degraded in the HCH-treated rhizosphere soil while 61.6 and 72.6% of the α- and γ-HCH, respectively, were recovered undegraded from the untreated rhizosphere soil. Degradation rate of β-HCH was quite slow in both HCH-treated and untreated rhizosphere soils. β-HCH is known for its thermodynamic recalcitrant nature. The half lives of α–, β- and γ-HCH were 13.2, 33.1 and 27.4 days, respectively in the untreated rhizosphere soil. But, half lives were significantly reduced in the HCH-treated rhizosphere soil and were 2.82, 22.7 and 2.7 days, respectively. The results indicate that *Kochia* rhizosphere has active microbial population which can degrade the soil applied HCH isomers. However pre-treatment of rhizosphere with HCH acclimatized these microorganism to HCH therefore significantly faster degradation of subsequently applied HCH isomers

was observed in the HCH-treated rhizosphere soil. Bhuyan et al. (1992) also observed similar results for the degradation of γ-HCH in rice rhizosphere. Soil from HCH-treated rhizosphere caused distinctly more rapid degradation of γ-HCH in mineral salts medium under aerobic condition than did the sample from the

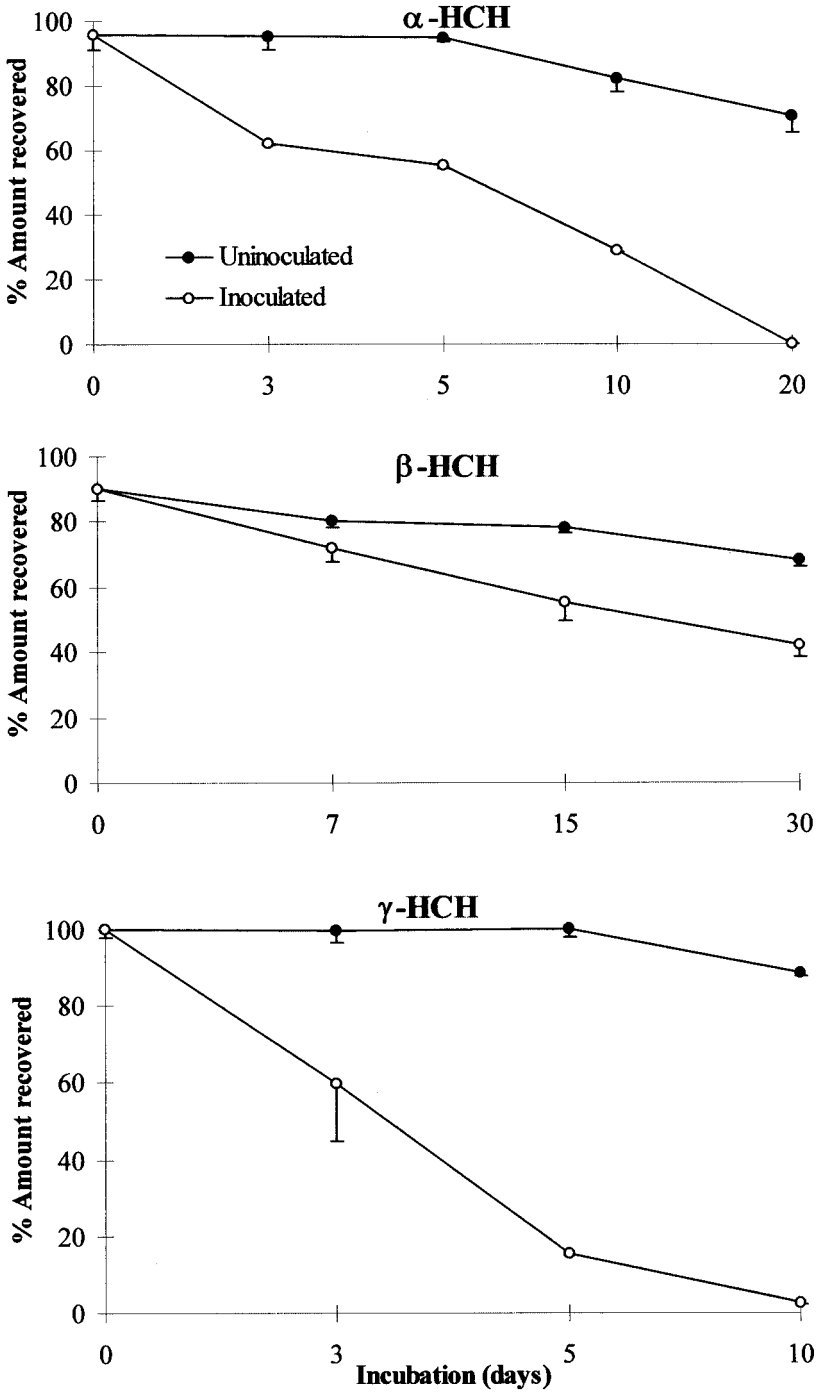

Figure 2. Degradation of HCH isomers in mineral salts medium by the rhizosphere soil suspension from HCH-treated *Kochia.*

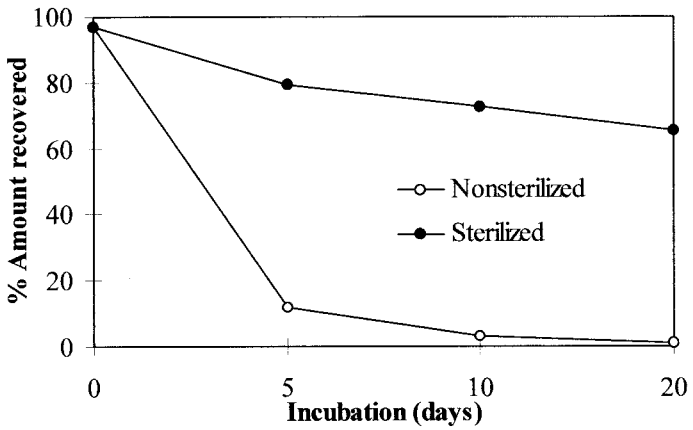

Figure 3. Degradation of γ-HCH in sterilized and nonsterilized rhizosphere soil from HCH-treated *Kochia.*

untreated rhizosphere. Within 10 days all the added γ-HCH disappeared from the medium inoculated with HCH-treated rhizosphere soil than an approximately 50% loss from the medium inoculated with HCH-untreated rhizosphere soil.

Subsequently, the rhizosphere soil suspension from the HCH-treated *Kochia* plant was inoculated into the mineral salts medium containing either of α–, β- and γ-HCH. The rhizosphere soil suspension was highly effective in degrading the HCH isomers in the mineral salts medium (Figure 2). Concentrations of α- and γ-HCH reached to negligible levels in the inoculated medium after 20 and 10 days, respectively. While during the corresponding period 77 and 88% of the respective HCH isomer was recovered undegraded from the uninoculated control medium. Degradation of β-HCH was slow and after 30 days of incubation nearly 88 and 55% of β-HCH was recovered undegraded from the uninoculated and inoculated medium, respectively. The faster degradation rate of only α– and γ-HCH isomers by the HCH-treated rhizosphere soil suspension probably indicates that micro-organisms utilise these isomers as the carbon source and therefore proliferate at the expense of α- and γ-HCH. However, degradation of β–HCH may be cometabollic and bacteria did not proliferate. Therefore bacteria become limiting for the degradation of β-HCH and complete degradation of β-HCH was not observed. Sahu et al. (1990), who studied the degradation of α–, β– and γ–HCH isomers in the rhizosphere soil suspension from HCH-treated sugarcane, showed similar observations. HCH-treated sugarcane rhizosphere soil suspension observed complete degradation of α- and γ-HCH isomers while degradation of β-HCH was quite slow. Subsequent studies using *Sphingomonas paucimobilis*, a bacteria isolated from HCH-treated sugarcane rhizosphere soil, proved that bacteria used α- and γ-HCH isomers as carbon source while degradation of β-HCH was cometabollic (Sahu et al. 1995).

In a follow-up experiment, sterilized rhizosphere soil from HCH-treated *Kochia* failed to promote accelerated degradation of γ-HCH (Figure 3). In nonsterile rhizosphere soil, concentration of γ-HCH decreased to negligible levels in 20 days while only 20% of the initially supplemented γ-HCH was lost from the sterilized rhizosphere soil. This further indicates that micro-organisms were involved in the degradation of HCH isomers.

Present study indicates that *Kochia* rhizosphere harbours active microbial population which can degrade HCH isomers aerobically. Further, these micro-organisms can be acclimatized to degrade HCH isomers at faster rate by prior treatment of *Kochia* rhizosphere with HCH. Therefore, *Kochia* plants can be used to decontaminate HCH residues in the contaminated soil.

Acknowledgment: I thank the Head, Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi for providing all necessary facilities to carry out this work.

REFERENCES

Anderson TA, Kruger EL, Coats JR (1994) Enhanced degradation of mixture of three herbicides in the rhizosphere of a herbicide tolerant plant. Chemosphere 28: 1551-1557

Anderson TA, Coats JR (1995) Screening rhizosphere soil samples for the ability to mineralize elevated concentrations of atrazine and metolachlor. J Environ Sci Health B30: 473-484

Bhuyan S, Sahu SK, Adhya TK, Sethunathan N (1992) Accelerated aerobic degradation of γ-hexachlorocyclohexane in suspension of flooded and non-flooded soils pretreated with hexachlorocyclohexane. Biol Fertil Soil 12: 279-284

Cunningham SD, Anderson TA, Schwab AP, Hsu FC (1996) Phytoremediation of soil contaminated with organic pollutant. Adv Agron 56: 55-114

Curl EA, Truelove B (1986) The rhizosphere, Springer, Berlin, p 280

Fang C, Radosevich M, Fuhrmann JJ (2001) Atrazine and phenanthrene degradation in grass rhizosphere soil. Soil Biol Biochem 33: 671-678

Knaebel DB, Vestal JR (1994) Intact rhizosphere microbial communities used to study microbial biodegradation in agricultural and natural soils. In Anderson TA, Coats JR (Ed) Bioremediation through rhizosphere technology, ACS symposium series number 563, p 56-70

Miya RK, Firestone MK (2001) Enhanced phenanthrene biodegradation in soil by selender oat root exudates and root debris. J Environ Qual 30: 1911-1918

Perkovich BS, Anderson TA, Kruger EL, Coats JR (1996) Enhanced mineralization of ^{14}C-atrazine in *Kochia scoparia* rhizosphere soil from a pesticide contaminated site. Pestic Sci 46: 391-396

Sahu SK, Patnaik KK, Sethunathan N (1990) Degradation of α–, β– and γ-hexachlorocyclohexane by rhizosphere soil suspension from sugarcane. Proc Indian Acad Sci (Plant Sci) 100: 165-172

Sahu SK, Patnaik KK, Bhuyan S, Sreedharan B, Kurihara N, Adhya TK, Sethunathan N (1995) Mineralization of α–, γ- and β-hexachlorocyclohexane by a soil bacterium under aerobic conditions. J Agric Food Chem 43: 833-837

Wade H, Senoo K, Takai Y (1989) Rapid degradation of γ-HCH in upland soil after multiple applications. Soil Sci Plant Nutr 35: 71-77