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SOILS, SEC 3 • REMEDIATION & MANAGEMENT OF CONTAMINATED OR DEGRADED LANDS • RESEARCH ARTICLE

Withania somnifera Dunal-mediated dissipation of lindane from simulated soil: implications for rhizoremediation of contaminated soil

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Abstract
Background, aim, and scope Lindane is an organochlorine chemical that has been used both as an agricultural insecticide and as a treatment for head lice and scabies. It is a neurotoxin that interferes with GABA neurotransmitter function. In humans, lindane primarily affects the nervous system, liver, and kidneys and may be a carcinogen and/or endocrine disruptor. Currently, India is the largest consumer and producer of lindane in the world. Due to its continuous use and indiscriminate industrial production, lindane-contaminated soils are widespread in the country. Apart from India, historical lindane production sites were found in Austria, France, Spain, Bulgaria and in China, Turkey, and the former USSR. Before 1984, lindane was also manufactured in the German Democratic Republic, Poland, Yugoslavia, Romania, and Hungary; since then, all production has been stopped in Germany, Japan, The Netherlands, the UK, and the USA. Because of its worldwide use for more than 50 years, lindane-contaminated soils can be found in most countries of the world. Although many countries have restricted or eliminated its usage, obsolete stock piles continue to pose a threat to various ecosystems and human health. Physical, chemical, and biological methods can all be used for the remediation of contaminated sites, but phytoremediation is now recognized as a cost-effective method for the decontamination of soil sites. The present study examines the potential of *Withania somnifera* Dunal (previously shown to accumulate lindane from contaminated industrial area; Abhilash et al., Chemosphere 72:79–86, 2008) to take up lindane (γ-HCH) and the subsequent plant-mediated dissipation of lindane from an artificially contaminated soil.
Materials and methods The study species was grown in four simulated concentrations (5, 10, 15, and 20 μg g^{-1}) of lindane. Each treatment was prepared in triplicate. In addition, two control treatments were established: vegetated control (non-contaminated soil planted with *W. somnifera*) and non-vegetated control [contaminated soil (prepared in above said concentrations) without plants]. Pots were harvested after 21, 50, and 145 days. Plant growth, biomass, chlorophyll, protein, carotenoids content, microbial biomass carbon, lindane concentrations in plant parts, residual lindane concentrations in soil, and percentage lindane dissipation from soil were determined after every harvest. Lindane accumulation potential of *W. somnifera* per acre was calculated based on the mean dry matter production of the plant multiplied by mean lindane accumulation potential and the number of plants that can be planted per unit area to optimum planting density.
Results Plant growth (root length, shoot length, and dry matter production) decreased with increasing lindane concentration. At 145 days, the dry matter production in 5, 10, 15, and 20 μg g^{-1} of lindane was reduced to 7%, 9%, 11%, and 20% of control plants, respectively. Similarly, there was a significant reduction in chlorophyll contents and soluble proteins in various treatments at each harvest. In contrast, carotenoids content increased with exposure time and lindane treatments. After 145 days, the accumulation of lindane in four spiked concentrations reached up to 8.4, 14.2, 26.8 and 45.0 μg g^{-1} dry matter, respectively.

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Regardless of the lindane treatment, maximum accumulation occurred in roots followed by stems and leaves ($p<$ 0.01). In contrast, lindane was not detected in the roots of control plants. However, low levels of lindane were detected in shoot and leaf (0.98 and 1.35 μg g^{-1} dry matter) matrices of control plants.

Discussion Although the growth of the plants was affected by lindane, *W. somnifera* survived in all spiked soils without any visible toxic symptoms. After final harvest, lindane concentrations in the 5-, 10-, 15-, and 20-μg g^{-1} treatments were reduced to 0.83, 2.0, 3.53, and 5.38 μg g^{-1}, respectively. This corresponds to a dissipation of 83%, 80%, 78%, and 73% in the four different lindane treatments. In contrast, a significantly ($p<0.001$) lower dissipation was observed in non-vegetated controls: 40%, 35%, 32%, and 30%, respectively. These differences in lindane dissipation between vegetated and non-vegetated soils were correlated with their respective microbial biomass carbon, suggesting that *W. somnifera* assisted in the enhanced dissipation of lindane due to an enhanced rhizospheric microbial activity.

Conclusions Based on the present study, it was estimated that *W. somnifera* can accumulate 764–944 mg of lindane per acre after 145-day cultivation. However, the plant-mediated dissipation of lindane (phytostimulation) is the major contribution of this species, leading to the enhanced remediation (rhizoremediation) of contaminated soil (>73%). However, other processes such as volatilization or adsorption cannot be discarded (Kidd et al., Plant Soil 302:233–247, 2008).

Recommendations and perspectives W. somnifera can be used for the remediation of lindane contaminated soils. However, suitable agronomic practices are essential for the successful implementation of this venture. Density of planting is a key factor determining the successful growth of plants. It is obvious that plants cannot grow well in contaminated area. Therefore, overcrowding will cause a negative effect on plants growth which will ultimately reduce their remediation potential. A spacing pattern of 1 × 1 m is suggested so that a maximum of 4,000 plants can be planted per acre (however, more agronomic trials are required to get an optimum planting density). Further, the accumulation and dissipation potential of plants can be enhanced by suitable soil amendments (e.g., addition of organic acids; White et al., Environ Pollut 124:71–80). However, field trials are needed to establish the on-site remediation potential of *W. somnifera*. Furthermore, additional investigations are needed to understand the catabolic degradation of lindane in *W. somnifera.*

Keywords Contaminated soil · Dissipation · Microbial biomass carbon · Phytoremediation · Phytostimulation · Rhizoremediation · *Withania somnifera* Dunal · Lindane

1 Background, aim, and scope

Lindane, the 'γ' isomer of hexachlorocyclohexane (γ-HCH), is an organochlorine compound primarily used as an insecticide and fumigant against a wide range of soil-dwelling and phytophagous insects. Other major uses are for personal hygiene as scabicide and pediculicide in the form of lotions, creams, or shampoos. However, agricultural uses are mainly responsible for the persistence of lindane residue in soil. Due to its worldwide use for more than 50 years, lindane-contaminated soil can be found in many parts of the world. Although many countries have restricted or eliminated its usage, obsolete stock piles continue to pose a threat to various ecosystems (WHO 1991; WWF 1999). Once lindane enters the environment, it can distribute globally (Simonich and Hitéis 1995; Willet et al. 1998; Li 1999; Walker et al 1999) and can persist in various environmental compartments (WWF 1999; Abhilash et al. 2008; Abhilash and Singh 2008a, 2009a; Abhilash 2009).

When compared with expensive chemical and physical treatments options, the in situ phytoremediation alternative is relatively inexpensive, does not require disturbing the contaminated soil, and the hazardous compounds tend to remain immobilized during the remediation process. Vegetation growing on contaminated soil aids the dissipation of pollutants in several ways (Bacci et al. 1990; Schroll et al. 1994; Schwitzguebel et al. 2002; Shimp et al 1993; Cunningham et al 1996; Schwitzguébel et al. 2006; Abhilash 2007). With respect to their direct roles in remediation processes, plants use several different strategies for dealing with environmental chemicals: phytoextraction, phytodegradation, phytovolatilization, and rhizodegradation (Schnoor 1997). Phytoextraction involves the removal and subsequent storage of contaminants by the plant and is often applied to the exclusion and storage of metals that may undergo speciation in plants, but cannot be metabolized. However, certain organic chemicals may also be treated in this manner due to inherent resistance to degradation. Conversely, phytodegradation describes processes in which plants metabolize the contaminants they take up. A further attenuation mechanism, referred to as phytovolatilization, involves the release of contaminants to the atmosphere following their uptake from the soil or water. An indirect mechanism, rhizodegradation refers to the transformation of contaminants by resident microbes in the plant rhizosphere (Cunningham et al. 1996; Abhilash 2007). Previous studies have proven that rhizodegradation is the dominant mechanism in the removal of organic contaminants from soil by suitable plant species (Schwab and Banks 1994; Kidd et al. 2008). In addition, contaminated soil sites are more acceptable to the public when vegetation is used in the cleanup operation. For these reasons, increasing attention has been given to the application of phytoremediation for soils contaminated by organic pollutants.

The objective of the present study was to investigate the phytostimulation potential of *Withania somnifera* Dunal in lindane-contaminated soil and the subsequent plant-mediated dissipation of lindane in soil. For this, *W. somnifera*-assisted dissipation of lindane in soil was compared with the dissipation of lindane in non-vegetated control. *W. somnifera* was chosen as a study species since earlier studies showed that this species can accumulate considerable amounts of lindane compared to other native species growing in a contaminated area (Abhilash et al. 2008; Abhilash and Singh 2009b). Furthermore, its rapid growth, secondary branching, large number of leaves, and rapid regeneration from pruned stems makes it a suitable candidate for phytoremediation (Fig. 1).

2 Materials and methods

2.1 Experimental design

W. somnifera-mediated lindane remediation study was conducted in four different experiments: (1) vegetated control (0 μg g^{-1} lindane), (2) non-vegetated control (0 μg g^{-1} lindane), (3) non-vegetated treatments (5, 10, 15, and 20 μg g^{-1} lindane), and (4) vegetated treatments (5, 10, 15, and 20 μg g^{-1} lindane). The top layer garden soil (collected from the Garden block of NBRI) was air-dried and sieved through a 2-mm mesh. The larger particles were removed to attain soil homogeneity. The pH of the soil samples was slightly alkaline (7.8±0.3); electrical conductivity was (EC) 198.56±6.5 (mS cm^{-1}) and total organic carbon was 0.985±0.15 (mg kg^{-1}). Twenty percent commercial grade lindane EC solution (98% purity; Kanoria Chemicals, Sonabhadra, UP) was dissolved in 1,000 ml cyclohexane to attain a treatment solution of 5, 10, 15, and 20 μg g^{-1} soil of lindane, respectively. These treatment solutions were mixed with 2.5-kg garden soil taken in a plastic tray, mixed well, and allowed to dry for 2 days. During this period, the soil samples were shaken several times a day in order to mix the lindane with the soil thoroughly and to increase the cyclohexane evaporation rate. Finally, the spiked soils were filled in earthen pots. All the experiments were conducted in triplicates so that a total of 90 pots were prepared for three different harvest periods (each experiment, pots were harvested in triplicate after 21, 50, and 145 days). High-quality seeds of *W. somnifera* were collected from Vatika Nursery, Lucknow. Seeds were soaked in deionized water for 3 h and soaked seeds were directly sown into treated pots (three seeds per pot). All pots were watered to maintain soil close to filed capacity, with the aim of minimizing leaching from the base of the pot. Leachate exiting the pots was trapped in individual trays kept under each pot and collected leachate was reapplied to the soil surface.

2.2 Measurements of plant growth and determination of photosynthetic pigments and protein content

Growth parameters were recorded at each harvest: fresh weight, root length, and shoot length. The fresh parts of the plants were immediately used for the estimation of chlorophyll and protein contents. Chlorophyll content in leaves was extracted in 80% chilled acetone and estimated by the method of Arnon (1949). Similarly, carotenoid concentration in the same extract was calculated by the formula given by Duxbury and Yentsch (1956). Protein content in the leaves was estimated by the method of Lowry et al. (1951) using bovine serum albumin as a standard protein.

2.3 Analysis of lindane

Plant and soil samples were collected at each harvest. Plant samples were washed several times in tap water in order to remove any adhered lindane particles, separated to different

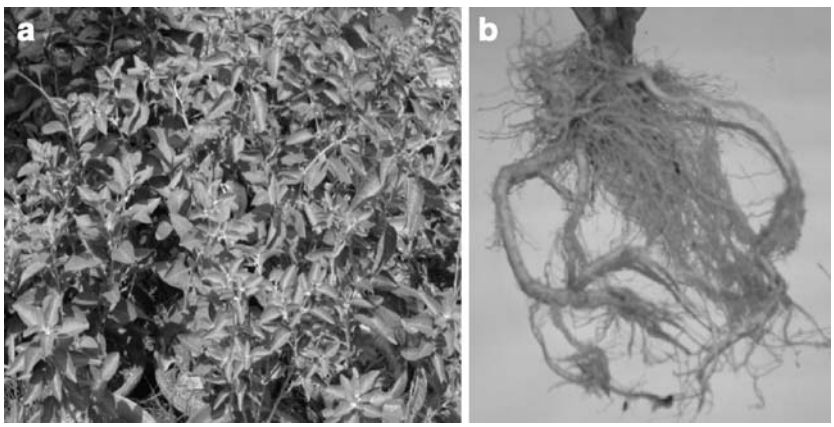

Fig. 1 *W. somnifera* Dunal. **a** Plant foliage, **b** Root system

parts: stem, leaf, and root. Leaf, stem, and root samples were dried at 35°C for 24 h, powdered, sieved (1–2 mm), and stored at 4°C for posterior analysis. Plant matrices (5 g) were gently ground with 0.5 g Florisil (deactivated with 3% acetone) in a pestle and mortar for 5 min, and 1 g $MgSO_4$ and 0.5 g NaCl were added to this mixture and ground firmly for five more minutes. This mixture was transferred into a glass column filled with neutral alumina (Al_2O_3) deactivated with 3% acetone (2 g) and anhydrous Na_2SO_4 (0.5 g). A mixture of *n*-hexane/ethyl acetate solvent 70:30 (*v*/*v*, 10 ml) was utilized for elution in the column and repeated with another 10 ml of the same solvent mixture. The detailed extraction and cleanup procedures for plant and soil samples have been published earlier (Abhilash et al. 2007, 2009); Abhilash and Singh 2008a, b.

Soil samples were air-dried, sieved through a 2-mm mesh, and ground before analysis. After the pretreatment, soil samples were frozen at −1°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). Quantification of lindane in all samples was done using external standard calibration on a Perkin-Elmer Clarus 500 gas chromatograph equipped with a capillary column and ^{63}Ni electron capture detector. Gas chromatography–electron capture detection conditions, column conditions, calibrations, quality assurances protocols, and data interpretation were described in previous publications (Abhilash et al. 2007, 2008, 2009).

Soil microbial biomass carbon (MBC) was determined by the method of Vances et al (1987).

3 Results and discussion

3.1 Effect of lindane on the growth of *W. somnifera*

The effect of different lindane concentrations on the growth of *W. somnifera* and the relative changes in growth compared to the respective control plant are summarized in Tables 1 and 2, respectively. There was no significant difference in growth of plants (at 95% confidence level) with low-level lindane exposure (5 μg g^{-1}); however, increasing lindane concentrations significantly reduced ($p<0.05$) the dry matter production, and this was most pronounced in plants grown in 20 μg g^{-1} of lindane. After 145-day exposure, biomass production in the four different treatments reduced 7% to 20% the control plants.

3.2 Effect of lindane on photosynthetic pigments and protein content

The changes in chlorophyll a, chlorophyll b, and total chlorophyll, carotenoids, and soluble proteins are shown in Table 3. Regardless of the exposure time and exposure concentrations, the experimental results indicated that there was a significant reduction ($p<0.05$) in chlorophyll a, b, and total chlorophyll content. Similarly, there was a

Table 1 Growth response of *W. somnifera* grown in lindane spiked soils (mean±SE)

Treatments	Harvesting period (days)	Root length (cm)	Shoot length (cm)	Biomass (g dry weight)
Control	21	6.8±0.02^{a}	11.0±0.22^{b}	0.30±0.01^{c}
	50	17.5±0.12^{b}	44.5±0.45^{c}	0.97±0.02^{c}
	145	28.4±0.33^{bc}	89.5±0.50^{c}	5.25±0.14^{c}
5 μg g^{-1}	21	7.3±0.02^{ab}	12.5±0.16^{b}	0.30±0.01^{c}
	50	18.5±0.11^{b}	42.0±0.28^{b}	0.91±0.09^{b}
	145	29.0±0.09^{bc}	91.2±0.33^{d}	4.90±0.08^{bc}
10 μg g^{-1}	21	7.0±0.13^{ab}	12.6±0.01^{b}	0.29±0.01^{b}
	50	17.0±0.22^{b}	42.0±0.58^{b}	0.93±0.02^{b}
	145	27.5±0.10^{b}	85.6±0.10^{b}	4.80±0.11^{b}
15 μg g^{-1}	21	7.6±0.04^{b}	12.3±0.24^{b}	0.28±0.04^{a}
	50	18.0±0.08^{b}	43.7±0.18^{b}	0.89±0.05^{a}
	145	25.7±0.18^{a}	80.5±0.15^{a}	4.68±0.29^{b}
20 μg g^{-1}	21	6.8±0.01^{a}	10.5±0.08^{a}	0.25±0.01^{a}
	50	16.5±0.28^{a}	40.2±0.70^{a}	0.85±0.02^{a}
	145	25.0±0.30^{a}	79.5±0.11^{a}	4.25±0.06^{a}

Means with different letters in a particular exposure day are significantly different at $p<0.05$ (ANOVA-DMRT)

Table 2 Relative changes in dry matter production of *W. somnifera**

Exposure days	Lindane treatments (μg g^{-1})			
	5	10	15	20
21	1.00 (=)	0.96 (−4)	0.93 (−7)	0.83 (−17)
50	0.94 (−6)	0.96 (−4)	0.92 (−8)	0.88 (−12)
145	0.93 (−7)	0.91(−9)	0.89 (−11)	0.80 (−20)

(+) increases in growth, (−) decreases in growth, (=) equal growth
*compared to the control plant dry matter as 1

significant reduction in the protein content of *W. somnifera* grown in the four different lindane treatments. The reduction was more important at final harvesting. On the contrary to chlorophyll contents, an increase in carotenoid content was observed in plants grown in simulated soil throughout the sampling periods.

Lindane might damage photosynthetic pigments and may also catalyze degradation of proteins through oxidative modification and increased proteolytic activity. Reduction in total chlorophyll content of *W. somnifera* during the entire exposure periods could be caused by the interaction of lindane to –SH group of enzymes of chlorophyll biosynthesis. The increased carotenoid level in *W. somnifera* could be part of the strategy adopted by the plant to counteract the toxic effect of lindane. Further, carotenoids are supposed to act as free radical scavengers by electron transfer to their double-bond structure and play a significant role in the protection of chlorophyll pigment under stress conditions by quenching the photodynamic reactions, replacing peroxidation and collapsing of membrane in chloroplasts. Reduction in the protein content of test plants might be due to the breakdown of soluble proteins or to the increased activity of catabolic enzymes which were activated and destroyed the protein. However, more studies are needed to establish the antioxidant defense system in *W. somnifera* to cope with the lindane toxicity, especially the strategy involving the activation of various enzymatic and non-enzymatic antioxidants as important components of antioxidant defense mechanism as well as catabolic pathway for the mineralization of lindane.

3.3 Uptake and accumulation of lindane by *W. somnifera*

It has been suggested that lipophilic organic pollutants including pesticides are strongly associated with the soil organic fraction and are not expected to be susceptible to plant uptake and subsequent translocation (Simonich and Hitéis 1995; Wild et al. 2005). The main accumulation pathway for such compounds is from air to leaf surface (Kipopoulou et al. 1999). However, there are few exceptions. Hulster et al. (1994) have observed that zucchini (*Cucurbita pepo* L. convar. *giromontiina* cv. Diamant F1) and pumpkins (*Cucurbita pepo* L. cv Gelber Zentner) can accumulate higher concentrations of polychlorinated dibenzo-*p*-dioxins and dibenzofurans from contaminated soil, and this was the main contamination pathway for this species.

Lindane concentrations in various plant matrices (root, stem, and leaf) of *W. somnifera* are shown in Fig. 2. The accumulation of lindane in plant parts is expressed as micrograms of lindane per gram of dry matter. The level of

Table 3 Changes in chlorophyll, carotenoids, and protein content (mean±SE)

Treatments	Harvesting period (days)	Chl a (mg g^{1})	Chl b (mg g^{1})	Total (mg g^{-1})	Caro (mg g^{1})	Pro (mg g^{-1})
Control	21	0.58 ± 0.01^{c}	0.35 ± 0.01^{bc}	0.93 ± 0.02^{c}	0.45 ± 0.03^{a}	13.45 ± 0.12^{c}
	50	0.67 ± 0.07^{c}	0.39 ± 0.02^{b}	1.06 ± 0.08^{c}	0.52 ± 0.01^{a}	18.54 ± 0.30^{b}
	145	1.21 ± 0.09^{cd}	0.47 ± 0.05^{c}	1.68 ± 0.32^{d}	0.61 ± 0.03^{a}	38.65 ± 0.18^{c}
5 μg g^{-1}	21	0.59 ± 0.03^{c}	0.35 ± 0.02^{bc}	0.94 ± 0.18^{c}	0.44 ± 0.04^{a}	13.25 ± 0.22^{b}
	50	0.65 ± 0.06^{c}	0.34 ± 0.03^{a}	0.99 ± 0.09^{bc}	0.55 ± 0.06^{b}	18.03 ± 0.16^{b}
	145	1.18 ± 0.86^{c}	0.44 ± 0.15^{b}	1.62 ± 0.22^{c}	0.64 ± 0.01^{b}	37.05 ± 0.13^{b}
10 μg g^{-1}	21	0.55 ± 0.04^{b}	0.33 ± 0.06^{b}	0.88 ± 0.16^{b}	0.45 ± 0.02^{a}	12.88 ± 0.05^{ab}
	50	0.63 ± 0.05^{bc}	0.33 ± 0.07^{a}	0.96 ± 0.10^{b}	0.55 ± 0.03^{b}	17.65 ± 0.18^{ab}
	145	1.15 ± 0.10^{b}	0.43 ± 0.01^{b}	1.58 ± 0.05^{b}	0.63 ± 0.01^{b}	36.90 ± 0.03^{ab}
15 μg g^{-1}	21	0.52 ± 0.02^{a}	0.34 ± 0.12^{b}	0.86 ± 0.09^{b}	0.47 ± 0.04^{a}	12.30 ± 0.04^{a}
	50	0.60 ± 0.01^{a}	0.32 ± 0.03^{a}	0.92 ± 0.11^{a}	0.58 ± 0.05^{bc}	16.79 ± 0.11^{a}
	145	1.12 ± 0.30^{b}	0.44 ± 0.04^{b}	1.56 ± 0.13^{b}	0.66 ± 0.01^{c}	35.78 ± 0.21^{a}
20 μg g^{-1}	21	0.50 ± 0.02^{a}	0.31 ± 0.02^{a}	0.81 ± 0.03^{a}	0.49 ± 0.01^{b}	11.98 ± 0.07^{a}
	50	0.58 ± 0.01^{a}	0.33 ± 0.01^{a}	0.91 ± 0.10^{a}	0.60 ± 0.03^{c}	16.33 ± 0.10^{a}
	145	1.05 ± 0.04^{a}	0.39±0.04a	1.44 ± 0.11^{a}	0.69 ± 0.02^{cd}	35.01 ± 0.11^{a}

Means with different letters in a particular exposure day are significantly different at $p<0.05$ (ANOVA-DMRT). Chl a=chlorophyll a; Chl b=chlorophyll b; Total=total chlorophyll; Caro=carottenoids; Pro=protein content

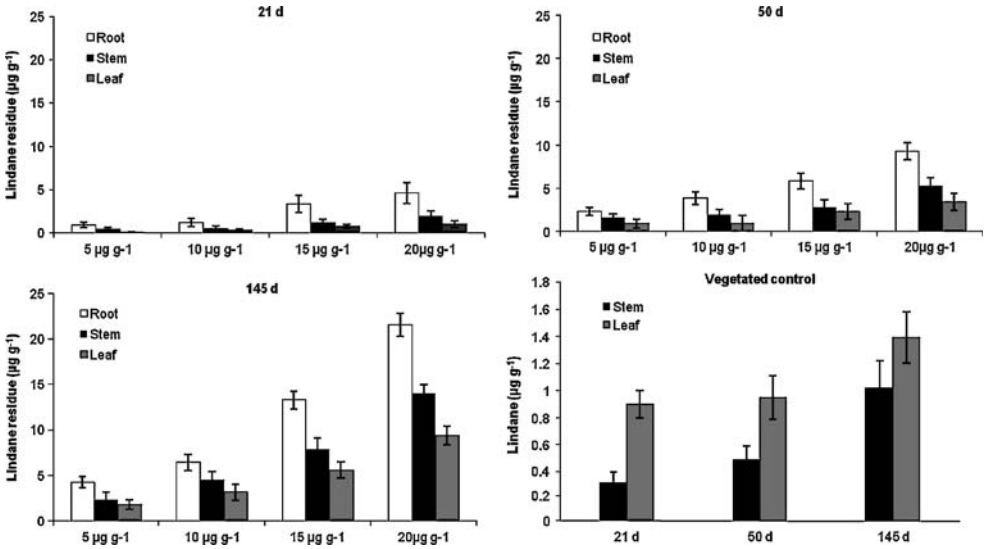

Fig. 2 Accumulation of lindane in different plant parts of *W. somnifera* Dunal during various harvesting periods (21, 50, and 145 days; results expressed as the micrograms of lindane per gram dry matter; ±SD, $n=3$)

pollutants in plant species depends upon their exposure and accumulation period (Moser et al. 1992; Krauthacker et al. 2001). Regardless of the spiked level, lindane concentrations significantly increased with the exposure time and lindane spiked concentration ($p \leq 0.05$). A linear relationship was exhibited between the uptake of lindane by *W. somnifera* grown in four different spiked levels of lindane. Regardless of the exposure time and lindane concentrations, maximum accumulation occurred in root matrix. Regression analysis reveals that root accumulation of lindane increased with the increment of their soil concentrations ($R^2=0.936$ to 0.993) and exposure periods ($R^2=0.955$ to 0.981).

Many previous studies have shown that most lipophilic organic compounds ($K_{ow} > 3.5$) partition to the epidermis of the roots (Paterson and Mackay 1994; Wang and Jones 1994), and the extent to which a lipophilic organic compound enters the plant roots from contaminated soil depends on the K_{ow}. Generally, the more lipophilicity results in the larger root concentrations (Trapp et al. 1990). Although a lack of information on the root uptake of lindane hitherto still exists, results of this work consistently showed that root accumulation of lindane in *W. somnifera* was increased with increasing lindane concentration. In our study, the effect of root lipid concentration on lindane adsorption and uptakes has not been elucidated and information was scant on the correlation between the root lipid content of various species and their specific lindane accumulation. However, results of our study proved that although lindane is hydrophobic, *W. somnifera* can take up considerable levels of lindane in its roots. However, additional studies are required to validate this. Lower concentration of lindane was detected in shoot and leaf samples of control plants. However, no residual concentration of lindane was detected in root samples of control plants (plants grown in non-spiked soils). Although air concentration of lindane had not been measured through the entire exposure period, it is clear that concentrations of lindane detected in control plants obviously entered from air. However, when compared to the treated plants, the accumulation of lindane in control plants was very low ($p < 0.001$). This difference in accumulation clearly suggests that soil–plant pathway was the major route of lindane accumulation in this experiment.

The phytoaccumulation potential of *W. somnifera* grown in four lindane treatments is presented in Table 4. Although the phytoextractability of lindane using *W. somnifera* is low, it can be enhanced by suitable substrate amendments

Table 4 Lindane accumulation potential of *W. somnifera*

Treatments	Accumulation/plant (µg g^{-1})	Percentage phytoextraction (whole plant)	Concentration removed from soil due to plant extraction (ng g^{-1})
5	41	0.33	17.0
10	68	0.27	27.0
15	125	0.33	500.0
20	191	0.38	760.0

Table 5 Field utilization potential of *W. somnifera*

Maximum dry matter production/plant at 145 days	5.25 g
No. of plants can be cultivated/acre	4,000 (1×1 m spacing)
Estimated dry matter/acre (g)	21,000 g
Maximum accumulation of lindane/dry matter	45 μg
Accumulation of lindane/acre	764–944 mg

like application of low-molecular-mass organic acids (White et al. 2003) organic amendments, fertilizer application, and inoculation of suitable plant-growth-promoting bacteria, etc. (Abhilash 2009).

Lindane accumulation potential of W. *somnifera* per unit area was calculated (Table 5). The total dry matter production per plant was used to project their dry matter production per unit area (acre). In order to evaluate the accumulation potential, the accumulation of lindane per gram dry weight of plants was multiplied by the total dry matter per area. Although the laboratory investigation differs from an on-site investigation in many ways (because of impact of various factors such as microclimate, pedobiology, etc.), our study provides some quantitative information using *W. somnifera* to remediate lindane from contaminated soil and lays the foundation for more detailed field trialing.

3.4 Bioconcentration of lindane in *W. somnifera*

Bioaccumulation is the ratio of contaminant concentration in plant species to their respective environmental media and is generally expressed using bioconcentration factor (BCF). However, it is very difficult to express the bioaccumulation of volatile organic pollutants, especially when the accumulation route is not clear. In order to overcome this issue, regression models are suggested for determining the bioconcentration of organic pollutants in plant parts (Mikes et al. 2009; Abhilash and Singh 2009b). Regression polynomials were used to depict the relations between pollutant concentrations in soil and plants. Here, the soil concentration of lindane was considered as a predictable variable, whereas the lindane concentrations in test plants were used 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 BAF and the '*y*'-axis intercept as the background tissue concentration. According to Mikes et al. (2009), the advantages of this interpretation are that all measured values contribute to calculated BCF and background concentration and all fluctuations can be adequately considered. Furthermore, the 'R^2' values describe how much of the concentration variance in plants is explained by the concentration variability in soil.

Form the BCF values presented in Table 6, it is very clear that there was a strong significant correlation exhibited between lindane concentrations in *W. somnifera* with their respective soil concentrations. The trend in BCF was found in the following manner: root > shoot > leaf. The decreasing order of BCF values in shoot and leaf samples of *W. somnifera* grown in spiked concentrations clearly indicate that major translocation of lindane had happened through the root. Further, increased BCF values in plant parts with high lindane treatments in soil favor this argument. In all the lindane treatments, the *y*-axis intercept of the regression model was negative or near to zero, indicating that the background concentration of lindane in plant samples irrespective of the spiked soil concentrations was very low or negligible.

Plant accumulation of persistent organic pollutants can involve several different mechanisms: (1) root adsorption,

Table 6 Bioconcentration of lindane in *W. somnifera*

Harvesting period (days)	BCF (slope of the curve)	Background concentration (intercept)	R^2
Root bioconcentration factor			
21	1.321	−0.75	0.936
50	2.283	−0.355	0.966
145	5.882	−3.315	0.946
Shoot bioconcentration factor			
21	0.513	−0.25	0.932
50	1.211	−0.1	0.864
145	3.823	−2.36	0.949
Leaf bioconcentration factor			
21	0.311	−0.185	0.988
50	0.884	−0.275	0.907
145	2.517	−1.305	0.953

(2) root uptake followed by transpirational translocation of pollutants from roots to shoots, (3) volatility of contaminants from soils followed by foliar adsorption, (4) contamination of plant foliage by pollutants laden soil, and (5) atmospheric deposition of airborne pollutants. It has been suggested that lipophilic organic pollutants partition to the epidermis of the root are not drawn into the inner root or xylem (Kipopoulou et al. 1999; Gao and Zhu 2004). Based on this assumption, many studies have focused on the foliar uptake and accumulation of pollutants, and information is scarce on the root uptake and subsequent translocation of persistent organic pollutants (Trapp et al., 1990; Simonich and Hitéis 1995; Kipopoulou et al. 1999; Mattina et al. 2003). As a consequence, there are difficulties to evaluate the translocation of pesticides from roots. However, few studies have reported that translocation is possible for many persistent organic pollutants. For example, Gao and Zhu (2004) have reported that the translocation of phenanthrene (K_{ow}, 4.57) and pyrene (5.18) from root to shoot was positive and this was the major pathway for the shoot accumulation of these compounds. Similarly, Mattina et al. (2002) have proven that *Cucurbita peppo* and *Spinacia oleracia* accumulate soil-bound chlordane and this is the major bioaccumulation pathway for these plants. Results of this work showed that although K_{ow} of lindane is 3.66, the translocation of this compound from root to aerial part was positive and was usually the major pathway of accumulation in aerial parts. Although the translocation of lindane from root to aerial part was suggested in this research, no attempts were made to evaluate the xylem or phloem flow of lindane necessary to transport the observed amounts in aerial parts of test plants.

3.5 *W. somnifera*-mediated dissipation of lindane from simulated soils

The plant-mediated dissipation of lindane from various spiked soils and from non-vegetated control plants are presented in Fig. 3. The presence of test plants remarkably accelerated the dissipation of lindane from soil. As seen from Fig. 2, the remaining concentrations of lindane in soil with plants were generally significantly lower than those of controls (non-vegetated spiked pots). Further, the graph shows the dissipation of lindane from soil irrespective of the presence of test plants as a function of time. Also, the graph clearly shows that the dissipation rate slightly decreased with increasing lindane treatments. This may be attributed to the fact that increasing lindane concentration reduces the plant growth which in turn resulted in the reduced root secretion of the plant. However, in the present study, no attempts were made to determine the plant rhizospheric secretion or their concentration with increasing lindane treatments in soil. The variation in the dissipation of lindane between the non-vegetated treatments and vegetated treatments was principally due to the plant effect.

W. somnifera is a medicinal plant (Solanaceae) known for several biochemical properties attributed to its characteristic steroidal compounds in roots, stems, and leaves, called withanolides and glycowithanaloides (Mishra et al. 2005; Lal et al. 2006; Sharma et al. 2007). Recent studies reveal that 3β-hydroxysterol glycosyltransferase in *W. somnifera* provide a wide range of biochemical properties. It is proven that UDP-glycosyltransterases (UGTs) has a significant role in secondary metabolism (Sharma et al. 2007). In higher plants, UGT-catalyzed glycosylation constitutes a prominent terminal modification in the biosynthesis of secondary metabolites and generates diverse natural glycosides (Bowles et al. 2006). They also produce glycosylate xenobiotics to cope up with environmental stresses through detoxification process. Biological functions of glycosylations in plants include storage, inter- and intracellular transport of metabolites, regulation of homeostasis, etc. (Sharma et al. 2007). Although the present study was not focused on enzymatic and metabolic adaptations of test plants grown in lindane treatments, it is assumed that comparatively better dissipation of lindane in *W. somnifera* rhizosphere may be due to its inherent biochemical peculiarities. However, detailed biochemical studies are needed to validate this.

The literature provides ample evidence that plant-mediated dissipation of organic pollutants from contaminated soil is mainly due to the rhizospheric effect (Arthur

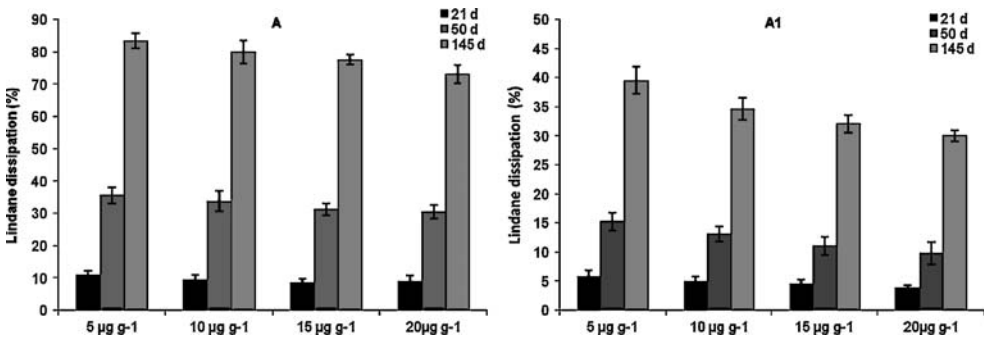

Fig. 3 Percentage dissipation of lindane in vegetated treatments (*A*) and non-vegetated treatments (*A1*) (±SD, $n=3$)

and Coats 1998; Miya and Friestone Mikes et al., 2009; Banks et al. 2003; Singh et al. 2004; 2006; Gao et al. 2006; Cofield et al. 2007; Henderson et al. 2007; Yi and Crowley 2007; Olson et al. 2007; Cofield et al. 2008; Kidd et al. 2008; Rezek et al. 2008). Liste and Alexander (2000) have reported an enhanced degradation of pyrene by nine plant species and noted that pyrene is reduced by 74% in planted soil compared to less than 40% in unplanted soil. Arthur and Coats (1998) have demonstrated that several plants (Kochia, musk thistle, catnip, foxtail barley, witchgrass, lambs quarter) rhizosphere exhibit enhanced ability to mineralize atrazine (Arthur and Coats 1998). In Kochia-rhizosphere soil, more than 60% of the applied atrazine is completely mineralized after 50 days. Rhizospheric soils from musk thistle and catnip mineralizes 33% and 24% of the applied atrazine. Miya and Firestone (2000) have reported enhanced phenanthrene degradation due to the addition of slender oat root exudates and root debris. Yoshitomi and Shann (2001) have found that the addition of root exudates stimulates the mineralization of ^{14}C-pyrene in an unplanted soil to the same degree as observed in actual rhizosphere. All this work indicates that plant root exudates have the potential to increase the dissipation of organic pollutants by promoting the growth of soil organisms.

The present study also showed the same trends. As expected, the dissipation of lindane from vegetated soil was higher than the dissipation of lindane in non-vegetated controls. The difference in dissipation of lindane in vegetated soil as well as their non-vegetated soil was also attributed by the difference in their MBC level. Soil microbial communities play a vital role in nutrient cycling, the decomposition of organic matter, carbon sequestration, and more general effects on xenobiotic degradation and, consequently, water retention of the soil (Singh et al. 2006). The evolution of microbial biomass carbon in vegetated controls (non-spiked soils with test plants), non-vegetated controls (non-spiked pots without test plants), and test plants grown in spiked soils are presented in Fig. 4. There was a marked difference in MBC content of all three experimental pots, and this variation could be attributed to plant effect due to their rhizospheric secretion. The plant rhizosphere is the soil close to the root system and under its direct influence (Fan et al. 2008). Therefore, it plays a very important role in phytoremediation. Compared to bulk soil, the rhizosphere may be modified due to the activity of root system (Darrah 1993; Kaye and Hart 1997; Adam and Duncan 2002). A plant may secrete 10–20% of its photosynthate in root exudates, which supports the growth and metabolic activities of diverse soil microbial communities in the rhizosphere (Donnelly et al. 1994; Cunningham and Ow 1996; Siciliano et al. 2003; Kaimi et al. 2006; Kidd et al. 2008). Some organic compounds in root exudates may

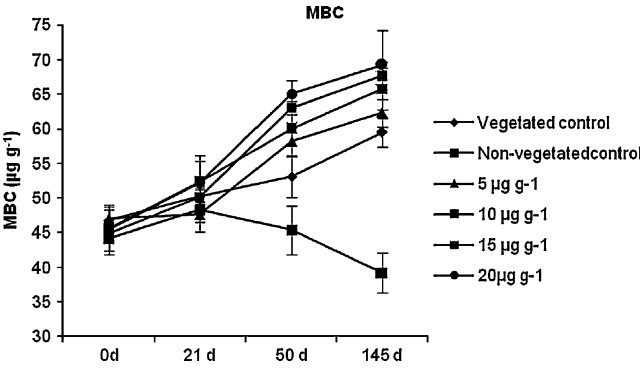

Fig. 4 Changes in microbial biomass carbon (*MBC*) in various experiments (vegetated control, non-vegetated control, and four different lindane treatments (results expressed as the micrograms MBC per gram soil; ±SD, n=3)

serve as carbon and nitrogen sources for the growth and long-term survival of microorganisms that are capable of degrading organic pollutants (Fan et al. 2008). Densities of rhizospheric bacteria can be as much as to four orders of magnitude greater than the microbial populations in the surrounding bulk soil.

Comparatively higher MBC load was detected in treated soil than for the respective control plants. Within the spiked concentrations, the MBC load was increasing with increasing lindane treatment. This may be due to the fact that increasing lindane concentration provides additional carbon source to microbes than the rhizospheric secretion and, therefore, amplifying the growth of bacteria. Hence, there was a significant difference in MBC load ($p<0.05$) between vegetated pots and vegetated spiked pots. Regarding the non-vegetated non-spiked pots, the MBC content was decreasing with increasing exposure periods. In non-vegetated non-spiked control pots, there were no sources for additional carbon, neither from the plant root exudates nor from organic contaminants. Therefore, the MBC content in non-vegetated non-spiked pots was decreasing rapidly with increasing exposure periods.

4 Conclusions

The present study supports the notion that phytostimulation (rhizoremediation) is a feasible technical approach for lindane-contaminated soil. *W. somnifera* was more or less tolerant to all concentrations of lindane in this experiment. The accumulation of lindane was linear with the exposure periods and exposure concentrations. Further, the presence of test plants considerably enhances the dissipation of lindane in soil, as compared to non-vegetated spiked soils. The microbial load was enhanced by test plants, which contributed to the enhanced dissipation of lindane. Hence, the lindane dissipation percentages in vegetated plants were

significantly higher than those in the non-vegetated spiked soils. However, other process such as volatilization or adsorption cannot be discarded (Kidd et al. 2008). Above all, it was estimated that *W. somnifera* can accumulate 764–944 mg of lindane per acre after 145-day cultivation. Therefore, the rhizoremediation potential of *W. somnifera* could be exploited for the on-site remediation of lindane-contaminated soils in India since *W. somnifera* is well adapted to the various agro-climatic regions of the country (Abhilash 2009).

5 Recommendations and perspectives

W. somnifera can be used for the remediation of lindane-contaminated soils. However, suitable agronomic practices are essential for the successful implementation of this venture. Density of planting is a key factor determining the successful growth of plants. It is obvious that plants cannot grow well in a contaminated area. Therefore, overcrowding will cause a negative effect on plant growth which will ultimately reduce the phytoextraction potential. A spacing pattern of 1 × 1 m is suggested so that a maximum of 4,000 plants can be planted per acre. Further, the accumulation and dissipation potential of plants can be enhanced by suitable soil amendments (e.g., addition of organic acids; White et al. 2003). However, field trials are needed to establish the on-site remediation potential of *W. somnifera*. Furthermore, additional investigations are needed to understand the catabolic degradation of lindane in *W. somnifera.* Recently, lindane and other HCH isomers (α- and β-HCH) have been nominated by the POPs Reviewing Committee for inclusion into the Stockholm Convention to address HCH contamination on a global level. Therefore, there is an urgent need to stop the production of lindane and remediate already contaminated soil sites.

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