

# Seasonal variation of HCH isomers in open soil and plant-rhizospheric soil system of a contaminated environment

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## Abstract

**Background, aim, and scope** Lindane, technically 1, 2, 3, 4, 5, 6-hexachlorocyclohexane ( $\gamma$ -HCH), is the most commonly detected organochlorine pesticide from diverse environmental compartments. Currently, India is the largest consumer and producer of lindane in the world. The production of lindane results in the generation of large quantities of waste HCH isomers (mainly  $\alpha$ -,  $\beta$ - and  $\delta$ -). All these isomers are toxic and have a long-range environmental transport potential. The aim of this study was to monitor the seasonal variation of HCH isomers in an open soil–plant–rhizospheric soil system of a contaminated industrial area. For this, selected plant species and their rhizospheric soil (soil samples collected at a depth range of 0–45 cm near to the root system) and open soil samples (soil samples collected (0–30 cm depth) from 1–1.5 m away from the plant root system) were collected for 2 years (two summer seasons and two winter seasons).

**Materials and methods** Seven plant species along with their rhizospheric soil and open soil samples were collected seasonally from different parts of the industry. Plant samples were separated into root, leaf and stem. HCH isomers in plant and soil samples were extracted by matrix solid-phase dispersion extraction (MSPD) and Soxhlet extraction, respectively, followed by GC-ECD. The seasonal difference in occurrence of HCH isomers in plant

samples with their respective soil-system was studied by multivariate statistical approaches.

**Results** The mean concentration of total HCH in plant samples, open soil and rhizospheric soil samples were found in the range of 14.12 to 59.29 mg kg<sup>-1</sup>; 38.64 to 104.18 mg kg<sup>-1</sup> and 8.38 to 26.05 mg kg<sup>-1</sup>, respectively. Cluster grouping reveals that *S. torvum* and *W. somnifera* can accumulate more HCH than other studied species.

**Discussion** There was a marked seasonal difference in the occurrence of HCH isomers in plant samples ( $p < 0.05$ ) and open soil samples ( $p < 0.01$ ). Comparatively higher levels of HCH isomers were detected from plant samples during summer, while higher levels of HCH isomers were detected from soil samples during the winter season. There was no significant difference in seasonal variation of HCH isomers in rhizospheric soil samples; however, total HCH in rhizospheric soil samples were 4 to 5-fold lower than the open soil samples. The total concentration of HCH isomers in roots is linearly related to their rhizospheric HCH level.

**Conclusions** HCH isomers were detected in open soil, plants and rhizospheric soil samples. Monitoring studies clearly revealed that the above-mentioned industrial area is contaminated with all major isomers of HCH. Occurrence of all these isomers in the study area point out the lack of sustainable management practices of this industry for protecting the area from hazardous waste. The analytical results confirmed that accumulation depends upon the plant species, soil and climatic conditions.

**Recommendations and perspectives** Recently,  $\alpha$ -,  $\beta$ - and  $\gamma$ -HCH have been nominated by the POPs Reviewing Committee for inclusion into the Stockholm Convention to address the HCH contamination on a global level. Therefore, there is an urgent need to stop the production of lindane and remediate contaminated soil sites. Based on the monitoring studies, the promising species like *W.*

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*somnifera* and *S. torvum* may be selected for the on-site phytoremediation of HCH-contaminated soil. The mismanagement of HCH residues from the organochlorine industry and their contemporary relevance often after decades of their deposition is one key example demonstrating the necessity to evaluate the waste deposits of the respective organochlorine productions and need for a strict waste management, and the necessity of an integrated pollution prevention and control strategy for the whole organochlorine industry including also the developing countries.

**Keywords** HCH isomers · India · Open soil · Phytoremediation · Plant samples · Rhizospheric soil · Seasonal monitoring · POPs contaminated site

## 1 Background, aim, and scope

The extensive and indiscriminate use of lindane ( $\gamma$ -HCH) and technical HCH over the last few decades has led to the widespread contamination of its four major isomers ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -HCH) into various environmental compartments. The severity of the problem is reflected from the fact that HCH residues continuously are detected in air, soil, vegetation and water samples and even in some pristine ecosystems like Arctic region, Antarctica, Pacific Ocean, etc. (USEPA 1980; Iwata et al. 1994; Willet et al. 1998; Walker et al. 1999; Lal et al. 2008). The chemical properties of HCH isomers such

as low water and high fat solubility, stability to photo-oxidation and low vapour pressure are the main factors attributing their persistence in the environment (Willet et al. 1998; Walker et al. 1999). Although the use of HCH has been discontinued for a considerable period of time in many countries, the residues continue to have a significant impact on a number of ecosystems (Abhilash 2009).

A second important environmental threat from HCH production is the HCH residues deposited at the production sites (Vijgen et al. 2006; Weber et al. 2008a). For the lindane production, approximately 90% of HCH isomers remained as by-products (so called HCH muck) and finally became hazardous waste (Li 1999; Vijgen 2006). It is estimated that 1.6 to 4.8 million tons of HCH residues were dumped or landfilled close to the respective production sites worldwide (Vijgen et al. 2006; Weber et al. 2008a). Literature provides evidence on some heavy contamination episodes by HCH due to formulation factories and their illegal muck dumping (Table 1). Such HCH dumpsites have been found all over the world and have been reported from Brazil (Rio de Janeiro; Oliveira et al. 2003); Spain (Pontevedra; Concha-Grana et al. 2006; Vega et al. 2007), Bilbao (Mohan et al. 2006); Germany (Chemnitz; Boltner et al. 2005; Bitterfeld; Kalbitz and Popp 1999; Wycisk et al. 2003); the US (North Carolina; Earth fax 2002), and India (Lucknow; CAPE <http://www.panna.org/campaigns/docsLindane/lindaneDirtySecret.pdf>). In Galicia (Spain), the stores of a pesticide factory located in the industrial estate of Torneiros until 1964 caused the contam-

**Table 1** Occurrence of HCH isomers in dumpsites/contaminated soil samples from various part of the world

Country	Location/specific sites/type of contamination	HCH isomers(mg kg <sup>-1</sup> )				Total HCH	References
		$\alpha$ -HCH	$\beta$ -HCH	$\gamma$ -HCH	$\delta$ -HCH		
Brazil	Cidade dos Merinos, Duque de Caxias, Rio de Janeiro	6,200	7,320	140	530	14,190	Oliveira et al.(2003)
Canada	Disposal sites	18,000	1,800	4000	1300	25,100	Philips et al. (2005)
Germany	Contaminated soil sites	1.33	15.43	0.02	0.24	17.02	Vijgen (2006)
	Waste disposal site at Muggenburger Straße	–	–	–	–	182,000	Sievers and Friesel 1989
	Waste disposal site at Kirchsteinbek	–	–	–	–	3.8	Sievers and Friesel 1989
	Contaminated site at Ochsenwerder Landscheideweg	–	–	–	–	9,140	Sievers and Friesel 1989
Spain	Accidental contaminated area near to Porrino	25	15	2.2	0.5	42.7	Vega et al.(2007)
	Former industrial area, Galicia	45,815	34,830	47.6	343	81,036	Concha-Grana et al. (2006)
	Former industrial area, Galicia	13,375.0	6,512.5	11.3	9.9	19,904.6	Calvelo-Pereira et al. (2006)
India	Dumped sites	79,940	44,850	990	–	125,280	Dadhwal et al. (2008)
	Surface soil from Hiranki, Delhi	0.0009	0.0111	0.1878	–	0.1998	Prakash et al. (2004)
	Surface soil from Barkhalsha (Haryana)	0.00098	0.0084	0.2018	0.0010	0.2122	Prakash et al. (2004)
	Near a lindane producing factory	38.10	463.40	3.50	3.70	508.70	Prakash et al. (2004)
USA	Former lindane manufacturing facility	–	–	–	–	83,628	Philips et al. (2006)

ination of an area of 45,000 m<sup>2</sup> affecting the soils and aquifer (Concha-Grana et al. 2006). Also, the deposited residues in Bitterfeld (Germany) highlight the relevance and risk of aquifer contamination from deposited HCH residues (Wycisk et al. 2003). In addition, some companies have tried to recycle the HCH waste isomers by decomposition to chlorobenzene which in turn have lead to PCDD/F contaminated residues and contaminated sites (Weber et al. 2006, 2008b). However, a number of illegal disposals of HCH muck remain unnoticed.

The HCH-contaminated-sites cases demonstrate the necessity for their remediation and highlight the importance of the waste management practise during HCH production as a prime example for the crucial role of waste management policy for production sites of chlorinated organics (Weber et al. 2008a, b). Recently  $\alpha$ -,  $\beta$ - and  $\gamma$ -HCH have been nominated by the POPs Reviewing Committee for inclusion into the Stockholm Convention to address the HCH contamination on a global level ([www.pops.int](http://www.pops.int)).

India started the production of technical HCH in 1952 (Abhilash and Singh, 2009). Technical HCH and DDT amounted to 70% of total insecticide production in the 1980s. Available data suggest that from 1948 to 1995, around one million tons of technical HCH was used in India. Residues of HCH isomers have been reported in soil (Agnihotri et al. 1996; Bhattacharya et al. 2003; Prakash et al. 2004; Singh et al. 2007; Abhilash and Singh 2008a), vegetation samples (Abhilash et al. 2008), drinking water (Mathur et al. 2003; Bakore et al., 2004), food products (Pandit and Sahu 2002; Kumari et al. 2005, 2006) and even from bottled waters (Prakash et al. 2004). Realising the widespread contamination by HCH and toxic nature of HCH isomers, the use of technical HCH was banned in India in 1977, but restricted use of lindane is still permitted. India has produced 6,353 tons of lindane for export and domestic usage during 1997 to 2006; that would mean about 60,000 tons of waste HCH isomers (muck) were generated during this period, primarily consisting of  $\alpha$ -,  $\beta$ - and  $\delta$ -isomers. However, no reports are available on the waste HCH management practices during this period and no systematic study has been carried out to locate the dumpsites created during this period of lindane production (Lal et al. 2008).

Therefore, it is crucial to monitor the environmental fate of ground water and soil pollution around the HCH waste isomer landfills/dumpsites containing the major quantities of HCH production (Vijgen 2006; Weber et al. 2008a). Soil and vegetations are interesting compartments for monitoring the occurrence of persistent organic pollutants. The vegetation has been used to indicate ubiquitous pollutant contamination levels (Holoubek et al. 2000) and to determine the global contamination of organic pollutants (Simonich and Hit eis 1995). In addition, it can be utilised as a kind of passive sampler for contaminated site monitoring. Many studies have used vegetation, which bio-accumulates organic pollutants

for monitoring studies (Kylin et al. 1994; Simonich and Hit eis 1995; Holoubek et al. 2000; Abhilash et al. 2008). Vegetation plays an important role in the global transport of organochlorine pesticides (OCPs) since they cover over 80% of the earth's land surface, have a high lipid content and their surface area is larger than the ground area they cover (Jones 1992). The aim of this work was to study the (1) seasonal variation of hexachlorocyclohexane isomers ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -HCH) in soil-plant system of a contaminated industrial area (2) and to suggest promising species for the phytoremediation of lindane contaminated soil.

## 2 Materials and methods

### 2.1 Study area and sample locations

Monitoring of HCH isomers in plants and their soils was initiated in January 2005 (winter season) and was confined to a lindane producing factory (India Pesticides Limited (IPL) situated at Chinhat industrial estate, around 20 km from Lucknow City in Uttar Pradesh. The study area is located between 27° 55' North latitude and 89° 3' East longitude. The industry started during 1993 and, apart from the lindane production; the industry also has an in-house formulation facility for producing various lindane formulations such as lindane EC, carbaryl lindane 4.4 granules, lindane 6% granules, lindane 6.5 WDP and lindane 1.3%. Between 10th January 2005 and 25th May 2006, four samplings were completed (two winter seasons; 10th January 2005 and 18th February 2006 and two summer seasons; 21st May 2005 and 19th June 2006). Seven species of the most abundant growing plants (*Lantana camara* (Verbenaceae); *Erianthus munja* (Poaceae); *Calotropis procera* (Asclepedaceae); *Withania somnifera* (Solanaceae); *Solanum torvum* (Solanaceae) and *Achyranthes aspera* (Amaranthaceae)) were collected in triplicate from the campus and nearby areas of IPL during the respective season. Before uprooting the selected plant species, soil samples were collected from the rhizospheric zone ( $n=3$ ) of each species with the help of a core sampler (for herbs, rhizospheric samples were collected from 0–15 cm depth range and for shrubby plants; the sampling depth was up to 45 cm). Similarly, the open soil samples ( $n=3$ ) of each species were collected from 1 to 1.5 m away from the plant species and collected up to a range of depth from 0–15 cm. The plant samples were washed, separated into different matrices like root, stem and leaves, air-dried and stored at 4 °C for subsequent analysis.

### 2.2 Analysis of HCH isomers from plant matrices

The plant samples were extracted by matrix solid-phase dispersion extraction (MSPD) as described below and

finally determined by a Gas-Chromatograph equipped with  $^{63}\text{Ni}$  Electron Capture Detector (ECD). Leaf, stem and root samples were dried at 35 °C for 24 h, powdered, sieved (1–2 mm) and stored at 4 °C for subsequent analysis. Bulb (onion) and fruit samples were chopped firmly and 5 g chopped samples were directly used for the extraction purposes. 5 g plant matrices were gently ground with 0.5 g Florisil (deactivated with 3% acetone) in a pestle and mortar for 5 min and 1 g  $\text{MgSO}_4$  and 0.5 g NaCl were added to this mixture which was then ground firmly for 5 more min. This mixture was transferred into a glass column filled with neutral alumina ( $\text{Al}_2\text{O}_3$ ) deactivated with 3% acetone (2 g) and anhydrous  $\text{Na}_2\text{SO}_4$  (0.5 g). A mixture of *n*-hexane-ethyl acetate solvent 70:30 (v/v, 10 ml), was utilised for elution in the column and repeated with another 10 ml of same solvent mixture. When necessary, further purification can be performed using a ‘co-column’ (deactivated  $\text{Al}_2\text{O}_3$ +anhydrous  $\text{Na}_2\text{SO}_4$ ) for clean up, coupled to the first one containing MSPD material. The resulting extract was concentrated, resuspended with *n*-hexane (1 ml) and kept in 4 °C for subsequent analysis (Abhilash et al. 2007; Abhilash et al. 2009; Abhilash and Singh 2008b). All concentrations of HCH given refer to plant dry matter.

### 2.3 Analysis of HCH isomers from soil matrix

Open and rhizospheric soil samples were air-dried, sieved through a 2-mm mesh, and ground before analysis. After the pre-treatment, soil samples were frozen at –18 °C until extraction. Samples of 10 g of air-dried soil underwent Soxhlet extraction over 24 h with 150 ml of toluene in a Soxhlet unit. The extracts obtained were then concentrated for subsequent cleanup, mixed with 20 ml of dichloromethane-*n*-hexane (1:1 v/v), transferred to a Florisil column, and eluted with 130 ml of the same solvent mixture. The elutes were concentrated and dissolved in 1 ml of toluene (Abhilash and Singh 2008a, c). A Perkin Elmer (Norwalk, CT, USA) gas chromatograph Model Clarus 500 series equipped with  $^{63}\text{Ni}$  electron capture detection (ECD) system and split–splitless injector was used. Nitrogen of purity greater than 99.99% was used as carrier gas at a programmed flow of 1.5 ml  $\text{min}^{-1}$ . For separation, a 35% diphenyl and 65% dimethyl polysiloxane capillary column (30 m×0.32 mm i.d., 0.5  $\mu\text{m}$ ) Elite 35 (Perkin-Elmer) was employed. All concentrations of HCH given refer to soil dry matter.

### 2.4 Statistical analysis

The data were subjected to analysis of variance (ANOVA) to test significant differences by using SPSS (14.0) software for Windows Programme (Illinois, USA) and followed to post hoc DMRT tests. The seasonal variation of HCH isomers in plant samples, rhizospheric and open soil

samples were compared by Student's *t*-test. Similarly, the significant difference between pesticide residue in different plant matrices were calculated by Student's *t*-test and degree of correlation between uptake of HCH isomers and different plant species were compared by linear regression and Pearson Coefficient of correlation. Cluster analysis was performed on the seasonal variation of HCH distribution in soil samples and their rhizospheric soil to group the matrices in terms of their contamination level.

## 3 Results and discussion

India Pesticide Limited (IPL) has started the production of lindane during 1993; with an installed production capacity of 300 metric tons per annum. As mentioned above, the production of one ton of lindane generates a waste (HCH muck) of around 6–10 tons of other isomers (primarily  $\alpha$ -,  $\beta$ - and  $\delta$ -HCH; Li 1999, Vijgen 2006). Consequently,  $\alpha$ -,  $\beta$ - and  $\delta$ -isomer mixtures were often dumped under unsuitable circumstances (Vijgen 2006). The lack of hazardous waste management strategies of this industry has created the widespread pollution of the nearby ecosystems. The industry has produced about 3,000 tons of lindane, which in turn resulted in the production of about 30,000 tons of waste HCH isomers (Dadhwal et al. 2009) and dumping HCH waste in the dump yard created 20 km away from the industry. This industrial dumping site serves as a sink of ‘muck HCH’ from where HCH residues can enter into various environmental compartments. Similarly, the industrial premise is also contaminated with all major isomers of HCH due to the ongoing production and formulation of lindane and also due to the transportation of muck HCH to the dumping site. Hence, these isomers can spread to far off regions due to leaching, volatilization and atmospheric transport.

### 3.1 Physico-chemical properties of soil samples

Physico-chemical properties of open and rhizospheric soil samples are presented in Table 2. pH and electrical conductivity (EC) of open soil samples were higher during winter than summer season, although, in the case of rhizospheric soil samples, there was no seasonal difference (slightly acidic throughout the sampling period). This may be attributed to the so-called plant–rhizospheric effect regularly supplying low molecular weight organic acids (root exudates) to the surrounding rhizospheric soil. However, the pH of open soil was slightly alkaline during winter and slightly acidic during the summer season. Similarly, the total organic carbon showed an opposite trend, i.e. higher concentrations during summer season than during the winter season. This change is mainly due to the



**Table 2** Seasonal variation in physico-chemical properties of soil samples

Properties	Open soil		Rhizospheric soil	
	Winter	Summer	Winter	Summer
pH	7.8–8.9	6.3–7.6	6.8–7.3	6.2–7.8
EC (mS cm <sup>-1</sup> )	93.2–185.26	109–189.4	98.4–167.7	103.4–155.3
TOC (mg kg <sup>-1</sup> )	0.796–1.260	1.127–1.857	0.995–1.454	1.35–2.122

enhanced decomposition rate of litter and humus and the subsequent release of humic as well as carboxylic acids during the summer season (Sharma et al. 2007).

### 3.2 Distribution of HCH isomers in plant species

The concentration of HCH isomers in plant matrices during different seasons are presented in Fig. 1. All seven plant samples analysed were capable of accumulating the four isomers of HCH in their tissues. The mean concentration of total HCH isomers in the plant samples varied between 14.12 and 59.24 mg kg<sup>-1</sup>. Maximum concentration of total HCH was detected during summer 2005 (60.65 mg kg<sup>-1</sup>) and minimum concentration was found during winter 2005 (13.23 mg kg<sup>-1</sup>). The total β-HCH (including all matrices of plant) varied from 10.25 to 25.06 mg kg<sup>-1</sup>, whereas α-HCH varied from 2.17 to 9.32 mg kg<sup>-1</sup>; γ-HCH varied between 1.75 and 10.56 mg kg<sup>-1</sup>; and δ-HCH from 0.06 to 5.69 mg kg<sup>-1</sup>. Regardless of the time of sampling, the maximum HCH accumulation was detected in *S. torvum* (51.58 mg kg<sup>-1</sup>) and minimum concentration was detected in *E. munja* (16.82 mg kg<sup>-1</sup>).

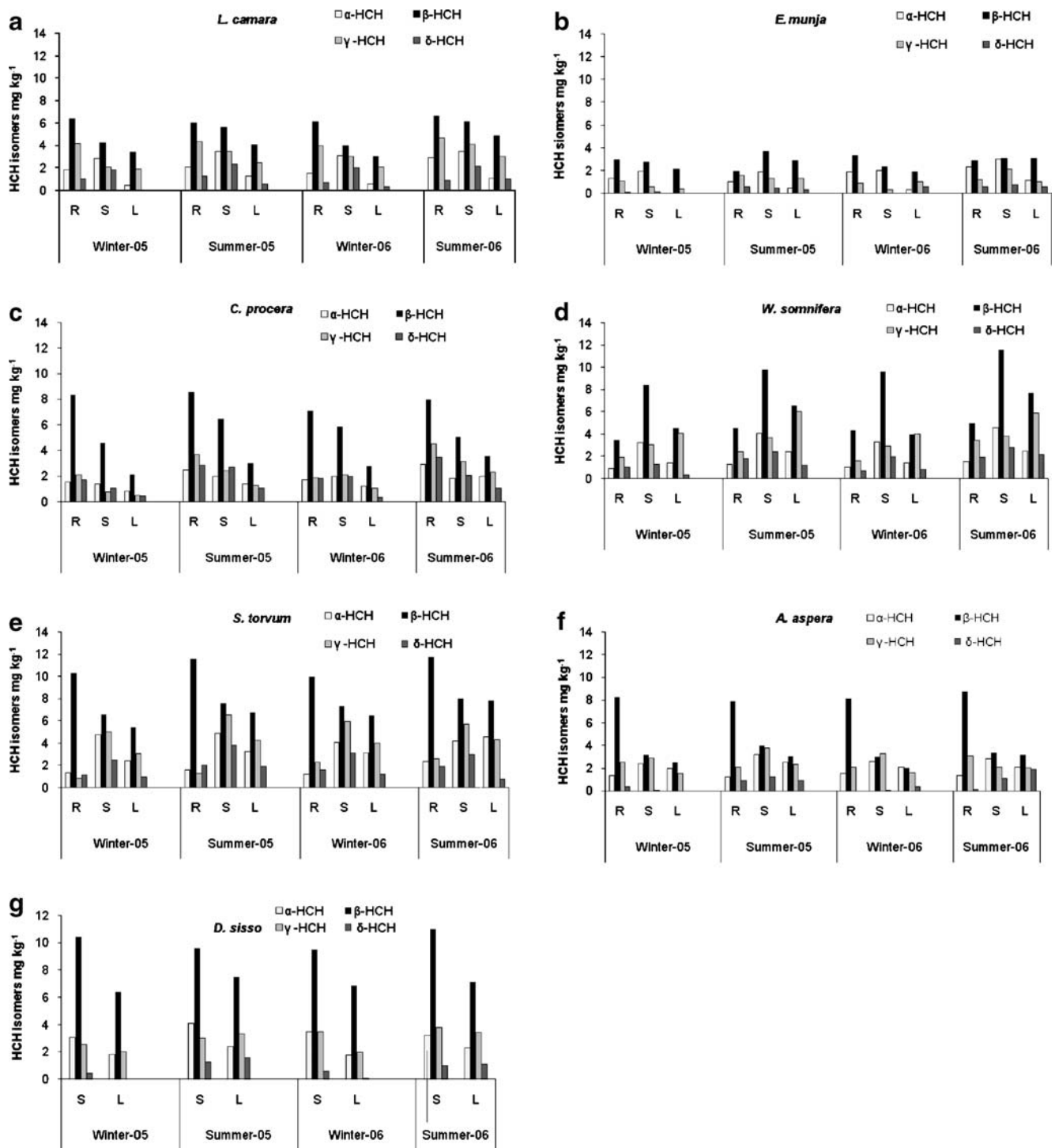
Cluster grouping of HCH isomers in various plant parts (Fig. 2) clearly indicates that the relative accumulation of each HCH isomer in each part of plants is isomer selective and also depends on plant species. The total HCH in the leaves of seven plant species ranged between 2.48 and 18.23 mg kg<sup>-1</sup>. Concentrations of α-, β-, γ- and δ-HCH isomers in the leaves of various plant species were found in the range of 0 to 4.55 mg kg<sup>-1</sup>; 1.87 to 7.81 mg kg<sup>-1</sup>; 0.38 to 5.99 mg kg<sup>-1</sup> and 0 to 2.14 mg kg<sup>-1</sup>, respectively. The highest concentration of total HCH was found in the leaf samples of *W. somnifera* during summer 2006. The mean accumulation potential of HCH isomers in different leaf samples were found in the following manner: *S. torvum* (14.87 mg kg<sup>-1</sup>)>*W. somnifera* (13.28 mg kg<sup>-1</sup>)>*D. sisso* (12.21 mg kg<sup>-1</sup>)>*A. aspera* (7.35 mg kg<sup>-1</sup>)=*L. camara* (7.33 mg kg<sup>-1</sup>)>*C. procera* (5.90 mg kg<sup>-1</sup>)>*E. munja* (4.04 mg kg<sup>-1</sup>). Concentrations of total HCH in stem matrix were found to be in the range of 4.63 to 22.76 mg kg<sup>-1</sup>. β-HCH was the predominant isomer in stem matrix (2.31 to 11.98 mg kg<sup>-1</sup>) followed by γ-HCH (0.32 to 6.5 mg kg<sup>-1</sup>), α-HCH (1.37 to 4.89 mg kg<sup>-1</sup>) and δ-HCH (0 to 3.78 mg kg<sup>-1</sup>). The maximum accumulation of 22.76 mg kg<sup>-1</sup> was detected in stem samples of *S. torvum* during summer 2005.

The overall accumulations of total HCH in the stem matrix of seven species were: *S. torvum* (20.66 mg kg<sup>-1</sup>)>*W. somnifera* (18.99 mg kg<sup>-1</sup>)>*D. sisso* (17.06 mg kg<sup>-1</sup>)>*L. camera* (13.34 mg kg<sup>-1</sup>)>*C. procera* (11.04 mg kg<sup>-1</sup>)>*A. aspera* (9.65 mg kg<sup>-1</sup>)>*E. munja* (6.35 mg kg<sup>-1</sup>). Concentrations of α-, β-, γ- and δ-HCH isomers in the root matrix of various plant species were found in the range of 0.87 to 2.90 mg kg<sup>-1</sup>; 1.95 to 11.76 mg kg<sup>-1</sup>; 0.82 to 4.7 mg kg<sup>-1</sup> and 0 to 3.48 mg kg<sup>-1</sup>, respectively. Maximum accumulation of total HCH was found in the root matrix of *C. procera* (18.82 mg kg<sup>-1</sup>) during summer 2006 and minimum concentration was detected in *E. munja* (5.05 mg kg<sup>-1</sup>) during summer 2005. Accumulations of total HCH isomers in root matrix were found in the following order: *S. torvum* (15.78 mg kg<sup>-1</sup>)=*C. procera* (15.40 mg kg<sup>-1</sup>)>*L. camera* (13.61 mg kg<sup>-1</sup>)>*A. aspera* (12.38 mg kg<sup>-1</sup>)>*W. somnifera* (8.95 mg kg<sup>-1</sup>)>*E. munja* (5.83 mg kg<sup>-1</sup>).

Mean concentration of total HCH (α-+β-+γ-+δ-HCH) in plant species (root+leaf+stem) were found in the following order: *S. torvum* (51.58 mg kg<sup>-1</sup>)>*W. somnifera* (41.68 mg kg<sup>-1</sup>)>*L. camera* (33.51 mg kg<sup>-1</sup>)>*C. procera* (32.07 mg kg<sup>-1</sup>)>*D. sisso* (31.29 mg kg<sup>-1</sup>)>*A. aspera* (30.38 mg kg<sup>-1</sup>)>*E. munja* (16.82 mg kg<sup>-1</sup>). The cluster grouping of plant species according to their accumulation potential is depicted in Table 3.

### 3.3 Concentration of HCH isomers in open soil samples

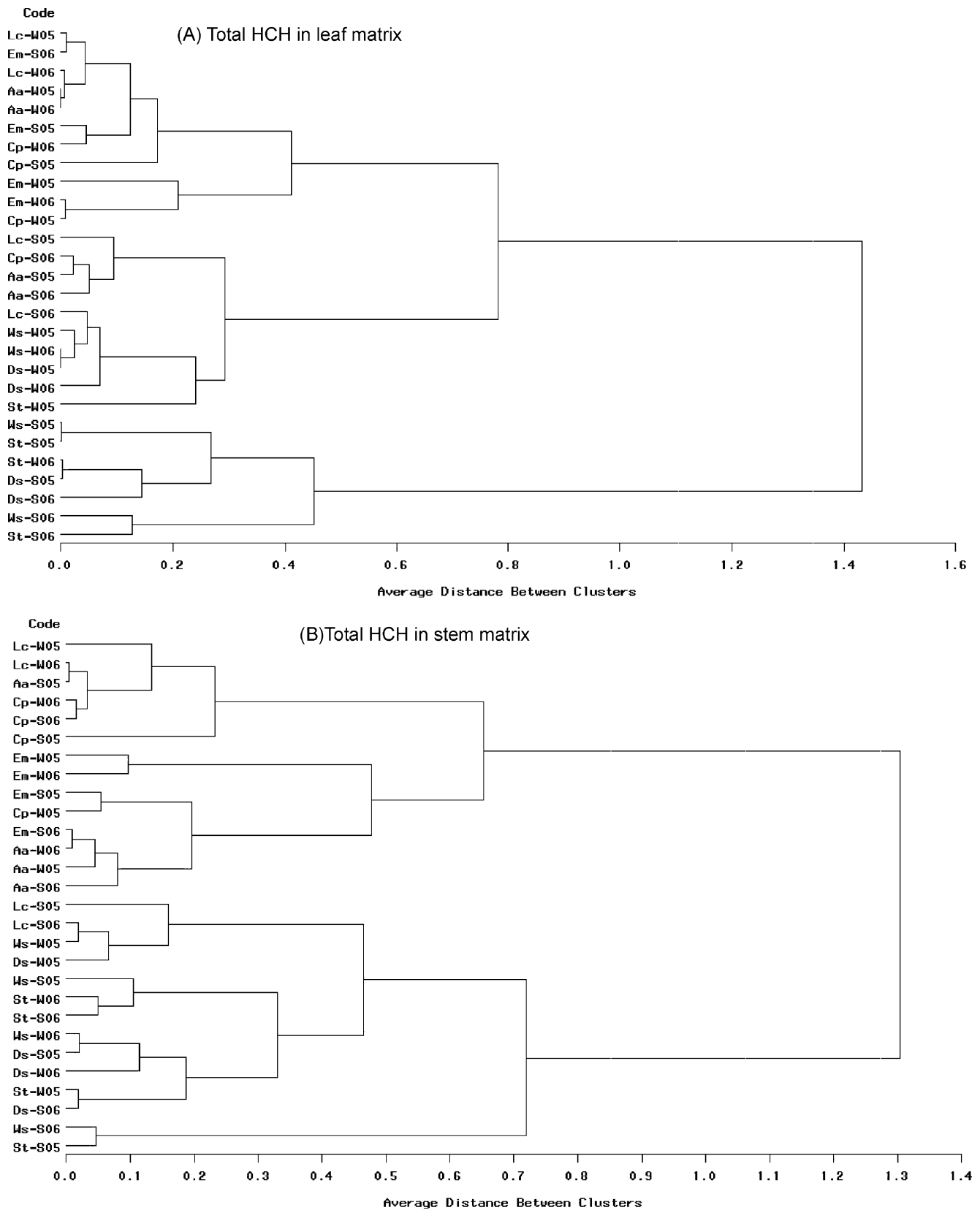
Open soil samples near the different plant species were analysed in order to evaluate the extent to which contamination of the total HCH isomers varied. The concentration of total HCH (sum of isomers α-HCH+β-HCH+γ-HCH+δ-HCH) in the analyzed samples varied from 38.64 to 104.18 mg kg<sup>-1</sup>. The maximum concentration of total HCH was detected (the soil sample near to *W. somnifera*) during winter 2005 and minimum concentration was detected during summer 2005 (the soil sample near to *E. munja*). The concentration of α-HCH ranged between 2.3 to 12.99 mg kg<sup>-1</sup>, β-HCH between 28.14 to 70.28 mg kg<sup>-1</sup>, γ-HCH between 4.5 to 15.15 mg kg<sup>-1</sup>, and those of δ-HCH between 0.59 to 7.23 mg kg<sup>-1</sup>. The relative distribution of different HCH isomers to the total HCH in the soil samples differed from that of the isomeric composition in original waste. Further, this isomeric ratio was entirely different from the ratio of HCH isomers in dumpsites reported



**Fig. 1** Seasonal occurrence and matrix wise distribution of HCH isomers in various plant species. ( $n=3$ ). The letters *R*, *S* and *L* denote root, stem and leaf, respectively

(Sievers and Friesel 1989; Kalbitz and Popp 1999; Oliveira et al. 2003; Wycisk et al. 2003; Calvelo-Pereira et al. 2006). In all the dumpsite cases,  $\alpha$ -HCH was the predominant isomer followed by  $\beta$ -HCH and  $\gamma$ -HCH. However, in the present study,  $\beta$ -HCH was the predominant isomer constituting half of the total HCH in all samples followed by  $\gamma$ -

HCH (8–16.5%) and  $\alpha$ -HCH (5–13%). The occurrence of a high level of  $\beta$ -HCH is attributed by its chemical stability against microbial degradation. Furthermore, the predominance of  $\beta$ -HCH can be explained by the isomerisation of  $\alpha$ -HCH to  $\beta$ -HCH and of  $\gamma$ -HCH via  $\alpha$ -HCH to the more stable  $\beta$ -HCH, which is energetically more favourable



**Fig. 2** Cluster grouping of plant matrices based on their HCH accumulation potential.  $N=3 \times 2$  winter season+2 summer seasons. The first two letters denote the scientific name and the last code denotes the respective season. *W05* winter 2005, *W06* winter 2006,

*S05* summer 2005, *S06* summer 2006, *Lc* *Lantana camara*, *Em* *Erianthus munja*, *Cp* *Calotropis procera* *Ws* *Withania somnifera*, *Aa* *Achyranthes aspera*, *Ds* *Dalbergia sisso*, *St* *Solanum torvum*

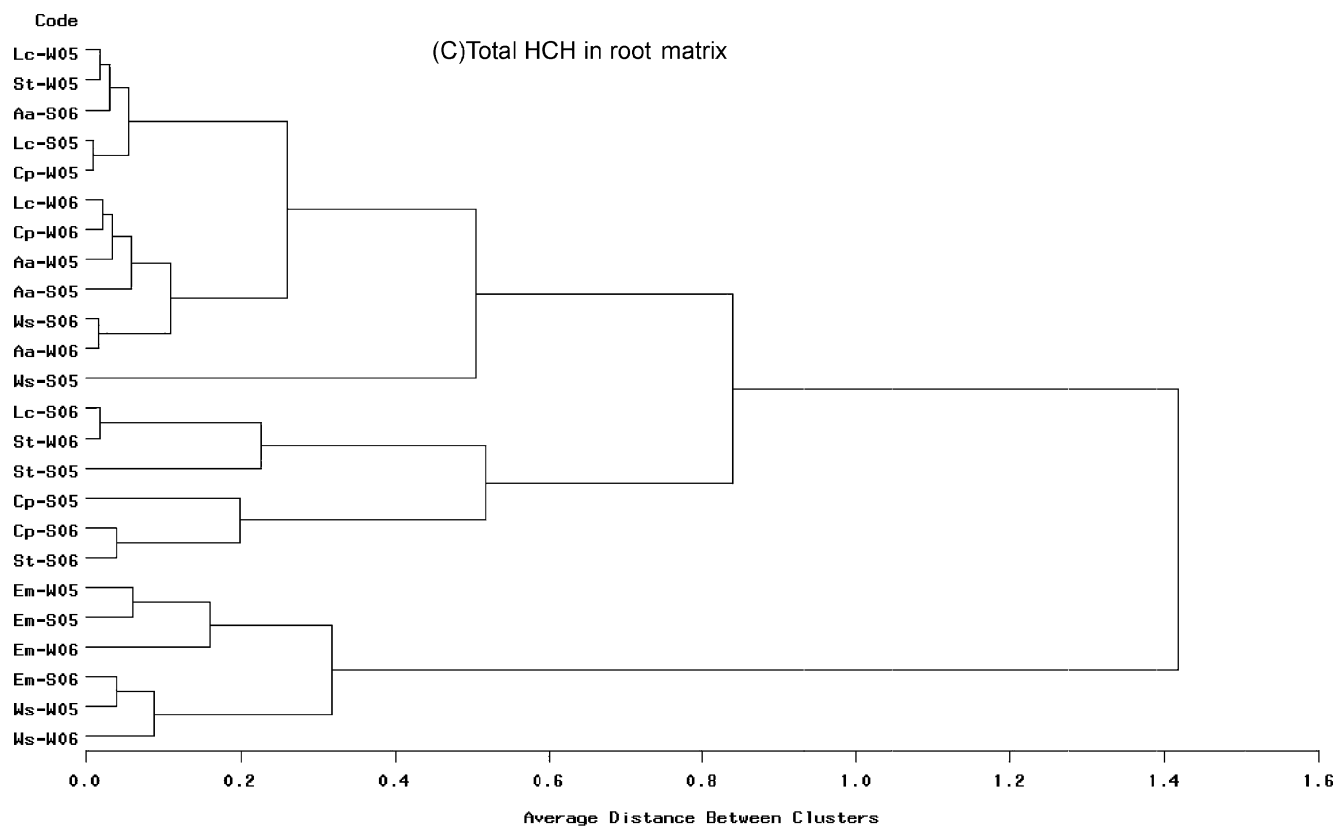


Fig. 2 (continued)

(Fielder et al. 1995; Wu et al. 1997; Manz et al. 2001). The comparatively higher level of lindane in soil samples indicates the possible contamination (spill over during formulation or transportation of lindane) due to the ongoing production. This can be further evidenced by the  $\alpha$ -HCH/ $\gamma$ -HCH ratio. In all the samples, the  $\alpha$ -HCH/ $\gamma$ -HCH ratio was  $<1$ , which indicates the recent input of pollution (Krauthacker et al. 2001).

Recently, Dadhwal et al. (2009) reported the higher concentration of HCH isomers from the above industrial area (total HCH of  $4,251.9 \text{ mg kg}^{-1}$ ). In contrast to our observations, higher concentrations of  $\alpha$ -HCH (66.5%) were detected in all samples, followed by  $\beta$ -HCH (29.2%). The percentage distribution of  $\delta$ -HCH (1.5%) and  $\gamma$ -HCH was very low (0.54%). Furthermore, the relative distribution of different HCH isomers in various soil samples were similar to the isomeric composition in muck HCH, indicating that most of the samples might be taken from direct muck HCH spill-over sites. Similar to our observations, noticeable percentage of  $\gamma$ -HCH (15.93%) and  $\delta$ -HCH (5.17%) were detected from the contaminated area of Canada (Philips et al. 2005; Philips et al. 2006). In the present study, all the soil samples were taken from the specific points where there were not any visible signs of muck HCH and care has been taken to avoid sampling from

the direct spill over sites. It is obvious that muck dumped/spill over sites are heavily contaminated with HCH isomers and the extent of HCH isomers contamination can be calculated up to a certain level by measuring the production quantity itself. Therefore, in the present study, we focused the contamination of HCH isomers in nearby soil system of an industrial premise and the extent to which these isomers enter into the natural soil and plant species growing there.

### 3.4 Concentration of HCH isomers in rhizospheric soil samples

The rhizosphere is the soil in the immediate vicinity of a root that is affected by root processes (Darrah 2006). The

**Table 3** Cluster grouping of plant species based on their accumulation potential

Cluster group	No. of plants	Species
I	2	<i>L. camara</i> ; <i>C. procera</i>
II	2	<i>A. aspera</i> ; <i>D. sisso</i>
III	1	<i>E. munja</i>
IV	2	<i>W. somnifera</i> ; <i>S. torvum</i>



total HCH isomers in rhizospheric soil samples ranged from 8.39 mg kg<sup>-1</sup> to 26.05 mg kg<sup>-1</sup> (Fig. 3). Regardless of the time of sampling, the concentration of total HCH isomers in rhizospheric soil samples was lower than the surrounding open soils ( $p < 0.01$ ). The mean concentration of four HCH isomers in rhizospheric soil was four- to fivefold lower than the open soil. The maximum HCH concentration was found in the rhizospheric zone of *W. somnifera* (during winter 2006) and minimum concentration was detected from the rhizospheric zone of *E. munja*. The mean concentration of HCH isomers in the rhizospheric zone of various plant species was found in the following order: *S. torvum* (24.61 mg kg<sup>-1</sup>) > *W. somnifera* (16.74 mg kg<sup>-1</sup>) > *L. camera* (11.92 mg kg<sup>-1</sup>) = *C. procera* (11.66 mg kg<sup>-1</sup>) = *A. aspera* (11.25 mg kg<sup>-1</sup>) > *E. munja* (9.11 mg kg<sup>-1</sup>).

The occurrence of low-level concentration of HCH isomers in the rhizospheric zone is mainly due to (a) enhanced root adsorption and subsequent plant uptake (b) and rhizodegradation. Statistical analysis proved that there is a linear relation between the total HCH concentrations in root samples to their respective rhizospheric soil. Many recent studies demonstrate significantly enhanced dissipation and or mineralization of persistent organic pollutants at the root–soil interface (Anderson et al. 1993; Anderson and Coats 1995; Kuiper et al. 2004; Chaudhry et al. 2005; Krutz et al. 2005). This rhizosphere effect is generally attributed to an increase in microbial density, diversity and or metabolic activity due to the release of plant root exudates, mucigel and root lysates (enzymes, amino acids, carbohydrates, low-molecular-mass carboxylic acids, flavonones and phenolics; Curl and

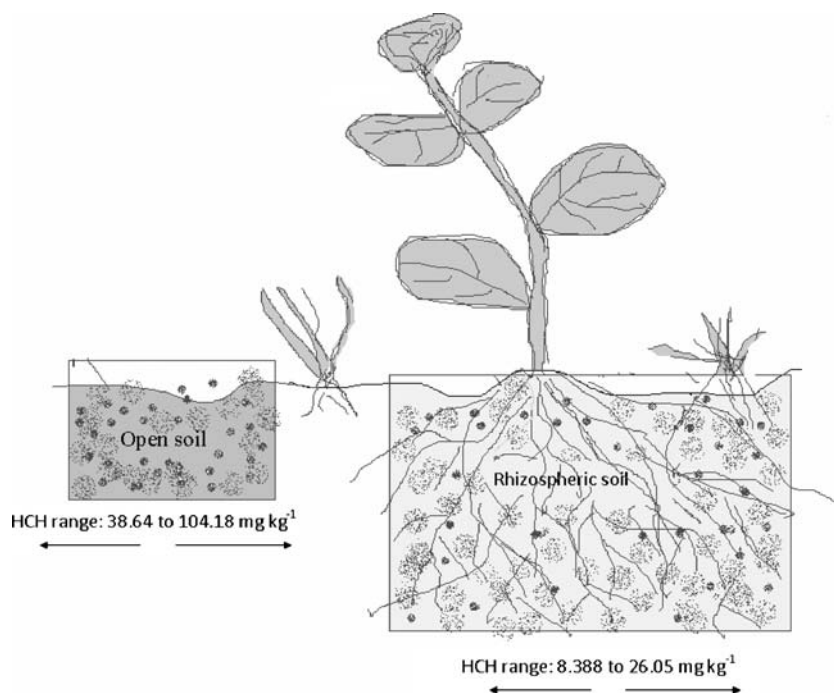
Truelove 1986; Kidd et al. 2008). Further, plants can also improve the physical and chemical properties of the contaminated soil, and enhance the bioavailability of the soil contaminants for the root-associated microorganisms.

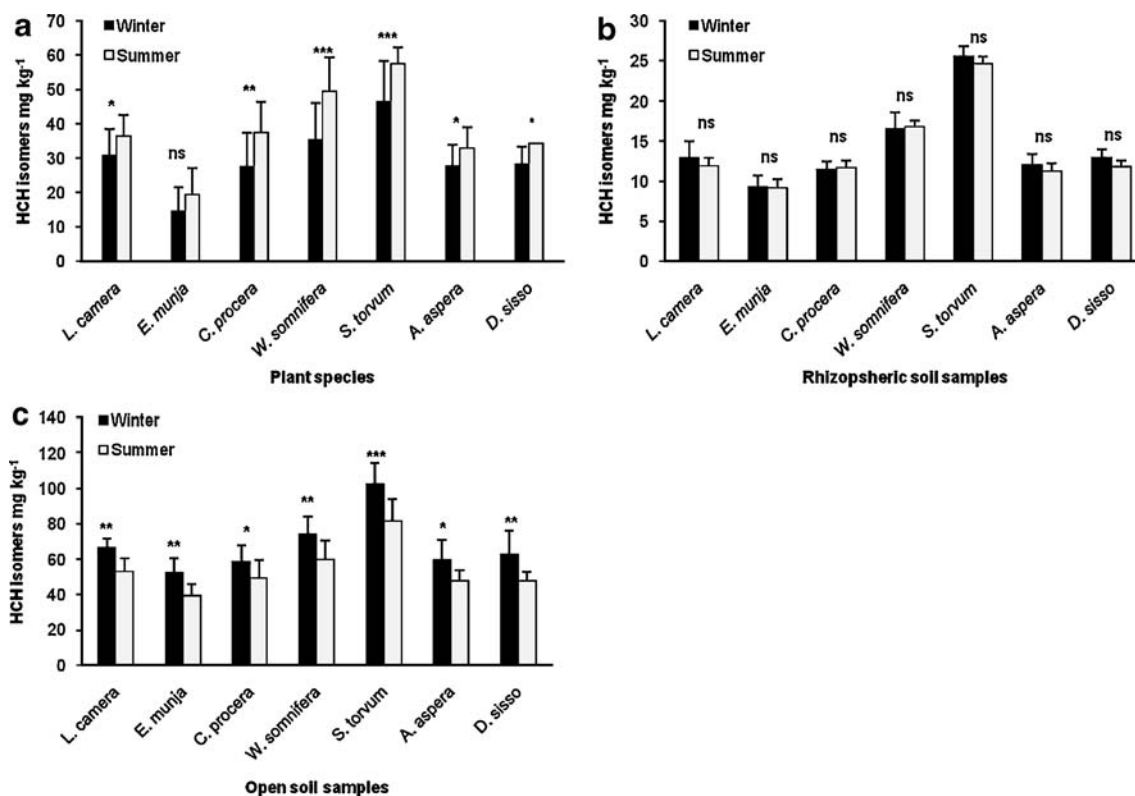
### 3.5 Seasonal variation of HCH isomers in soil–plant system

The statistical evaluation confirmed that there is a significant difference in the occurrence of total as well as individual HCH isomers in open soil, plant species and their respective rhizospheric soil samples. The seasonal variation of HCH isomers in various plant species and their respective soil systems are depicted in Fig. 4. From this figure, it is very clear that maximum HCH accumulation has occurred during summer season. According to a variance analysis (two-way ANOVA), differences at a significant level of 0.05, 0.01 and 0.001 were found among the seasons for the accumulation of  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ - and total HCH isomers in root, stem, leaf matrices of tested species (data not shown). The differences were more prominent for stem and leaf matrix during summer season and root matrix for winter season. As mentioned earlier, HCH isomers enter into plants through both soil–plants as well as air–plants pathways. We presume that the elevated level of HCH isomers in plant species during summer (Fig. 4a) is mainly due to the enhanced evaporation of HCH isomers -either directly from the open soil matrix or after dissolution of the substances from the pore water

The concentrations of HCH isomers in open soil samples support the above interpretation. Similar to the plant HCH concentration, there was a distinct variation in the seasonal

**Fig. 3** Pictorial representation of the range of HCH isomers in rhizospheric and open soil samples





**Fig. 4** Seasonal variation of HCH isomers **a** plant species, **b** rhizospheric soils, **c** open-soil samples. *NS* not significant, \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.00$  (*t*-test)

occurrence of HCH isomers in open soil samples. However, this variation was in opposite trend with more HCH isomers during the winter season than during summer (Fig. 4c). The occurrence of higher concentration of HCH in open soil during winter season is presumably mainly due to the low-level of evaporation of HCH isomers from open soil. Interestingly, there was no significant seasonal variation in occurrence of HCH isomers in rhizospheric soil (Fig. 4b). It may be expected that the elevated level of dissolved organic matter in rhizospheric soil compared to the open soil samples promotes plant uptake of the HCH isomers. Hence, there was a constant level of HCH isomers in the rhizospheric soil of plants species.

According to Cunningham et al. (1996), soil has three phases: solid, liquid and vapour. Plants obtain contaminants from the soil most readily from the liquid phase, but in some cases vapour phase uptake is most important. Vapour-phase movement of organics into plants from the surrounding soil can be an important uptake mechanism for some compounds, even those with relatively low vapour pressure. Many soil insecticides rely on this delivery mechanism (Cunningham et al. 1996).

The occurrence of persistent organic pollutants in soil-plant system depends upon several environmental factors, such as temperature, soil type, pH, and organic carbon content, morphological and biochemical properties of the

plant species. Further, the intensity of evaporation, degradation and translocation of pollutants from root to plant system all depend upon the substance-specific parameters, such as water solubility, vapour pressure and Henry's law constant. All these properties affect both the concentrations in the soil-plant system and long-range transport of these pollutants.

### 3.6 Bioconcentration of HCH isomers

Bioaccumulation is the ratio of contaminant concentration in plants or animals to their respective environmental media and is for plants generally expressed by using a bioconcentration factor (BCF), whereas also biomagnification accounts for bioaccumulation in animals. Regression polynomials were used to depict the relations between pollutant concentrations in soil and plants. Here, the soil concentrations of HCH were considered as a predictable variable and the HCH concentrations in test plants as an estimate. The slope of the linear regression between the soil and the test plant concentrations can be interpreted as the root or shoot BCF, the ordinate intercept as the background tissue concentration. The correlation of the rhizospheric concentration of HCH isomers with the plant root matrix resulted in a goodness-of-fit of linear regression  $R^2 = 0.788$  to  $0.998$ ; (Table 4); however, the correlation was insignificant for

**Table 4** Bioconcentration factors of HCH isomers calculated on the basis of plant concentrations and their rhizospheric soil samples

Plant species	Root BCF	Background concentration	$R^2$
<b><math>\alpha</math>-HCH</b>			
<i>L. camara</i>	0.283	0.611	0.899
<i>E. munja</i>	0.857	0.071	0.862
<i>C. procera</i>	0.245	0.397	0.853
<i>W. somnifera</i>	0.063	1.273	0.788
<i>S. torvum</i>	1.685	0.666	0.979
<i>A. aspera</i>	0.286	0.959	0.998
<b><math>\beta</math>-HCH</b>			
<i>L. camara</i>	1.457	2.740	0.996
<i>E. munja</i>	4.53	0.744	0.946
<i>C. procera</i>	3.788	1.454	0.854
<i>W. somnifera</i>	6.748	1.099	0.969
<i>S. torvum</i>	11.13	0.544	0.782
<i>A. aspera</i>	3.850	1.401	0.822
<b><math>\gamma</math>-HCH</b>			
<i>L. camara</i>	0.920	1.332	0.978
<i>E. munja</i>	0.712	1.685	0.799
<i>C. procera</i>	0.305	1.515	0.789
<i>W. somnifera</i>	2.027	0.236	0.970
<i>S. torvum</i>	3.253	0.176	0.783
<i>A. aspera</i>	0.557	0.270	0.832
<b><math>\delta</math>-HCH</b>			
<i>L. camara</i>	0.071	0.871	0.998
<i>E. munja</i>	0.165	0.282	0.864
<i>C. procera</i>	0.476	0.254	0.866
<i>W. somnifera</i>	0.895	0.160	0.842
<i>S. torvum</i>	0.005	0.740	0.791
<i>A. aspera</i>	0.003	0.764	0.965

BCF (bioconcentration factor) of HCH isomers in root matrix of the various plant species calculated using liner regression between HCH isomers in root and their respective rhizospheric soil concentration. Slopes and intercepts of the regression are interpreted as BCF and background concentrations, respectively. ' $R^2$ ' is the Pearson's correlation coefficient; the correlation is significant ( $p < 0.05$ ) for  $R > 0.75$

HCH isomers in rhizospheric soils with the aerial plant parts. As from this table, it is very clear that ordinate intercept (the background concentration of HCH isomers) was always above zero, suggesting that there is a certain background level of HCH isomers in root samples in the area of investigation, independent of the rhizospheric concentration. Further, the correlation between open soil samples and root matrix was insignificant ( $R^2 = 0.488$  to  $0.678$ ); however, the correlation was significant for aerial parts (leaf,  $R^2 = 0.812$  to  $0.985$ ; stem,  $R^2 = 0.883$  to  $0.988$ ). Similarly, the ordinate intercept was always greater than one indicating that there was a significant background concentration of HCH isomers in aerial parts independent

of soil concentration. The occurrence of HCH isomers in both roots and aerial plant parts clearly suggests that plants may either take up HCH isomers from the air through the surfaces of above-ground parts such as leaves, stems or bark or from the soil through the roots, or both, respectively. Therefore, HCH measured in above-ground parts may have entered the plant by a combination of pathways.

#### 4 Conclusions

The integrated study of the behaviour of organic contaminants in the rhizospheric soil-plant system is essential for assessing the risk of transfer to the trophic chain and for the development of phytoremediation techniques that can be applied to contaminated soils. Further, the contaminant levels in vegetation samples can be used as indicators of environmental pollution as plants can adhere particles from rhizospheric soil and absorb compounds through their roots; furthermore, they can absorb volatile compounds and trap atmospheric deposition in their leaves. Our monitoring studies clearly showed that the above-mentioned industrial area is contaminated with all major isomers of HCH. Occurrence of all these isomers in the study area point out the lack of sustainable waste management practices of the industry in the area investigated. Among the four isomers,  $\beta$ -HCH was being predominantly detected in all samples. Cluster grouping of plant species according to their uptake potential proved that both *S. torvum* and *W. somnifera* can accumulate considerable amount of HCH isomers in their root, shoot and stem matrix than any other species. The results reflect the importance of these plants in monitoring purposes and their potential to attenuate soil contamination by HCH isomers.

#### 5 Recommendations and perspectives

There is an urgent need to stop the production of lindane and to remediate contaminated soil sites. Based on the monitoring studies, promising species like *W. somnifera* and *S. torvum* may be selected for the on-site phytoremediation of low level HCH contaminated soil. Compared to *S. torvum*, *W. somnifera* is more suitable for phytoremediation studies because of (a) larger biomass than *S. torvum*; (b) rapid growth (c) easy culture and harvesting (spines present in leaves and stem of *S. torvum* is not easy for culturing); (d) better adaptation to a diverse range of soil. However, the phytoremediation of highly contaminated sites are very difficult, especially for the decontamination of dumped sites and obsolete stockpiles due to the phytotoxicity of contaminants elevated at high concentrations. There

are also some microbial species (e.g. *Sphingobium indicum*, Lal et al. 2008) reported for the bioremediation of HCH isomers. However, many onsite studies reveal that the survival and performance of the isolated microorganisms in real contaminated sites was not promising. The development of suitable HCH waste management strategy is important for the decontamination of dumped sites, especially for the decontamination of impacted sites in developing countries. The conversion of waste HCH to TCB (trichlorobenzenes) is also a technological option; however, this process has a high potential to generate huge amounts of unintentionally produced PCDD/PCDF (Sievers and Friesel 1989; Weber et al. 2006, 2008a), which in turn can become an additional environmental liability. Therefore, the following points should be considered for the successful remediation of HCH contaminated sites and those of a similar nature:

1. Detailed assessment of the contaminated sites from HCH and other organochlorine production and their contemporary relevance for the ground water, biota and potential human exposure.
2. Evaluate and monitor the remediation capacity within the natural processes at the sites (natural attenuation) and possibly increase this remediation capacity by, for example, suitable substrate modifications (e.g. sugarcane bagasse Abhilash and Singh 2008c) or by proper selection and modification of plants and organisms. In this respect, the insertion of foreign genes in suitable plants may be evaluated to optimise the remediation potential of the plants.
3. The utilisation of soft remediation processes like phytoremediation, bioremediation and natural attenuation processes need to be carefully monitored for toxic degradation products and their mobility (Weber et al. 2008a). It was recently demonstrated, e.g. for a PAH contaminated site, that the toxicity of the soil increased during bioremediation, although the target 16 EPA PAH had decreased (Andersson et al. 2009). For HCH-contaminated sites, more water-soluble degradation products like chlorinated cyclohexenols and cyclohexenediols (Raina et al. 2007, 2008) need to be taken into consideration.
4. For contaminated sites with immediate impact and a risk for human health and the environment, instant securing and remediation measures need to be applied utilizing best available technology (BAT) standards.

Furthermore, the mismanagement of HCH residues from the organochlorine industry and their contemporary relevance (for other cases often after decades of their deposition, Vijgen 2006; Wycisk et al. 2003) is one key example demonstrating the necessity to evaluate the waste deposits of organochlorine productions. We highlight the need of strict waste

management in these factories, the necessity of an integrated pollution prevention, and a control strategy for the organochlorine industry also in developing countries.

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