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Title: Effects of irrigation with HCH-contaminated water on crop performance and HCH accumulation in plant and soil

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Abstract

Lindane production is very ineffective since, for each ton of lindane obtained, between 6 and 10 tons of hexachlorocyclohexane (HCH) isomers and other toxic compounds are also produced. Due to the disposal of these residues, contaminated zones still exist. Many dumpsites are close to rivers and water reservoirs. The current study examines the consequences of irrigating pea, maize and alfalfa, with water containing different HCH concentrations on the accumulation of HCH in plant material and soils. The experiments were conducted on pots under controlled conditions using drinking water (as reference) and water with several HCH concentrations: 0.5 μg L^{-1} (the maximum threshold allowed for human consumption), 2.5 μg L^{-1}, 5 μg L^{-1}, and 20 μg L^{-1}. Results showed that both surface and overhead irrigation with these HCH concentrations did not cause any toxicity effects on the considered crops. However, under overhead irrigation with HCH concentrations higher than 5 μgL^{-1} HCH is absorbed by maize leaves and its concentration in plant biomass overpassed the EU maximum residue level of 10 μg kg^{-1} (EU, 2017). In the case of fodder maize, an HCH concentration of 0.84 μg L^{-1} in irrigation water produced a HCH concentration in plant above 20 μg kg^{-1} dry matter, the upper limit established in the Spanish legislation, that limit the use for animal feeding. In the case of alfalfa, HCH was detected in treatments with the highest HCH concentration (13 μg L^{-1}) under surface irrigation, but concentration was below the EU maximum residue level. In conclusion, in overhead irrigated systems, water with HCH concentrations below 5 μg L^{-1} does not produce HCH accumulation in pea and maize grain above the maximum residue levels; however, for fodder maize, the HCH concentration in irrigation water should be controlled to avoid HCH accumulation in plants above the limit for animal feeding.

Keywords: overhead irrigation, surface irrigation, Hexachlorocyclohexane, Organochlorines, Foliar uptake, Environmental risk.

1. Introduction

The 1,2,3,4,5,6-hexachlorocyclohexane (HCH, $C_6H_6Cl_6$) is a non-natural organochloride compound with nine isomers. It is an organic pollutant with acute toxicity persistence in the environment (UNEP, 2009; Vijgen et al., 2011). Even though nowadays HCH is prohibited, it was widely used as an insecticide, scabicide, pediculicide and parasiticide since the 1940's (Breivik et al., 1999; Saleh et al., 1982). The compound was available in two formulations: technical-grade HCH (a mixture of different isomers) and lindane (>99% γ-HCH). All the HCH isomers are toxic, being lindane the most toxic, affecting the nervous and endocrine systems and causing severe diseases such as cancer (Bradley et al., 2016). The γ-HCH production is based on chlorination of benzene in the presence of UV light, and it is very ineffective since for each ton of lindane obtained, between 6 and 10 tons of other HCH isomers and compounds (benzene, chlorobenzenes, and chlorophenols) are also produced (Vijgen, 2006). Due to the disposal of these residues, contaminated zones exist, especially in areas located in the proximities of production facilities (Calvelo Pereira et al., 2008), although this pollutant can be transported by the air, thus contaminating other sites (van Pul et al., 1999). Therefore, the identification and rehabilitation of land affected by HCH contamination is a priority worldwide (Triphati, 2019; Vijgen et al., 2022).

In Europe, the production of lindane has caused the extensive contamination of soils and waters with thousands of tons of toxic and persistent products (Vega et al., 2016). Production factories were often situated near rivers and river floods have contributed to the diffuse mobilization of these contaminants. The expected increase in the number of floods derived from climate change would contribute to an intensification of the release of these persistent pollutants towards rivers. In Spain, the manufacture of lindane was associated with four factories that generated 200,000 tons of HCH wastes, approximately (Fernández et al., 2013; Vega et al., 2016). Nearly 65% of this production was generated by the INQUINOSA factory located in Sabiñánigo (Aragón, Spain) that manufactured or formulated lindane products from 1975 to 1992 (Fernández et al., 2013). Generated wastes (approximately 115,000 tons) were mainly dumped in two unlined landfills (Bailin and Sardas). The Dense Non-Aqueous Phase Liquid (DNAPL) from these two dumpsites constitute a serious risk for agricultural crops due to the proximity of the Gállego River (Fernández et al., 2013), which supplies water for irrigation to more than 60,000 ha of cultivated land.

Residues of HCH have been detected in roots and aboveground tissues of different plant species grown in contaminated soils. Calvelo-Pereira et al. (2008) reported that the main mechanism of HCH adsorption by plants was not only by the roots, but also by adsorption through the aerial biomass from either volatilization or atmospheric deposition. Absorption from irrigation water has also been reported; in an experiment for determining the leaf sorption of different pollutants, Calderón-Preciado et al. (2013) spiked lettuce shed leaves with solutions of different pollutants and observed that lindane was absorbed almost completely by cuticula. Benimeli et al. (2008) reported that concentrations of lindane from 100 to 400 μg kg^{-1} in the soil did not affect the vigor and germination of maize seeds. Although there are many efforts to assess, and remediate the HCH waste legacy (Vijgen et al., 2022), no information on the effects of irrigation with HCH-contaminated water on crop absorption is available in the literature and HCH can be introduced into the food chain from HCH contaminated irrigation water. The European (EU, 1998, Council Directive 98/83/EC) and the Spanish legislation (BOE, 2023, Real

Decreto 3/2023) set a threshold of 0.5 μg L^{-1} for total pesticides in water for human consumption, but there is no regulation or thresholds for pesticides in irrigation water.

In this context, the aims of the current study were to assess the impact of different HCH concentration levels in the irrigation water on (i) HCH accumulation in the grain and plant biomass of three crops (alfalfa, maize and pea); (ii) grain yield and biomass; (iii) HCH levels in the soil at harvest time, and iv) to compare overhead versus surface irrigation, thus performing a first evaluation of the risk of this element entering the food chain through contaminated irrigation water.

2. Materials and Methods

2.1. Experiments location and setup

Different experiments were performed in the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) (41º 42' 47.3'' N, 0º 49' 39.9''W), located about 15 km North of Zaragoza (Spain). To avoid contamination risks in the environment, the experiments were conducted in pots separated from the soil surface by a plastic film (Supplementary material – Figure S1).

Three crop species were considered: alfalfa (*Medicago sativa* L.), maize (*Zea mays* L.) and pea (*Pisum sativum* L.). In 2015, experiments were carried out with pea and maize-grain; in 2016, experiments were conducted with fodder maize, whereas, in 2017, alfalfa was used.

Pots were filled with clay loam textured soil (270 g kg^{-1} sand, 380 g kg^{-1} silt, and 350 g kg^{-1} clay), and compacted to reach the original soil bulk density (1.45 g cm^{-3}). The crops were planted in pots of different size depending on the species (Supplementary material - Figure S2). In the case of pea, pots were 0.9 m length, 0.4 m width and 0.5 m depth, whereas for alfalfa and maize, pots were 0.6 m length, 1 m width and 0.76 m depth.

Pea was sown in February 2015 at 0.18 m between rows and 0.05 m between plants (111 seeds m^{-2}). Maize was sown on 22 June 2015 (LG3540 Waxy cultivar) and 31 May 2016 (Pioneer P0725 cultivar) at 0.7 m between rows and 0.15 m between plants (10.5 seeds m^{-2}). Alfalfa was sown on 30 May 2016 at a sowing density of 450 seeds m^{-2}

2.2. Irrigation treatments

In 2015, four treatments were considered for both pea and maize, T0: irrigation water without HCH (drinking water from the Zaragoza network); T1: irrigation water from the Gállego river at La Sotonera reservoir (collected on a 15-day basis); T2: irrigation water with HCH concentration of 0.5 μg L^{-1} (the threshold for drinking water), and T3: irrigation water with HCH concentration of 5 μg L^{-1} (10 times greater than the drinking water threshold). The HCH concentration refers to the sum of the α-, β-, γ-, δ- and ε- HCH isomers concentration.

The T0 treatment acted as a reference with no HCH content while T1 represents the actual irrigation water quality in the Gállego river at La Sotonera reservoir (the irrigation water storage reservoir). Since 2015, this reservoir is strictly refilled during periods in which HCH concentration is below the drinkability threshold (HCH < 0.5 μg L^{-1}). The T2 treatment represents the upper limit of HCH concentration in La Sotonera reservoir, and T3 represents a situation with an exceptionally high concentration of HCH in the irrigation water (10 times greater than the upper limit for drinking water).

All treatments were irrigated using a tailored overhead system (explained below) to avoid HCH dissemination in the environment.

In years 2016 and 2017, the T0 and T2 treatments applied in the 2015 experiments were maintained, and three new treatments were incorporated to understand the influence of the irrigation system, overhead *vs.* surface irrigation on the absorption of HCH by plants. The new treatments were, T4: overhead irrigation with water with HCH concentration of 2.5 μg L^{-1} (5 times the threshold for drinking water); T5: overhead irrigation with water with HCH concentration of 20 μg L^{-1} (40 times the threshold for drinking water); and T6: surface irrigation with water with HCH concentration of 20 μg L^{-1}. For all crops and years, four repetitions per treatment were considered.

The overhead irrigation treatment, simulating a sprinkler irrigation system, was built with an aerial dripper arrangement. For this purpose, a metallic structure was installed to support the irrigation system of each treatment. To reproduce both, irrigation time and drop size of the sprinkler systems used in commercial crop fields, regulated drippers were used. A network of driplines was built with emitters spaced 10 cm along each dripline and emitter flow set at 0.06 L h^{-1} (Supplementary Material Figure S3). In the case of pea, 160 drippers per treatment were used, the duration of a single irrigation event was 3 hours and the applied dose per event was 19.5 mm. In the case of maize and alfalfa, 220 drippers per treatment were used, the duration of each irrigation event was 3.5 hours and the irrigation dose per event was 25 mm. In this way, the overhead system simulated the wetting time of the crop (irrigation time) and the irrigation dose of a typical sprinkler irrigation in a commercial field. The dripper network was at 1.5 m height from the soil surface for pea and at 3 m height for alfalfa and maize crops (Supplementary Material Figure S3). The overhead system was designed to irrigate, simultaneously, the four replicates of each treatment using water from the same primary tank. The structure was surrounded by plastic curtains that were unfolded during each irrigation event to avoid water drifts caused by the wind (Supplementary material - Figure S2). In the case of the surface irrigation treatment, a hose with an opening valve connected each tank with each pot (Supplementary Material Figure S3) with an irrigation dose per event of 25 mm. Irrigation was scheduled according to the common practice in the region. Crop water requirements were estimated using the FAO methodology (Allen et al., 1998), using the reference evapotranspiration (ET_o) and the crop coefficients supplied by the regional advisory service for irrigation. The number of irrigation events and the total irrigation doses applied to each crop are reported in Table 1.

The irrigation water used in treatments T2, T3, T4, and T5 was prepared using DNAPL coming from the Bailin dumpsite. DNAPL was collected in a 50 L tank for each experiment and analyzed for α−, β−, δ−, γ−, and ε−HCH isomer concentrations. Then, the tank was transported to the experimental site and transferred (after an intense mixing) to 1.5 L numbered opaque glass bottles, then bottles were stored at 4 ºC. For each irrigation event, the adequate amount of DNAPL, from the same bottle, was added to each tank to obtain the HCH concentration (sum of α−, β−, δ−, γ−, and ε−HCH isomers) assigned to each treatment. Because HCH is very oily and does not mix well with water, a maximum volume of 1.2 L of DANPL was used from each bottle and the remaining was analysed again for HCH isomers concentration. The average HCHs concentration in irrigation water for each treatment and experiment is presented in Table 2 and the average HCH isomer concentrations are detailed in Tables S1 to S4 (Supplementary Material).

2.3. Sampling and determinations

At maturity, all pea and maize plants from each repetition were collected separately and grain and rest of above biomass weight were determined. For maize, grain humidity was measured to determine the grain yield referred to 14% humidity. For alfalfa, forage dry yield was determined at each harvest. At the end of each experiment, a soil sample from each replicate was collected.

Waters were analyzed for α–, β–, δ–, γ–, and ε–HCH isomer concentrations by gas chromatography (Agilent 7890A) in the Aragon Government Laboratory at Bailin (Fernández et al., 2013). Soil and fresh plant samples were analyzed for α–, β–, δ–, γ–, and ε–HCH isomer concentrations using a Varian CP-3800 Gas Chromatograph coupled with a Varian Saturn Ion Trap 2000 GC/MS/MS System with a quantification limit (QL) of 1 μg kg^{-1} that was improved to 0.5 μg kg^{-1} for maize grain and soils in 2016. Plant samples were not dried prior to analysis to avoid losses of HCH isomers by volatilization.

2.4. Statistical analysis

Plant biomass, crop grain yield and soil and plant HCH concentrations were subjected to analysis of variance (ANOVA) to evaluate the effect of the treatments imposed. The assumptions of normality and homoscedasticity were checked using Shapiro-Wilks and Bartlett tests, respectively. When needed, mean separation was performed using the Tukey's HSD test at 0.05 significance level. Statistical analyses were carried out using the R Statistical Environment v.3.6.1 (R Core Team, 2019).

3. Results

The targeted HCH concentrations in irrigation water (T2 and T3) were reached adequately in the pea-2015 and maize-2015 experiments (Table 2). However, HCH concentrations did not reach the target values (Table 2) in maize-2016 (≈20 times lower) and alfalfa-2017 (30% lower). In maize-2016, the DNAPL initial analysis gave a HCH concentration higher than the measured later in the numbered bottles. As there was a delay in the analysis of these samples, the HCH concentration of the irrigation water could not be corrected in time. In alfalfa-2017, the numbered DANPL bottles were analyzed with a higher frequency allowing to correct the amounts of DNAPL added to the tanks, but low concentrations in the three bottles used in the last irrigation events reduced the target HCH values by 30%.

On average, in the 2015 experiments (Supplementary material Table S2), isomer δ was the most abundant in the irrigation water, 54% (pea-2015) and 58% (maize-2015), while in 2016 (Supplementary material Table S3) isomers β (38%) and δ (35%) were present in similar percentages and in 2017 (Supplementary material Table S4) isomers δ (43%) and γ (34%) presented the highest proportion.

3.1. Pea experiment

Grain yield ranged from 4,757 kg ha^{-1} (T0) to 5,214 kg ha^{-1} (T1); whereas plant biomass varied between 16,077 kg ha^{-1} (T0) and 18,124 kg ha^{-1} (T1). However, no significant differences were detected among treatments for both grain yield and plant biomass (Table 3).

No HCH isomers were detected in the samples of pea grains from any treatment (Table 4) and the average HCH concentration in the plant was below 1 μg kg^{-1}, although δ–HCH was detected (1.5 μg kg^{-1}) in plant biomass of a T3 replicate, the treatment with the highest HCH concentration (Supplementary Material-Table S6). HCH isomers were not detected in soil samples, except for two replicates of the T3 treatment that showed concentrations of the δ isomer slightly over the detection limit of 1 μg kg^{-1} (Supplementary material - Table S7).

3.2. Maize-grain experiment 2015

Grain yield ranged from 3,565 kg ha^{-1} (T3) to 5,313 kg ha^{-1} (T2); whereas plant dry biomass varied between 6,376 kg ha^{-1} (T2) and 9,476 kg ha^{-1} (T0). No significant differences were detected among treatments for both variables (Table 3).

The average HCH concentration in grain and maize plants in the 2015 experiment was below 1 μg kg^{-1}, except for plants from the T3 treatment (Table 4). In grain samples, only one replicate from the T3 treatment showed the presence of HCH isomers over the quantification limit: β-HCH (2 μg kg^{-1}) and δ-HCH (1 μg kg^{-1}) (Supplementary material - Table S8). In the samples of fresh above biomass, HCH isomers appeared in different replicates of T3: β-HCH (2 to 6 μg kg^{-1}), δ-HCH (3 to 12 μg kg^{-1}) and ε-HCH (2 to 5 μg kg^{-1}), but only one replicate was over the limit of 10 μg kg^{-1} for the sum of isomers. When converted to concentration per dry matter, the sum of HCH isomer concentrations reached 59.1 μg kg^{-1} (Supplementary material - Table S9).

In the case of soil, only the β and δ isomers were detected in samples of the T3 treatment. The value of the β-HCH was 1 μg kg^{-1} in three replications, whereas δ-HCH varied between 3 and 4 μg kg^{-1} (Supplementary material - Table S10).

3.3. Maize-fodder experiment 2016

In 2016, maize dry biomass ranged from 13,915 kg ha^{-1} (T5) to 17,012 kg ha^{-1} (T6) (Table 5) with no significant differences among treatments.

No presence of HCH was detected in grain samples from any treatment (Table 4, Supplementary material Table S11). In contrast, plant biomass from the T5 treatment presented significant concentrations of β–, δ– and ε–HCH that added up to 37.4 μg kg^{-1} in fodder maize dry matter (Supplementary material - Table S12). Soil samples did not show the presence of HCH isomers in any of the treatments (Supplementary material - Table S13).

3.4. Alfalfa experiment

Table 5 shows the average total dry biomass of the five alfalfa cuts in 2017 for each of the treatments. Dry biomass ranged from 10,605 kg ha^{-1} in T0 to 14,174 kg ha^{-1} in T6. The plant biomass generated in T6 was significantly greater than that generated in the rest of the treatments.

HCH isomers were detected in fresh biomass samples of 2nd, 3rd, 4th and 5th cuts in T5 and T6 (Supplementary Material - Table S14), the highest concentrations were detected in T6, reaching values up to 9.9 μg kg^{-1} (sum of α, β, δ and ε isomers). The HCH concentrations (β–, δ–, γ–, ε– and total HCH) were significantly higher under surface irrigation than under overhead irrigation (Table 6), suggesting losses of HCH compounds during sprinkler irrigation.

Only the soils from the two treatments with the highest concentration of HCH (T5 and T6) showed detectable contents of HCH isomers, ranging from 1.1 to 3.9 μg kg^{-1} for the sum of α- β–, δ– and ε–HCH isomers. The isomer with the greatest presence in these soil samples was δ-HCH (Supplementary Material - Table S15).

4. Discussion

All standards and guidelines for agricultural irrigation are mainly aimed at protecting health by controlling human and livestock exposure to pathogenic organisms and a limited number of toxic chemicals (Lazarova and Bahri, 2005). Similarly, selecting the irrigation technique relies on meeting plant water requirements optimally, while minimizing the sanitary risks. In contrast, little attention has been given to the potential risks stemming from foliar sorption of organic micro-contaminants as a possible pathway to the human food chain and the ensuing repercussions for both the population and the environment (Calderón-Preciado et al., 2013).

In the current study, yields of grain maize and pea were not affected by irrigation water with HCH concentrations up to 5 μg L^{-1} and biomass of fodder maize and alfalfa was not affected when these crops were irrigated with waters with HCH concentration up to 20 μg L^{-1}. The differences encountered among treatments on alfalfa biomass cannot be ascribed to the differential concentrations of HCH in the irrigation water and might have been caused by different levels of plant establishment within the pots. Therefore, in the current study, there was no toxic effect of HCH on the development and growth of the three crop species considered.

Previous research has shown that plants can accumulate contaminants when they come with irrigation water (Mishra et al., 2009). In fact, it has been observed (Calderón-Preciado et al., 2013) that sprinkler irrigation induces the foliar sorption of micro-contaminants and their accumulation in the leaves of lettuce. However, the results from the current study suggest that this depends on the plant species and on the concentration of pollutants in the irrigation water. In the case of pea-2015, no HCH isomers were detected in pea grains (Table 5), even when plants were overhead irrigated with water containing 5.2 μg L^{-1} of HCH (treatment T3), 10 times the limit for drinking water. Moreover, only one plant biomass sample from the T3 treatment showed the presence of the δ-HCH isomer (the isomer with the highest concentration in irrigation water, Table S1) at 1.5 μg kg^{-1}, which is below the maximum threshold (10 μg kg^{-1}) established by the European Union (EU, 2017) for vegetables.

In the case of the maize 2015 experiment, β and δ isomers were detected in grains from one sample and β, δ and ε isomers in different plant samples coming from the treatment with the highest HCH concentration (T3, 5.2 μg L^{-1}). HCH isomer concentrations in grain were well below the EU maximum threshold in all treatments. Although in plant samples, the δ isomer was above the 10 μg kg^{-1} EU threshold in some of the T3 replicates (Supplementary material Table S9), the average value did not surpass this threshold (Table 5).

In maize in 2016 experiment, the targeted HCH concentrations in the irrigation water were not reached, and actual HCH concentrations were 1/20 of the initially targeted concentrations (Table 2). No HCH isomers were detected in grains in any of the treatments. However, the T5 treatment (HCH concentration of 0.84 μg L^{-1}) surpassed the maximum residue level of 10 μg kg^{-1} for the sum of HCH isomers except lindane established by the European Union (EU, 2017). If the maize is used for fodder, HCH concentration in T5 was above the 20 μg kg^{-1} dry matter established in the Spanish legislation for the sum of HCH isomers except lindane (BOE, 1994, Real Decreto 280/1994).

The HCH concentration in irrigation water for maize 2016 T5 treatment (0.84 μg L^{-1}) was slightly higher than in the T2 treatment (0.66 μg L^{-1}) and much lower than in the T3 treatment (6.63 μg L^{-1}) of maize in 2015; however, the HCH accumulation in plant was higher than in 2015. The higher sensitivity to absorption of HCH of maize in 2016 in relation to the 2015 experiment is attributed to 2 different reasons. First, to a higher number of irrigation events applied in 2016 (45 events) in comparison to 2015 (29 events), associated to a larger maize cycle in 2016; the higher number of irrigation events in 2016 increased the total mass of HCH applied to the crop, indicating that absorption through leaves is an accumulative process. Second, to a higher proportion of the most stable β isomer in irrigation water in 2016 (37.8%) in comparison to 2015 (9.6%).

Finally, in alfalfa, plant biomass from the treatments with the highest concentration of HCH showed the presence of the δ isomer, and some of them also the α and ε isomers. Nevertheless, they never surpassed the maximum residue levels established by the European Union (EU, 2017) and were much lower than the values reported for crops grown in a contaminated site in China (Zhang et al., 2013).

In the case of maize in 2016, no HCH isomers were detected in plant samples of the T6 treatment (surface irrigated) with the same HCH concentration in irrigation water (13.2 μg kg^{-1}) than in the T5 treatment (overhead irrigated). This behavior would indicate a differential HCH absorption between leaves and roots in maize, being predominant the adsorption through the leaves. Urrego-Pereira et al. (2013) measured contact angles of water drops with leaf surfaces lower than 90º, indicating a high maize leaf wettability, that would explain the absorption of HCH with water through maize leaves. In contrast to maize, HCH concentrations in alfalfa in T6 (surface irrigated) where higher than in T5 (ovehead irrigated) despite both treatments receiving the same HCH concentration in irrigation water. This behavior indicates that in alfalfa the adsorption of water through the leaves is smaller than in the case of maize. According to Urrego-Pereira et al. (2013) this could be due to a higher hydrophobicity of alfalfa leaves than those of maize.

The differences in HCH concentrations (β–, δ–, γ–, ε– and total HCH) between surface and overhead irrigated treatments in alfalfa (Table 6) suggested some HCH compounds losses through water drifts that usually occur under sprinkler irrigation systems in the Ebro Valley Basin (Spain) and/or volatilization process.

The differences among crops in the accumulation of HCH isomers in plant might have been caused by the different capacity of each species for accumulating residues, as reported in other studies (Mishra et al., 2009).

With the results of the current experiments, some tentative threshold values for HCH in irrigation water can be established for different crops under surface and overhead irrigation in the Gállego River basin and similar areas. In overhead irrigated systems, HCH concentrations below 5 μg L^{-1} in the irrigation water would not result in HCH concentrations in pea and maize grain above the EU threshold value for food (10 μg kg^{-1}). In the case of overhead irrigated fodder maize, a HCH concentration in irrigation water of 0.84 μg L^{-1} produced accumulation of HCH above 20 μg kg^{-1} in plant dry matter, the upper limit established in the Spanish legislation, so they could not be destined for animal feeding. A safe limit for fodder maize would be 0.08 μg L^{-1}, the one in treatment T2. However, if fodder maize is surface irrigated, values up to 0.84 μg L^{-1} are not of concern.

In the four experiments carried out, HCH isomers were detected in the soil at harvest under the treatments with the highest HCH concentration in the irrigation water. The most detected isomer was δ, likely due to the composition of the irrigation water. In all cases, the reference levels of HCH isomers in the soil were not surpassed (Calvelo Pereira et al., 2010). In those treatments with the highest concentrations of HCH, more than 90% of the δ-HCH applied with the irrigation water was recovered from the soil, whereas the absorption by plants was low. Nevertheless, the concentrations of HCH found in the current study were lower than those reported for agricultural soils in Galicia (Calvelo Pereira et al., 2010), Europe, North America and Asia between 1990 and 2007 (Bidleman et al., 2006; Falandysz et al., 2001; Toan et al., 2007). The variability reflects the level and time of exposure to the contaminants suffered in those sites and the degradation by biotic and abiotic processes depending on soil properties (Saleh et al, 1982; Cousins et al., 1999; Kumar et al., 2006). Despite the results of this study, it is important to indicate that assessing the environmental effects of high concentrations of HCH in irrigation water requires performing field experiments over longer periods to consider the degradation, retention and bio-accumulation processes of HCH isomers in the soil matrix.

5. Conclusions

Applying water with different concentrations of HCH did not cause toxicity effects on alfalfa, maize and pea plants, thereby it did not alter their growth and yields. However, in overhead irrigation when HCH concentrations in irrigation water were high, maize absorbed some isomers and plant biomass HCH concentration was over the maximum residue limit of 10 μg kg^{-1} allowed by the EU in plant-based foods, surpassing also the limit for animal feeding. In addition, most of the soil samples from the treatments with relatively high HCH concentrations in the water showed accumulation of HCH, although in most cases they did not reach the levels of contaminated sites. This study indicated that when the criteria established for filling the La Sotonera reservoir (HCH below the drinkability threshold of 0.5 μg L^{-1}) are met, no problems of lindane contamination should occur on crops and soils irrigated with the water from this reservoir.

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CRediT authorship contribution statement

Jose Manuel Miras: Formal analysis, Writing – Original draft preparation- Review & Editing; **Raquel Salvador**: Conceptualization, Methodology, Writing - Review & Editing, Funding acquisition**, Monica Guillén**: Resources, Field work; **Farida Dechmi:**

Conceptualization, Methodology, Writing - Review & Editing, Funding acquisition, Supervision; **Dolores Quilez**: Conceptualization, Methodology, Data Analysis, Writing - Review & Editing, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Table 1. Number of irrigation events and total irrigation doses applied to the different crops and years.

Species	Year	Dates	# Irrigation events	# Irrigation events with HCH	Seasonal irrigation depth (mm)
Pea	2015	24 Feb – 18 May	11	7	214.5
Maize (grain)	2015	24 Jun – 27 Oct	33	29	825.0
Maize (fodder)	2016	30 May – 02 Oct	46	45	940.5
Alfalfa	2017	01 Jun – 27 Oct	56	56	1176.0

Table 2. Actual irrigation water HCH concentration in the different treatments for the different crops and years.

	Year	T0 Control	T1 Sotonera	T2 0.5 µg L^{-1}[a]	T3 5 µg L^{-1}[a]	T4 2.5 µg L^{-1}[a]	T5 20 µg L^{-1}[a]	T6 20 µg L^{-1}[a]
Pea	2015	<0.1	<0.1	0.52	5.24			
Maize	2015	<0.1	<0.1	0.66	6.63			
Maize	2016	<0.1		0.02		0.084	0.84	0.84
Alfalfa	2017	<0.1		0.34		1.69	13.24	13.45

[a] Target HCH concentration in the irrigation water for a given treatment

Table 3. Average (n=4) pea fresh grain yield and plant biomass and maize grain yield (14% humidity) and dry biomass in 2015 for each treatment.

	Pea – 2015		Maize - 2015	
Treatment	Grain yield (kg ha^{-1})	Fresh plant biomass (kg ha^{-1})	Grain yield (kg ha^{-1})	Dry plant biomass (kg ha^{-1})
T0	4757	16077	3736	9476
T1	5214	18124	4086	8837
T2	4948	17970	5313	6376
T3	4784	17220	3565	7453
p[1]	ns	ns	ns	ns

[1] Probability level of the treatment effect after ANOVA. ns: not significant, $p > 0.05$

Table 4 Average (n=4) concentrations of the α-, β-, γ- δ- and ε-HCH isomers and their total sum (excluding lindane) in the grains and rest of the plant for pea and maize and in alfalfa plant (4th cut) in the different treatments.

	HCH concentration irrigation water ($\mu g\ kg^{-1}$)	**HCH isomers ($\mu g\ kg^{-1}$)**					**Sum α, β, and ε isomers ($\mu g\ kg^{-1}$)**
		α	**β**	**γ**	**δ**	**ε**	
	2015 Pea grain and plant						
T0	<0.1	< 1	< 1	< 1	< 1	< 1	< 1
T1	<0.1	< 1	< 1	< 1	< 1	< 1	< 1
T2	0.52	< 1	< 1	< 1	< 1	< 1	< 1
T3	5.24	< 1	< 1	< 1	< 1	< 1	< 1
	2015 Maize grain						
T0	<0.1	< 1	< 1	< 1	< 1	< 1	< 10
T1	<0.1	< 1	< 1	< 1	< 1	< 1	< 10
T2	0.66	< 1	< 1	< 1	< 1	< 1	< 10
T3	6.63	< 1	< 1	< 1	< 1	< 1	< 10
	2015 Maize leaves + stems + cobs						
T0	<0.1	< 1	< 1	< 1	< 1	< 1	< 10
T1	<0.1	< 1	< 1	< 1	< 1	< 1	< 10
T2	0.66	< 1	< 1	< 1	< 1	< 1	< 10
T3	6.63	< 1	2.5	< 1	6.8	1.9	4.4
	2016 Maize grain						
T0	<0.1	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
T2	0.02	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
T4	0.084	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
T5	0.84	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
T6	0.84 (F)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	2016 Maize leaves + stems + cobs						
T0	<0.1	< 1	< 1	< 1	< 1	< 1	< 1
T2	0.02	< 1	< 1	< 1	< 1	< 1	< 1
T4	0.084	< 1	< 1	< 1	< 1	< 1	< 1
T5	0.84	< 1	4.7	<1	5.6	1.4	6.1
T6	0.84 (F)	< 1	< 1	< 1	< 1	< 1	< 1
	2017 Alfalfa 4th cut						
T0	<0.1	< 1	< 1	< 1	< 1	< 1	< 1
T2	0.34	< 1	< 1	< 1	< 1	< 1	< 1
T4	1.69	< 1	< 1	< 1	1.9	< 1	<1
T5	13.24	< 1	< 1	<1	< 1	< 1	< 1
T6	13.45 (F)	< 1	< 1	< 1	< 1	2.6	2.6

Table 5 Average (n=4) maize dry biomass in 2016 and total (5 cuts) dry biomass for alfalfa in 2017 for each treatment. Different letters in the columns indicate significant differences among treatments at $P < 0.05$.

	Maize-2016	Alfalfa-2017
Treatment	Dry biomass (kg ha^{-1})	Dry biomass (kg ha^{-1})
T0	15451	10605 a
T2	14588	11631 ab
T4	14527	11701 ab
T5	13915	12498 b
T6	17012	14174 c
p[1]	ns	0.0001

[1] Probability level of the treatment effect after ANOVA. ns: not significant, $p > 0.05$

Table 6. Average (n=16) concentrations of the α-, β-, γ- δ- and ε-HCH isomers and their total sum in alfalfa plants in the T5 (sprinkler irrigated) and T6 treatments (surface irrigated).

	HCH isomers ($\mu g\ kg^{-1}$)					**Sum** α, β, and ε **isomers ($\mu g\ kg^{-1}$)**
Treatment	**α**	**β**	**γ**	**δ**	**ε**	
T5	0.1325	0	0.706	0.0500	0	0.888
T6	0.2125	0.075	1.150	2.944	0.156	4.537
p^1	ns	<0.01	<0.05	<0.001	<0.01	<0.001

[1] Probability level of the treatment effect after ANOVA. ns: not significant, $p > 0.05$