Uptake of α-HCH by wheat from the gas phase and translocation to soil analyzed by a stable carbon isotope labeling experiment

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ABSTRACT

Hexachlorocyclohexane isomers (HCH) are persistent organic pollutants which cause serious environmental pollution. Phytoextraction is one of the strategies of phytoremediation, which was considered as a promising method for the clean-up of HCH contaminated field sites. To understand the uptake and translocation mechanisms of HCH in soil-plant system, the uptake of HCH from the gas phase was investigated in a tracer experiment with ^{13}C-labeled α-HCH. The results provide new insights on the uptake mechanism of HCH and allow the elucidation of transport pathways of POPs from the leaves to the rhizosphere. A higher dissipation of α-HCH in planted set-ups versus unplanted controls indicated next to intensive biodegradation in the rhizosphere the removal of HCH by root uptake, accumulation and possible transformation within plants. Analyzing the carbon isotopic composition (δ^{13}C) of α-HCH in the soil of unplanted controls revealed a change of 15.8 – 28.6 ‰ compared to the initial δ^{13}C value, indicating that a soil gas phase transportation of α-HCH occurred. Additionally, higher δ^{13}C values of α-HCH were observed in bulk and rhizosphere soil in non-labeled treatments compared to unplanted controls, revealing the uptake of α-HCH from the gas phase by the leaves and the further translocation to the roots and finally release to the rhizosphere. This uptake by the leaves and the subsequent translocation of α-HCH within the plant is further indicated by the observed variations of the δ^{13}C value of α-HCH in different plant tissues at different growth stages. The uptake and translocation pathways of α-HCH from the gas phase need to be considered in phytoremediation.

Key words: Hexachlorocyclohexane isomers, carbon isotope composition, uptake, translocation

1. Introduction

Hexachlorocyclohexane isomers (HCHs) are persistent organic pollutants (POPs) which are ubiquitous distributed in the environment. However, only γ-HCH (Lindane) possess insecticidal properties and was extensively used as pesticide [1,2]. The purification of Lindane out of the technical HCH resulted in a large amount of waste containing other isomers, especially α-HCH and β-HCH, and the global spread of these isomers has caused abundant contaminations in soil and groundwater [3,4]. Although it was banned in 2009 by the Stockholm Convention [5], the existing HCH contamination still causes serious environmental problems due to its bioaccumulation and spread along the food web [6]. Plant uptake of HCHs from soil and water is one of the first steps for HCHs to enter the food web. This effect attracts attention regarding to the important role of plants in terrestrial ecosystems and the risks to human health through food chain migration [6]. The accumulation of HCHs was already detected in the human milk [7], blood [8], and fatty tissues [9,10]. Therefore there is a special interest to understand the uptake mechanisms of HCH into plants.

Phytoremediation has been regarded as a promising option for the clean-up of contaminants in soil [11–13]. In that context, phytoextraction is one of the most important strategies in phytoremediation [14]. The uptake mechanism of HCHs by plants is essential for the phytoextraction and was already well studied before [15–17]. The uptake of semivolatile organic compounds (SOCs) into plants mainly occurs via two pathways: (i) passive or active uptake from the soil into the plant roots followed by the subsequent transport from the roots to the shoots; (ii) uptake from the gas phase or deposition of air-born particles on leaves and possible subsequent translocation into other plant parts [18]. Numerous studies were conducted to evaluate the pathway-specific uptake of SOCs by plants based on the specific physiochemical properties of the

respective organic compounds [18–20]. For the uptake via plant roots, the extent of uptake appears to be proportional to the octanol–water partition coefficient (K_{ow}) [21]. The hydrophobic α-HCH is a lipophilic compound with a log K_{ow} value of 3.9 ± 0.2 [22]. Therefore, HCH should be strongly adsorb on the root epidermis and hydrophobic domains and, additionally, exhibit only a relative low trend for translocation via the transpiration stream from the soil to the leaves. However, the uptake of α-HCH and other high hydrophobic POPs such as DDE ($logK_{ow}$ = 6.51) by plant roots was confirmed in recent studies [23] indicating that plants could have the ability to accumulate pollutants despite their high hydrophobicity. Referring to the uptake of α-HCH via the air (gas phase), the extent of uptake depends on the octanol–air partitioning coefficient (K_{OA}) [21]. Previous studies demonstrated that SOCs with a log K_{OA} smaller than 8.5 can be absorbed by leaves from the gas phase and an equilibrium distribution between leaf and air can be reached. However, SOCs with a log K_{OA} between 8.5 and 11 can still be absorbed by the leaves but the absorption is limited by the equilibrium distribution. For pollutants with a log K_{OA} higher than 11, only an absorption through deposition of soil particles on the leaf surfaces is possible [24]. Since the log K_{OA} of α-HCH is 7.26 [25], one main uptake pathway of HCH is the absorption by leaves from the gas phase [17].

Compound specific stable isotope analysis (CSIA) is a promising method to characterize the transformation of organic pollutants in complex environmental systems making use of isotope fractionation concepts. The fundamental principle of this method based on the fact that light isotopes (e.g. ^{12}C) require less energy for the bond cleavage compared to a bond substituted with the respective heavy isotope (e.g. ^{13}C). This process is known as kinetic isotope fractionation and results in the enrichment of the heavier isotopes in the residual (non-degraded) pollutant fraction. The extent of the carbon isotope enrichment can be used to qualitatively and quantitatively assess

pollutant transformation. The isotope fractionation of α-HCH transformation under aerobic conditions was recently studied and a moderate carbon isotope fractionation ($\varepsilon = -6.3 \pm 0.1‰$ to $-2.3 \pm 0.03‰$) was observed [26]. Physical processes such as evaporation, absorption and dilution are unlikely to influence the isotope fractionation to a major extent as no bond cleavage occurs during these processes. In this study, we apply for the first time isotope fractionation concepts to identify the uptake processes in soil-plant systems by conducting stable carbon isotope labeling experiments. The uptake of POPs form air was already demonstrated in non-contaminated controls of soil pot experiments /as well as in experiments using closed chamber systems to expose plants to air containing organic pollutants [27,28]. However, the uptake of organic pollutants by gas phases in most cases is too low for detection and the release of HCH from root to soil was not recognized. The analysis of the isotopic composition is a sensitive technique for the detection of substances with labeled atoms. Therefore, this technique provides the advantage that even a slight uptake of organic contaminants by plant tissues from the gas phase can be detected according to the changes of isotope composition in a tracer experiment, which indicated the uptake by plant tissues took place.

In this study we conducted experiments to evaluate the uptake of α-HCH by wheat via the gas phase and its subsequent translocation through the plant to the rhizosphere using ^{13}C-labeled α-HCH. To our best knowledge, this is the first report on the uptake and transport of POPs employing stable isotope tracer experiments. The combination of ^{13}C tracer experiments and the analysis of stable carbon isotope compositions provide a new perspective on the uptake mechanism of HCHs by plants and allow the elucidation of transport pathways of POPs from plant leaves down to the rhizosphere.

2. Materials and methods

2.1 Chemicals

The α-HCH (analytical purity, 99 %) was obtained from a chemical collection which has been described as number P25 in our previous study [29] and has a $\delta^{13}C$ value of -28.5 ‰. The fully ^{13}C-labeled α-HCH was synthesized and separated in our lab as described elsewhere (details are given in S2, SI). The chemical and isotopic purity of ^{13}C-labeled α-HCH was 97% as analyzed by GC-MS. The solvents n-hexane (≥99.9%, ROTISOLV HPLC, ROTH) and acetone (ROTISOLV® ≥99.9%, UV/IR-Grade) were used for HCH extraction. Dichloromethane (DCM, ≥99.9%, ROTISOLV HPLC, ROTH) was used for cleaning up the soil and plant extracts. Florisil (for chromatography, ROTH, 100-200 mesh) was used as stationary phase for liquid column chromatography. Glass wool (untreated, SUPELCO Analytical) and sea sand were used for packing the column [30]. To track the elution of HCHs from the Florisil column, 7,12-dimethyl-benz[α]anthracene (p. A, Reagent Grade) was applied as a fluorescence tracer [30].

2.2 Seeds and Plants Exposure to α-HCH in Soil.

Wheat (cultivar "Quintus" of *Triticum aestivum L.*) was used as the test plant. The seeds were obtained from the breeder Saaten-Union GmbH (Isernhagen, Germany). The soil was provided by LAV Technische Dienste GmbH (Markranstädt, Germany). The major physicochemical properties of the sandy soil are as follows: TOC = 2.2 % (w/w), pH = 7.9, soil texture = sandy silt soil with 11 % clay, 49 % silt, 40 % sand. The soil is classified as Luvisol (World Reference Base). The parent material is loess formed in a temperate zone located in the Saxony (Germany).

The experiment was conducted in the glass house of the research station Bad Lauchstädt of the UFZ. The sandy soil was air-dried and sieved through a 2 mm mesh sieve before usage. Then, 50

g soil of each pot was spiked with 210 mg α- HCH solution in acetone individually. After evaporation of the acetone, the spiked soil was mixed with 950 g non-spiked soil. Before filling of pots with soil (1 kg soil per pot), the soil was homogenized thoroughly and afterwards equilibrated at room temperature for several days at 60 % water holding capacity. No drainage of water was observed during the experiment. The final concentration of α-HCH was theoretically 30 mg kg^{-1}. In each pot, 5 seeds were grown uniformly and at last 3 seedlings were left after germination. During the whole period of wheat growth, the water content of the soil was maintained at 60 % of the maximum water content of soil. Hoagland nutrition was used to water the pots every day to maintain the 60% of the maximum water content. The treatments for the individual experiment were shown in Table 1:Soil amended with 30 mg kg^{-1} of non-labeled α-HCH planted with wheat (NT), soil amended 30 mg kg^{-1} of ^{13}C-labeled α-HCH planted with wheat (LT); soil not amended with α-HCH planted with wheat (PC); soil amended with 30 mg kg^{-1} of non-labeled α-HCH without wheat (NC); and soil amended with 30 mg kg^{-1} of ^{13}C-labeled α-HCH without wheat (LC). Each treatment was prepared in 12 replicates and LC and NC was prepared in 3 repeats. The scheme of experimental set up is shown in S1(SI). The PC pots without α-HCH amendment were used to detect the uptake of α-HCH by the leaves of the plants. The NC pots were used to detect the evaporation and a possibly occurring transformation of α-HCH. The NT pots were used to detect the changes of the isotopic composition of α-HCH in order to quantify the uptake and to demonstrate the translocation mechanisms. The experiments with ^{13}C-labeled α-HCH were used to provide labeled HCH in the gas phase by evaporation and phytovolatilization. The intention of this study was a semi quantitative analysis of gas phase transport using a new method based on the isotope composition.

2.3 Sampling of Soil and Plants.

The total growth time of the wheat was 102 days starting from 1th April to 5th July 2019. At tillering (20th May), jointing (4th June), heading (13th June) and harvest stage (5th July), soil and plant were sampled. The soil samples were separated into bulk soil and rhizosphere soil. Therefore, the whole soil and root system was gently crushed and loosely held soil was separated by shaking. This fraction is referred to as the bulk soil. The remaining tightly held soil particles were considered as the rhizosphere soil. This rhizosphere soil was removed by shaking in a plastic bag as described previously [31]. The soil samples were lyophilized for further treatment. Before planting of the soil pots, an aliquot of the initial soils was taken (named as original soil) and used to determine the initial α-HCH concentration and isotopic composition. After separation of rhizosphere soil from the root system, plant samples were washed thoroughly four times with sterilized water and then separated into root, stem, leaf, spike and grain. Afterwards, plant samples were lyophilized and ground for further treatment.

2.4 Extraction and Clean-up of α-HCH from Soil and Plant Samples.

Methods for extraction and clean-up of HCHs from soil and plants were recently published elsewhere [30]. Details of these methods are shown in S3 (SI).

2.5 Analytical Methods.

Concentration Analysis. An Agilent 6890 series GC (Agilent Technologies, USA) equipped with a flame ionization detector (FID) was used to determine the concentration of α-HCH throughout the study. Samples were separated with a HP-5 column (30 m × 320 µm × 0.25 µm, Agilent 19091J-413, USA) using helium as carrier gas with a flow rate of 2.2 mL min^{-1}. The oven temperature was held at 45 °C for 5 min, increased at 8 °C min^{-1} to 180 °C and then at 2 °C min^{-1}

to 195 °C, finally increased at 8 °C min^{-1} to 220 °C and hold for 2 min. Samples were measured using splitless mode with an injector temperature of 250 °C. The injection volume was 1 µL and each sample was measured in triplicates.

Isotope Analysis. The carbon isotopic composition was reported as δ notation in parts per thousand (‰) relative to the international standard scale (Vienna PDB, International Atomic Energy Agency (IAEA)) according to eq 1.

$$\delta^{13}C_{sample}\,(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \qquad (1)$$

R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and the standard, respectively. The $^{13}C/^{12}C$ isotope ratio of the VPDB defines as the $\delta^{13}C$ value of zero as 0.01123720.

The isotopic composition of carbon ($\delta^{13}C$) in α-HCH was analyzed using gas chromatography-combustion- isotope ratio mass spectrometry (GC-C-IRMS) employing a GC (7890A, Agilent Technologies, USA) connected through a GC-IsoLink and a ConFlo IV interface (Thermo Fisher Scientific, Germany) to a MAT 253 IRMS system (Thermo Fisher Scientific, Germany). Chromatographic separation of samples was done with a Zebron ZB1 column (60 m × 0.32 mm × 1 µm; Phenomenex, Germany) using helium as carrier gas with a flow rate of 2 mL min^{-1}. The oven temperature was initially held at 40 °C for 5 min, increased at 10 °C min^{-1} to 175 °C and then at 2 °C min^{-1} to 200 °C and hold for10 min, and finally increased at 15 °C min^{-1} to 300 °C and held for 2 min. All samples were injected in splitless mode with an injector temperature of 250 °C and were analyzed at least in three replicates. The quality of the isotopic data was regularly controlled by co-injecting α-HCH which was used for spiking of the soil.

Isotope Dilution Calculation. To quantify the amount of α-HCH which was incorporated into the plant by the uptake from the gas phase, a modified isotope dilution analysis using the atom percentage (AT %) of the sample and the labeled material was applied and was performed based on the following equation [32]:

$$\mathrm{Con.}\left(^{13}C\right) = \mathrm{Con.}\,(S) \times \frac{\mathrm{AT\%(Sx)} - \mathrm{AT\%(Si)}}{AT\%(^{13}C) - \mathrm{AT\%(Si)}} \qquad (2)$$

Con. (^{13}C) and Con. (S) are the concentrations of ^{13}C-labeled α-HCH and the total concentration of α-HCH in the plant sample, respectively. AT%(S_x) is the carbon atom percent of α-HCH in the soil and plant samples. AT%(S_i) is the carbon atom percent of the original non-labeled α-HCH (AT%(S_i) = 1.072948%). AT%(^{13}C) is the carbon atom percent of the fully ^{13}C-labeled α-HCH (AT%(^{13}C) = 99.9 %) in the plant sample. For the calculation the precise carbon atom percentage by CSIA was used (see above).

3. Results and Discussion

3.1 Experimental conditions in the greenhouse

The experimental setup is shown in S1 (SI). Non-labeled α-HCH and planted control pots were separated from ^{13}C-labeled α-HCH pots in two individual desks with a distance of 2.9 m. On each table three unplanted control pots were placed. α-HCH belongs to the semi-volatile organic compounds with vapor pressure 5.30×10^{-3} Pa. Consequently, α-HCH could evaporate into the air. The mean value of air velocity during the run time of the experiment was 1.8 ± 0.9 m/s and only on several days this value was higher than 5 m/s. The mean relative air humidity in the green house was 61.9 ± 18.9 % and the mean temperature was 16.1 ± 8.9 °C. Further details are shown in the SI. In the green house, α-HCH could evaporate from the soil and then transported by the

air flow and gas phase diffusion. The water content of the soil was set to 60% of the maximum water content and kept constant over the entire experiment. The low wind speed and the soil moisture make a soil particle transport unlikely and the uptake of α-HCH by the leaves of the plants could only take place by adsorption of α-HCH from the gas phase in the glass house.

3.2 Concentration of α-HCH in soil and wheat tissues at different sampling times in different treatments

The initial concentration of non-labeled α-HCH in soil in the NT set-up before planting was 25.50 ± 1.20 mg/kg. This concentration decreased in the bulk soil to 1.41 ± 0.08 mg/kg at the tillering stage followed by a constant decrease over the whole wheat growth period until it reached 0.54 ± 0.01 mg/kg at the harvest stage (Fig.S3a). A comparable reduction of α-HCH was observed for the non-labeled α-HCH in the soil of the NC set-up. Here, the concentration of α-HCH decreased in the bulk soil from the initial concentration of 25.50 ± 1.20 mg/kg to 3.12 ± 0.24 mg/kg during the tillering stage and reached finally a concentration of 1.38 ± 0.12 mg/kg at the harvest stage (Fig.S3a). Consequently, in comparison to the soil of the NT set-up a higher concentration of non-labeled α-HCH was observed in the soil of the NC treatment. This effect might be explained by a possibly occurring dissipation of α-HCH due to volatilization from or transformation in soil or due to the uptake, transformation and evapotranspiration of α-HCH by plants. Thus, the higher dissipation of α-HCH in the bulk soil of the NT set-up suggested higher losses of α-HCH due to the uptake, transformation and evapotranspiration by the plants.

An obvious lower concentration of α-HCH in the rhizosphere soil in comparison to the bulk soil of the NT set-up was observed for all stages of the wheat growth (Fig.S3a), suggesting a

higher removal of α-HCH in the rhizosphere which is possibly caused by a higher activity and diversity of microorganism in the rhizosphere. A similar observation was reported previously [23].

The concentration decrease of α-HCH in the soil samples was accompanied with a constant increase of the α-HCH concentration in different plant tissues over the whole period of wheat growth in the NT set-up (Fig. S3b). For example, the α-HCH concentration in the root increased from 1.29 ± 0.32 mg/kg at the tillering stage to 6.56 ± 0.24 mg/kg at the jointing stage followed by an increase to 7.49 ± 0.34 mg/kg at the flowering stage. Hence, the results indicated an accumulation of α-HCH in the different tissues during the plant growth. However, a decrease of the α-HCH concentration in all wheat tissues was found in the harvest stage. This may be related to a lower supply with the stream of water which decreased the delivery of HCH from soil in the harvest stage.

A similar trend of α-HCH concentration changes in soil and different plant tissues was observed in the ^{13}C-labeled set-up LT. Also here, the α-HCH concentration of the soil decreased from the tillering to the harvest stage (Fig. S3c-d). Differences in concentration between both experiments (NT and LT) showed the typical variability which can be expected for parallel soil experiments.

According to the mass balance, evaporation is the dominant process for the loss of α-HCH in all the treatments (Table. 2). The concentration of α-HCH in the plant represents at most 0.1 % of the total amount of α-HCH, and clearly demonstrates that plant accumulation of α- HCH alone does not represent an important route of HCH decontamination. The results were consistent with former reports [23], and suggest that the most viable approach for the remediation of HCHs is based on rhizoremediation or rhizodegradation techniques. However, recent research showed that

α-HCH can be transformed in plants [33] and although the uptake was rather low, phytotransformation needs to be considered in particular when contaminants become degraded in plants during uptake processes.

3.3 Mass spectrometry analysis of HCH in soil and plants

Besides analyzing the α-HCH concentration also the mass spectrum and the isotopic composition of α-HCH was investigated. In the non-labeled set-up NT, α-HCH showed a mass spectrum with the typical major mass peaks m/z 181 and m/z 219 (Fig. S4) indicating a natural abundance of the isotopic composition of α-HCH. In contrast, the analysis of plant (Fig. S5) and soil (Fig. S6) samples of the ^{13}C-labeled LT treatment revealed the presence of ^{13}C-labeled α-HCH by an overall shift of m/z 6 ($^{13}C_6$- α-HCH) compared to non-labeled α-HCH (major mass peaks: m/z 187 and m/z 225). In addition, the appearance of the metabolite pentachlorocyclohexene (PCCH) at a retention time of 1915 s indicates that α-HCH was degraded. However, no α- HCH was detected in soil and plant samples of the PC set-up (Fig. S7b and S8c), indicating that the uptake of HCH via the gas phase was below the GC-MS detection limit of 50 ppb. To further verify the obtained results the samples were additionally analyzed via GC-IRMS. Interestingly, the GC-IRMS analysis revealed the presence of a small amount of ^{13}C-labeled α-HCH in plant tissue samples of the PC treatment (Fig. S8a). However, this ^{13}C-labeled α-HCH was not detectable in the corresponding bulk soil sample (Fig. S7a) and thus indicates that an uptake of α-HCH by the plant must have occurred via the gas phase.

3.4 Carbon isotope ratio of soil at different sampling times

The carbon isotopic composition (δ^{13}C) of α-HCH in soil samples of the NC set-up ranged from -12.8 ± 0.2‰ to 0.1 ± 1.0‰ (Fig.2). Compared to the initial δ^{13}C value of α-HCH which

276 was used to spike the soil (-28.5 ± 0.3‰), the $\delta^{13}C$ value α-HCH in the soil samples increased to
277 15.8 ‰ at the tillering stage, to 28.6‰ at the jointing stage, to 22.2‰ at the flowering stage and
278 to 22.1‰ at the harvest stage (Fig.1).This increase of the $\delta^{13}C$ values cannot be explained by
279 isotope fractionation alone. A simulation of the extent caused by isotope fractionation which is
280 typically observed for aerobic degradation with a moderate fractionation factor (ε = -2.3 to -
281 6.1‰) causing an isotopic shift of approximately 3‰ would result in a degradation of more than
282 95% of the amended α-HCH [26]. The changes of isotope composition in this study was an order of
283 magnitude higher than that in biodegradation experiments showing that isotope fractionation
284 could not be the mechanism resulting in this isotope enrichment. Hence, a significant
285 contribution of isotope fractionation caused by biodegradation could be ruled out to explain the
286 observed enrichment. Based on a previous study, the increase of $\delta^{13}C$ value caused by the
287 transformation of α-HCH in soil is around 2-3 ‰ [33]. Compared to the increase of $\delta^{13}C$ in this
288 study, the increase of $\delta^{13}C$ caused by the transformation could be neglected. Thus the enrichment
289 can only be explained by the uptake of ^{13}C-labeled α-HCH from the ^{13}C-labeled treatments via
290 the gas phase and therefore the observed changes in the isotopic composition of α-HCH can only
291 be explained by a mixing of ^{13}C-labeled with non-labeled HCH. Physical processes such as
292 evaporation, sorption, absorption and advective gas phase transport could not change the isotope
293 composition of α-HCH to this extent [34] and diffusion would result in a discrimination of heavier
294 isotope values with increasing distance [35]. However gas phase diffusion over a longer distance is
295 unlikely in the greenhouse even with slow advective gas flow (see wind velocity, weather
296 conditions).

297 Higher enrichment of $\delta^{13}C$ values of α-HCH by up to 42.5 ± 2.6‰ and 87.1 ± 1.0‰ were
298 observed in the bulk and the rhizosphere soil in the NT set-up compared to that in the NC set-up

(Fig. 1).The $\delta^{13}C$ value of the bulk soil increased from -19.3 ± 0.7‰ to 42.5 ± 2.6‰ whereas the enrichment of $\delta^{13}C$ value in the rhizosphere soil was even larger ranging from 7.5 ± 0.1‰ to 87.1 ± 1.0‰ which leads to the hypothesis that the enrichment of $\delta^{13}C$ values in the soil was influenced by plant. The most likely explanation is that ^{13}C-labeled α-HCH absorbed by plant tissues, most likely leaves as they represent the largest surface and then transported to the rhizosphere. We hypothesized that HCH are translocated with photosynthates in the vascular system probably within the phloem down to the roots. They could be excreted from the roots to soil with exudates or passively. The concertation of ^{13}C HCH in roots were always much higher than in the rhizosphere or bulk soil providing a concentration gradient even for diffusive release. To evaluate this hypothesis, we monitored the carbon isotope composition of α-HCH in plant tissues which is shown below. However, the translocation of neutral species with a large Kow as has been suggested to be slow [36], thus our results were surprising and need future evaluation.

3.5 Carbon isotope ratio of different wheat tissues at different sampling times

The translocation and accumulation of non-labeled HCHs from soil to plant and within plants cannot influence the isotope composition of non-labeled HCHs as shown by a previous study [33]. The transformation of HCH in a soil-wheat system resulted in the maximal $\delta^{13}C$ enrichment of 6 ‰. However, in the present study a change of $\delta^{13}C$ value of up to 182.5 ± 1.4‰ was observed in plant tissues in the NT setup, indicating that the increase of the $\delta^{13}C$ value could only be due to the uptake and translocation of ^{13}C-HCH in the plant.

At the tillering stage, the $\delta^{13}C$ values of α-HCH in shoot and root in the NT setup increased to 133.3 ± 2.6‰ and 33.2 ± 3.4‰, respectively. The $\delta^{13}C$ values of α-HCH ranged from 45.8 ± 0.6‰ to 182.5 ± 1.4‰ at the jointing stage, from -35.6 ± 0.2‰ to 75.2 ± 0.8‰ at the flowering stage,

and from -18.5 ± 4.6‰ to 107.3 ± 22.3‰ at the harvest stage (Fig. 2). In all stages, the results showed that in the upper parts of the plants (e.g. leaf or stem) the $\delta^{13}C$ value of α-HCH was higher in comparison to the lower plant parts (e.g. roots), indicating that the uptake of ^{13}C-labeled α-HCH occurred in all plant tissues, especially in upper plant tissues via gas phase preferentially by leaves as they provide the largest surface and then ^{13}C-labeled α-HCH was probably translocated to lower plant tissues with photosynthates in the vascular system.

Regarding the roots, the $\delta^{13}C$ values of α-HCH were higher in the first two stages and then decreased in the flowering and harvest stage. Similar results were observed for stem and leaf. The $\delta^{13}C$ values of spike and grain were higher than other tissues in the flowering and harvest stage (Fig.2). The results suggest that during the tillering and jointing stage an intensive uptake of ^{13}C-labeled α-HCH from gas phase by the leaves took place and the ^{13}C-labeled α-HCH was then translocated into the lower plant parts most likely through the phloem transport. In the later flowering and harvest stages, the relative amount of ^{13}C-labeled α-HCH was lower in stem, root and leaf. This might be explained by the growth of spike and grain and the connected refilling process. Thus, the translocation of the^{13}C-labeled α-HCH from the leaves, stems and roots into the spike and grain caused the observed higher $\delta^{13}C$-value of α-HCH in spike and grain in later stages.

3.6 Isotope dilution during the uptake of α-HCH

For estimating the amount of α-HCH incorporated via air born transport we calculate an isotope balance assuming that isotope fractionation can be neglected as the change of the isotopic composition caused by transformation reactions makes up to less than 6 ‰ [33]. Hence, it was assumed that the observed enrichment of the isotopic composition of α-HCH in the NT set-up

was exclusively caused by the uptake of ^{13}C-labeled α-HCH from the gas phase. The amount of uptake or absorption of ^{13}C-labeled α-HCH was calculated based on the isotope dilution model assuming that the isotopic composition of the samples was altered by the addition of fully labelled ^{13}C-labeled α-HCH (99.9 atom % ^{13}C) to the non-labeled spiked α-HCH (atom 1.07 % ^{13}C).

The concentration of ^{13}C-labeled α-HCH in the soil samples of the NC and NT treatments ranged from 0.16 ± 0.01 to 0.76 ± 0.02 µg/kg (Fig. 3a). In general, higher amounts of ^{13}C-labeled α-HCH for individual growth stages were observed in the NC set-up in comparison to the NT set-up. This observation shows a similar trend as the concentrations of non-labeled HCH in both treatments and could therefore also be related to the higher dissipation of α-HCH in the bulk soil of the NT set-up due to the uptake, transformation and evapotranspiration by the plant.

In contrast, the concentration of ^{13}C-labeled α-HCH in plant samples of the NT treatment was significantly higher than the concentration in the soil. At the tillering stage, the concentration of ^{13}C-labeled α-HCH ranged from 90.23 ± 5.44 µg/kg in the root to 102.27 ± 1.81 µg/kg in the leaf (Fig. 3b). This concentration is more than 500 times higher than the corresponding concentration in the bulk soil (0.16 ± 0.01 µg/kg), indicating that the uptake of ^{13}C-labeled α-HCH into the plant is rather dominated via gas phase transport by plant tissues. The highest concentration of ^{13}C-labeled α-HCH was observed in the jointing stage with 549.95 ± 4.9 µg/kg in the root, 667.51 ± 4.29 µg/kg in the stem and 582.08 ± 125.03 µg/kg in the leaf (Fig. 4b), suggesting that the uptake of ^{13}C-labeled α-HCH by plant tissues was particularly evident in this stage. In every stage, the concentration of non-labeled HCH was higher in root compared to other plant tissues showing the main pathway of uptake from soil via roots to upper plant tissues. But the higher concentration of ^{13}C-labeled α-HCH was observed in the upper plant tissues, further indicating

366 more obvious uptake of ^{13}C-labeled α-HCH by above ground plant tissues via gas phase possibly
367 by leaves as they have the largest surface and then translocation of ^{13}C-labeled α-HCH to roots.
368 From jointing to harvest stage, the concentration of ^{13}C-labeled α-HCH in plant tissues decreased
369 and spike and grain had higher values, indicating the refilling and intern-translocation process in
370 plant to spike and grain. Nevertheless translocation of hydrophobic contaminants like HCH in
371 the plants as we found is surprising and has not been fully elucidated and this requires deeper
372 investigation to understand the transport mechanisms.

373 ## 4. Conclusion and outlook

374 In conclusion, a higher dissipation of α-HCH in soil in NT (Non-labeled α-HCH treatment)
375 compared to NC (non-labeled α-HCH unplanted control) was a result of uptake, transformation
376 and evapotranspiration by plant. The carbon isotope composition (δ^{13}C) of α-HCH in the soil of
377 NC increased by 15.8– 28.6‰ compared to the initial value of α-HCH, indicating a translocation
378 via gas phase from the ^{13}C-labeled pots. The increase of the δ^{13}C values in soil and plant system
379 in NT indicated the uptake of ^{13}C-labeled HCH by all plant tissues, especially the upper plant
380 tissues and then translocation to roots and subsequent release to the rhizosphere soil. The present
381 ^{13}C tracer experiment demonstrates the complex interaction of the plant affecting the
382 transportation of hydrophobic contaminants in the environment. Therefore, the present study
383 gave a new view about identifying the uptake mechanism of α-HCH in a pot experiment. The
384 uptake and translocation mechanisms of α-HCH from the gas phase should be taken into account
385 when using phytoremediation approaches and analyzing the function of green infrastructure in
386 urban areas.

387 ## Declaration of competing interest

There are no conflicts to declare.

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507 Table 1. The abbreviation of the treatments

Treatment	Abbreviation
Soil amended with 30 mg kg^{-1} of non-labeled α-HCH planted with wheat	NT
Soil amended 30 mg kg^{-1} of ^{13}C-labeled α-HCH planted with wheat	LT
Soil amended with 30 mg kg^{-1} of non-labeled α-HCH without wheat	NC
Soil amended with 30 mg kg^{-1} of ^{13}C-labeled α-HCH without wheat	LC
Soil not amended with α-HCH planted with wheat	PC

508

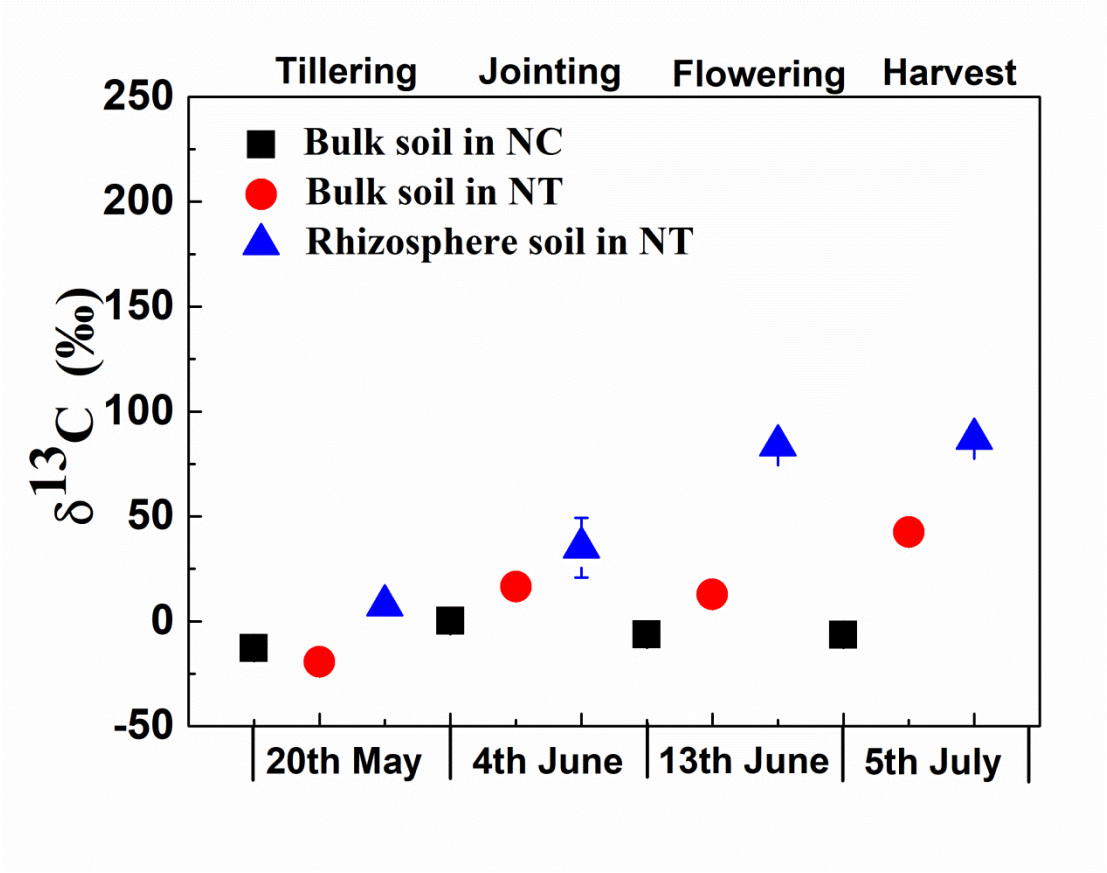
509

510 Table 2. Mass balance of non-labeled α-HCH in soil-plant system at all stages of plant growth

	Loss of HCH (%)		
Growth phase	Evaporation and possible transformation in soil	Uptake by plant	Transformation in or evapotranspiration by plants
Tillering	92.88	0.00	7.11
Jointing	94.58	0.06	5.41
Flowering	96.08	0.10	3.92
Harvest	96.66	0.06	3.34

511 The loss of HCH in the present study included the evaporation and possible transformation in soil and uptake by plants, the
512 transformation in or evapotranspiration by plant. The loss of evaporation and transformation in soil was calculated using the
513 concentration in the NC set-up (unplanted control experiment). The uptake by the plant was calculated using the wheat biomass
514 and concentration in the NT experiment (non-labeled α-HCH treatment).

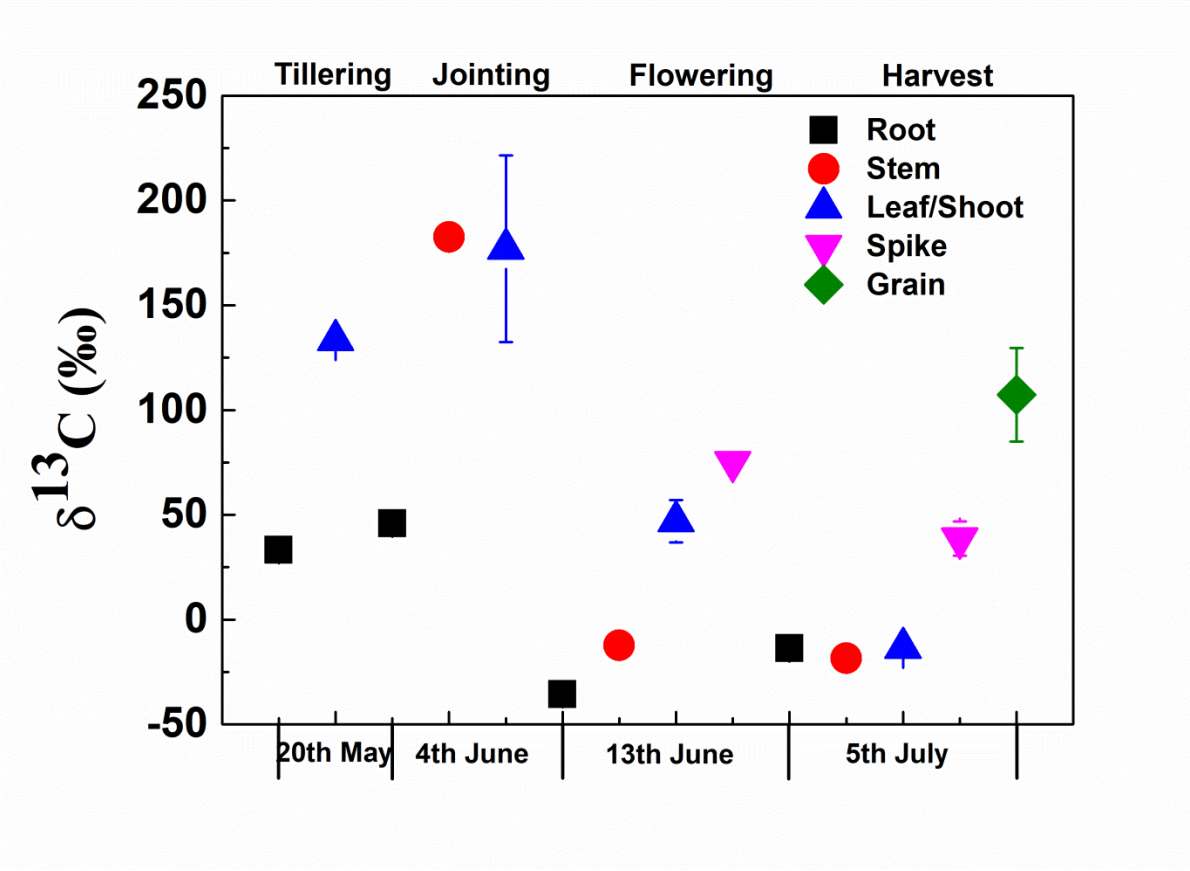
515



516

517 Fig. 1. Carbon isotope ratio of α-HCH in soil samples at different wheat growth stages for the
518 NT set-up (non-labeled α-HCH treatment). Error bars represent SD of 3 different pots in same
519 treatment.

520



521

522 Fig. 2. Carbon isotope ratio of α-HCH in wheat tissues at different growth stages in the NT set-
523 up (non-labeled α-HCH treatment). Error bars represent SD of 3 different pots in same treatment.

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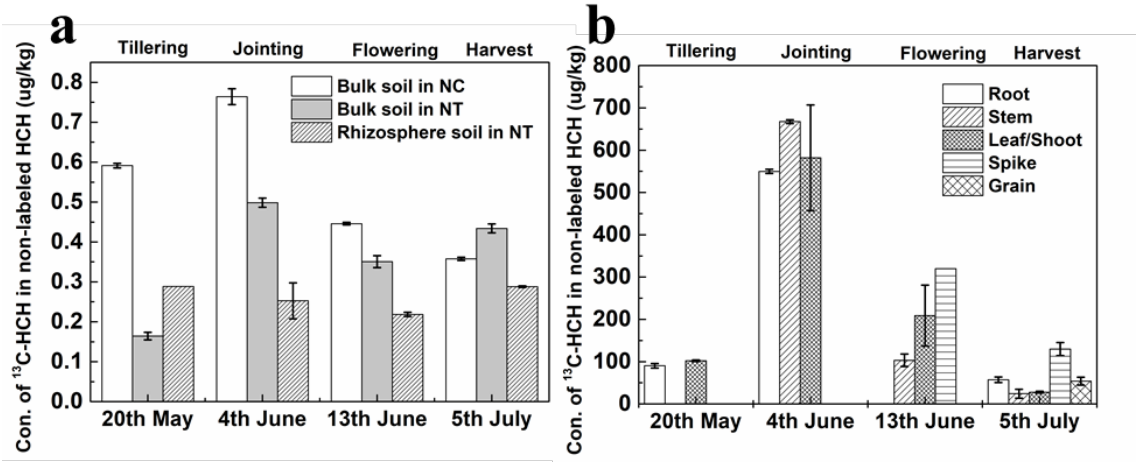

526

527 Fig. 3. Concentration of ^{13}C-α-HCH in soil samples at different wheat growth stages of the NC
528 (non-labeled α-HCH unplanted control) and the NT (non-labeled α-HCH treatment) treatment (a)
529 and concentration of ^{13}C-α-HCH in plant samples at different wheat growth stages for the NT
530 (non-labeled α-HCH treatment) treatment (b), which was calculated using the atom percent of
531 carbon. Error bars represent SD of 3 different pots in same treatment.

532