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RESEARCH ARTICLE

Response of vegetative and reproductive organs of *Cucurbita pepo* L. Subsp *pepo* 'Allegria' exposed to aged dieldrin contamination in regards to soil concentration and plant development

Félix Colin · Grégory J. V. Cohen ·
Marie-Cécile Affholder · Florian Delerue ·
Olivier Atteia

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Abstract

Aims Dieldrin is an Organochlorine Pesticides (OCPs) which, due to its persistence in agricultural soils, continues to be a source of contamination for crops, especially *Cucurbita pepo* L. subsp *pepo* (*C. pepo* L.). The latter are able to absorb dieldrin from contaminated soils and transfer it from the roots to the shoots. However, dieldrin allocation has been partially studied, especially in the shoots. This study aims at characterizing dieldrin distribution in the different plant organs according to soil contamination and plant growth.

Methods This was achieved by growing *C. pepo* L. 'Allegria' in soil mixtures presenting 5 levels of dieldrin concentration (<1 to 145 µg kg^{-1}) and by harvesting them after different exposure periods (30, 90 and 140 d after transplant).

Results The results showed (i) the predominance of the root impregnation path, (ii) that dieldrin concentration in the vegetative organs of *C. pepo* L. 'Allegria' increased with soil concentration while (iii) it stabilized for reproductive organs at soil concentrations exceeding 50 µg kg^{-1} DW. The bioconcentration factors (BCFs) for vegetative organs were classified as follows: stems (70) > roots (47) > leaves (19). Furthermore, the mass distribution of dieldrin was more dependent on variations in organ mass fraction with plant development than variations of dieldrin concentration in the different organs.

Conclusions These results are of main importance for (i) the identification of dieldrin contaminated agricultural plots using phytoscreening methods as well as for (ii) their phytomanagement providing crucial allocation information for phytoextraction purposes.

Keywords Organochlorine Pesticide · Soil contamination · Cucurbits organs · Bioconcentration factor · Contaminant exposure time

Introduction

It was thought until recently that the predominant pathway for lipophilic Organic Compounds (OCs) such as OrganoChlorine Pesticides (OCPs), Polycyclic Aromatic Hydrocarbons (PAHs) or PolyChlorinated Biphenyls (PCBs) to accumulate in vegetation was aerial deposition and not root uptake followed

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F. Colin · G. J. V. Cohen (✉) · M.-C. Affholder ·
F. Delerue · O. Atteia
Univ. Bordeaux, CNRS, Bordeaux INP, EPOC, UMR 5805, F-33600 Pessac, France
e-mail: gregory.cohen@ipb.fr

F. Colin
Bordeaux Métropole, Esplanade Charles de Gaulle, 33000 Bordeaux, France

M.-C. Affholder
Univ. Bordeaux, INRAE, BIOGECO, Allée Geoffroy St-Hilaire – bât. B2, CS 50023, 33615 PESSAC cedex, France

by translocation since these substances are highly hydrophobic and thus cannot easily solubilize in the soil solution (Inui et al. 2011; Schwab and Dermody 2021; Trapp and Legind 2011). However, numerous studies have shown the opposite investigating the contamination of plants by various OCs (Al-Nasir et al. 2020; Gao and Zhu 2004; Jiries et al. 2022; Mikes et al. 2009; Murano et al. 2010a; Namiki et al. 2013; Otani et al. 2007; Saito et al. 2012; Samsøe-Petersen et al. 2002; Trapp 2002). Among plants, those from the Cucurbitaceae family are able to absorb, accumulate and translocate OCs from roots to shoots (Mattina et al. 2007; Murano et al. 2010b; Namiki et al. 2013, 2015, 2018; White 2002). There are differences in accumulation between Cucurbitaceous species (Mattina et al. 2006; Otani et al. 2007), subspecies and cultivar with *Cucurbita pepo* L. ssp. *pepo* (*C. pepo* L.) being one of the most accumulative in roots and shoots (Chhikara et al. 2010; Inui et al. 2008; Isleyen et al. 2012, 2013; Isleyen and Sevim 2012; White et al. 2003b).

Uptake and accumulation of OCs from the soil to the plants can be divided into 4 steps: (i) desorption of OCs from soil particles and dissolution into soil pore water, (ii) root absorption, (iii) translocation to aerial parts, and (iv) storage and accumulation (Inui et al. 2008; Piutti et al. 2022; Schwab and Dermody 2021). Thanks to their root exudates composition, *C. pepo* are able to desorb and solubilize contaminants bind to Soil Organic Matter (SOM), which facilitates their absorption by the roots (Colin et al. 2022; Mattina et al. 2007; Richardson et al. 1982; Wang et al. 2004; White et al. 2003a, 2006b; White and Kottler 2002). Concerning translocation to aerial parts, the Major Latex-like Proteins (MLPs) produced in root cells for various biological functions (disease resistance, salt stress tolerance, leaf formation, enzymatic activity and plant hormone response) have the structural capacity to bind OCs and transfer them to shoots through the xylem sap (Fujita and Inui 2021a, b; Garvin et al. 2015; Inui et al. 2013; Iwabuchi et al. 2020). Finally, the contaminants distribution in the different plant organs results from these four steps. However, this distribution has only been partially studied (Donnarumma et al. 2009; Lunney et al. 2004; Mattina et al. 2004) although it is of primary importance for the cultivation and trade of edible parts of these plants and/or for phytomanagement consideration for contaminated soil.

So far, OCPs distribution in plant organs was mostly studied on specimen grown only for a few days (< 30) (Kelsey et al. 2006; Namiki et al. 2013; Saito et al. 2012) and often without distinction between the different aerial organs which were pooled as shoots (Inui et al. 2008, 2011). Few studies have been conducted on various OCPs and varieties of *C. pepo* L. grown for up to 60 d (Mattina et al. 2000, 2003, 2004; White 2001; White et al. 2006a), but only with one or two different soil concentration levels. Therefore, these studies did not allow to correctly characterize OCPs distribution in the different plant organs nor its time evolution. Indeed, distribution of contaminant in plant organs can vary with time because (i) the relative mass fractions of the different organs change as plants grow (Poorter et al. 2015) and (ii) partitioning of contaminants between the different organs can also vary with plant development (Jeke et al. 2015). Besides, due to short growing periods, fruits were rarely studied but when they were lower OCPs concentrations were measured in comparison with the other organs (Mattina et al. 2004), highlighting allocation differences. Moreover, the results obtained in former studies were sometimes acquired from plants cultivated on artificially contaminated soil (Namiki et al. 2013; Otani et al. 2007) which is not relevant for historical contamination because of soil aging processes inducing lower contaminant bioavailability (Inui et al. 2011; Simonich and Hites 1996; Trapp and Legind 2011). In sum, there is a lack of knowledge about the distribution of OCPs in separated plant organs (roots, stems, leaves, flowers and fruits) (i) at different development stage and (ii) for different contaminant concentrations of historically contaminated soils.

This study focuses on dieldrin, an OCP classified as a Persistent Organic Pollutant (POP) by the Stockholm Conventon in 2001. Dieldrin was worldwide used in agriculture between the 1950s and the end of 1970s (Hashimoto 2005; Jorgenson 2001). In France, dieldrin was banned in agriculture in 1972 (INRS 2007). However, fifty years after its prohibition, dieldrin is still present in arable soil and continue to contaminate crops, particularly *C. pepo* L. (Namiki et al. 2015, 2018; Saito et al. 2012; Tsiantas et al. 2021), with concentrations potentially exceeding European Maximum Residue Levels (MRL) fixed by regulatory institutions at 50 µg kg^{-1} (fresh weight, FW) for zucchini (Affholder et al. 2023; *Official Journal of the European Union* 2008).

Considering the above elements, the main objective of this study was to characterize the transfer and allocation of dieldrin from historically contaminated soil to *C. pepo* L. organs (stems, roots, leaves, flowers and fruits) in function of (i) soil concentration and (ii) plant development. To our knowledge, this is the first study investigating dieldrin contamination in all organs of *C. pepo* L. grown on historically contaminated soil.

Materials and methods

In order to characterize the transfer and allocation of dieldrin from historically contaminated soil to the different *C. pepo* L. organs (roots, stems, leaves, flowers and fruits), two growing experiments were conducted. The first one focused on the influence of soil concentration while the second one dealt with the influence of plant development, both for the determination of the concentration and distribution of dieldrin in vegetative (roots, stems, leaves) and reproductive organs (flowers and fruits). For the first experiment, the plants were grown in pots with soil presenting five levels of contamination and harvested at 90 d. For the second one, another set of plants were grown in pots with soil presenting the highest dieldrin concentration level and harvested after 30 and 140 d of contaminant exposure.

Soil preparation

For the first experiment, the gradient of dieldrin concentration in soil was prepared by mixing two agricultural soils: one historically contaminated with dieldrin and the other one with dieldrin concentration below the Limit of Detection (LOD) of about 1 µg kg^{-1} Dry Weight (DW). Both soils presented the same texture (85% sand, 5% silt, 10% clay) and neutral pH (6.7 ± 0.1) but the uncontaminated soil contained more organic matter (14 ± 1%) than the contaminated one (9 ± 1%). For each soil, approximately 300 kg were collected between 0 and 20 cm depth, roughly sieved (1 cm mesh), homogenised and air-dried on plastic sheets. Then, the two soils were mixed to obtain five levels of dieldrin concentration, with the following mass proportions (%contaminated-%uncontaminated): 0-100, 30–70, 50–50, 75 – 25 and 100-0. For each concentration level, five culture pots were filled with 15 kg of homogenised soil mixture, for a total of 25 pots. For each culture pot, three soil samples of approximately 30 g were collected to measure the dieldrin concentration. For the second experiment, 10 additional pots were similarly prepared with the fully contaminated (100-0) soil mixture. Finally, the 35 pots were placed in the agricultural field historically contaminated and buried at two third of their height to limit the increase of soil temperature. They were regularly inspected to cut off any roots that might emerge from the drainage holes.

Seedling, transplantation, crop maintenance and harvesting

Following local farmers' practices, seeds of the *C. pepo* L. var. 'Allegria' (CLAUSE, France) producing long, cylindrical and shiny dark green fruit were sown in 200 mL plastic cups containing uncontaminated potting soil (Bio Motte 20, PROVEEN). After 2 weeks of pre-growth in a greenhouse, the plantlets were individually transplanted into the pots. Depending on the meteorological conditions, especially the occurrence of rainfall events, the crops were watered two or three times a week with local groundwater (dieldrin concentration below 0.1 µg L^{-1}). Once a week, the crops were fertilized with an organic fertilizer (Bio-grow, Biobizz®, NPK: 4-3-6) at a concentration of 2 mL per liter of groundwater.

The 25 plants growing on the dieldrin concentration gradient (first experiment) were harvested 90 d after transplant while the 10 other plants (second experiment) were harvested at 30 d (five plants, just before the production of the first fruit), and 140 d (five plants) after transplanting. When present, the fruits were harvested at marketable size (between 15 and 20 cm long) throughout plant life. For both experiments, after harvesting, the plants were divided into roots, stems, leaves and flowers which were cleaned with tap water to remove any soil particles and dried at 30 °C until mass stabilization for biomass determination. Since flowers are renewed frequently during the growth of plants, the masses obtained for this organ correspond to the ones measured at the time of harvest and not the cumulative one. Flowers from the same soil condition were pooled to obtain sufficient mass for analyses.

Dieldrin analysis in soil and *C. pepo* L. organs

For soil, dieldrin analyses were conducted following the protocol described in Colin et al. (2022). For *C. pepo* L. fruits, each fruit was analyzed individually in fresh weight (FW) with the protocol described in Affholder et al. (2023). This protocol was adapted for the dried organs which were ground to a fine powder with a knife mill (Pulverisette 11, Fritsch). The following grinding cycle was applied: 5 s at 2000 rpm, 10 s at 6000 rpm and finally 10 s at 10,000 rpm. Liquid-solid extractions were performed with 5 g of this powder mixed with 10 mL of n-heptane (n-Heptane, 99+%, residues analysis, ACROS Organic, UK) in 50 mL glass flask and agitated during 48 h (Roller 10, IKA™). Supernatant was recovered after 5 min of centrifugation at 1500 rpm and placed in a 2 ml vial. Prior analysis, 50 ng of Phenanthrene d-10 (Restek, France), used as internal standard, was added to the sample. dieldrin was analyzed using Gas Chromatography (7820 A & 8860, Agilent, USA) coupled with Mass Spectrometer (5977E & 5977B, Agilent, USA) systems (GC-MS), as described in Colin et al. (2022).

Data analysis and statistics

All statistical analysis was produced using R software (R Core Team 2022). Concerning soil, an ANOVA was performed to confirm the difference of dieldrin concentration between the different soil mixture. For plants, the analysis was conducted discriminating vegetative and reproductive organs considering their different behavior and the fact that these organs were harvested at different growth times. In order to study potential relationship between the concentration of dieldrin in soil and *C. pepo* L. organs, the Bio-Concentration Factors (BCFs) were calculated for each organ of each harvested plant (except for the uncontaminated soil because of dieldrin concentration < LOD) of the first experiment. They were calculated as follows (Mattina et al. 2003):

$$\mathrm{BCF_{organ}} = \mathrm{C_{organ}}/\mathrm{C_{soil}} \qquad (1)$$

with C_{organ} and C_{soil} the DW dieldrin concentration in the plant organ and in the soil, respectively. Then, differences of BCFs with dieldrin concentration in soil and between vegetative organs were tested with two-way ANOVAs. The variance explained by both factors and their interaction (i.e. their effect size η^2) were calculated with the "effectsize" package in R. The BCFs were square-root transformed to respect the conditions of application of linear modelling.

In order to compare the results obtained for the fruits with the other plant organs, the fruits weight and dieldrin concentration were transformed into DW. Since the *C. pepo* L. fruits contain 95% water (Rana 2017), the fresh biomass was multiplied by 0.05 and the dieldrin concentrations divided by 0.05. No statistics were conducted on flowers as there was only one value for each condition ($N = 1$). When several fruits were harvested from the same plant, averaged values were calculated and considered for analysis. In all cases, one-way ANOVAs with Tukey posthoc multiple comparisons, in case of significant effect, were performed. To respect the conditions of applications of linear modelling (normality and homoscedacity of model residuals), proportion data (dieldrin and biomass repartition) were arcsin transformed before analyses. For other variables (organ biomass, dieldrin concentration and amount of dieldrin), values were square-root transformed.

Results

Influence of soil concentration on dieldrin distribution

The concentration of dieldrin in soil for each contamination level is presented in Fig. 1. They increased significantly ($p < 0.001$) in agreement with soil mixture preparation and resulted in the following dieldrin concentration gradient: < LOD, 30 ± 2, 54 ± 3, 83 ± 4 and 145 ± 2 µg kg^{-1} DW for the 0-100, 30–70, 50–50, 75 − 25 and 100-0 soil mixtures, respectively. This validated the dieldrin concentration gradient set up.

Vegetative organs

The Fig. 2 presents the results obtained during the first experiment on vegetative organs for plants harvested at 90 d in function of soil dieldrin concentration. The

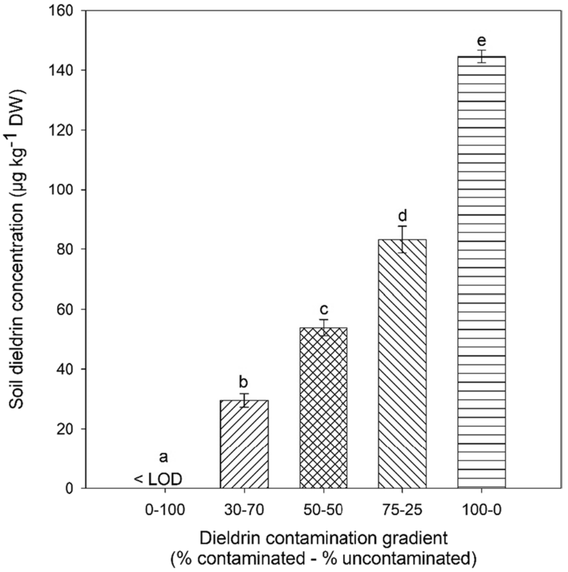

Fig. 1 Soil dieldrin concentration in the culture pots. Different letters above the boxes indicate significant difference in regards to dieldrin soil mixture. Error bars correspond to the standard error of the mean ($N = 5$)

total plant biomass (in DW) significantly decreases from 13.6 ± 0.6 to 7.6 ± 0.7 g with the increase of dieldrin concentration in soil, mainly due to leave biomass. Indeed, the leaves biomass decreased significantly with increasing soil dieldrin concentration, from 8.3 ± 0.5 to 3.9 ± 0.5 g (Fig. 2-1). A slight significant decrease in biomass was also recorded for the stems (3.6 ± 0.2 to 2.4 ± 0.1 g) while no difference ($p = 0.4$) was detected for roots (1.5 ± 0.1 g) according to soil concentration. However, considering the respective mass fraction of these different organs, no significant variation ($p = 0.08$) with soil contamination was detected for stems (Fig. 2-2), but slight variations in proportions for the roots ($p < 0.01$) and leaves ($p < 0.05$) were noted. Together, these results indicated that plants growing on the most contaminated soil (75 – 25 and 100-0 mixtures) were smaller than the others, but showed similar organ mass proportions trends with leaves (51–61%) > stems (27–32%) > roots (12–17%).

The concentration of dieldrin in the vegetative organs relative to soil concentration is presented in

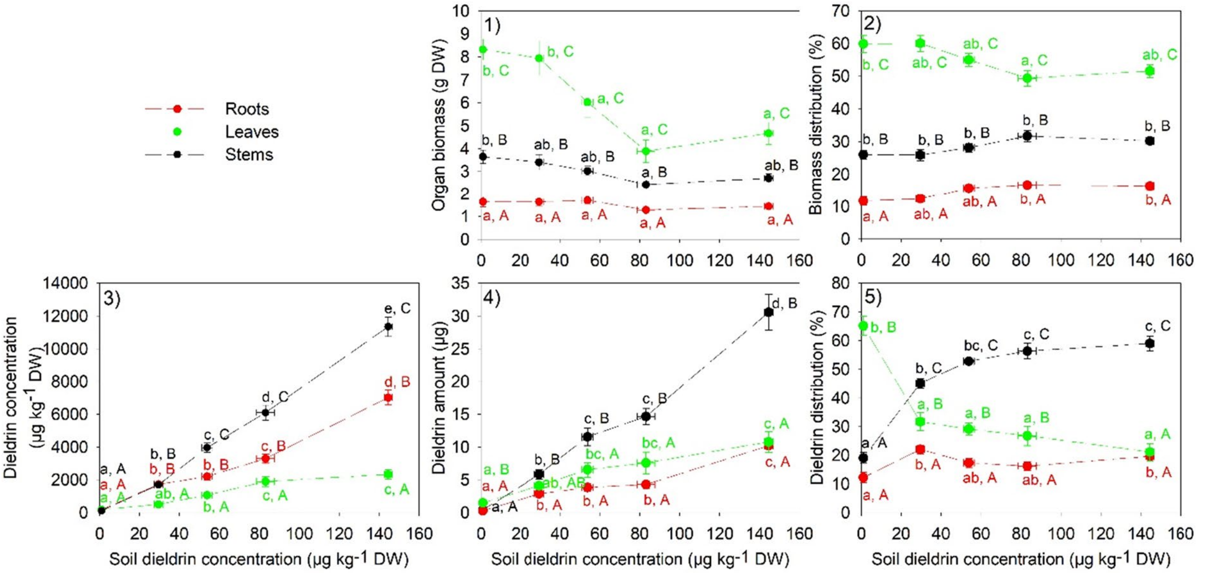

Fig. 2 Biomass (1), biomass distribution (2), dieldrin concentration (3), dieldrin amount (4) and dieldrin distribution (5) in the vegetative organs (roots, stems, leaves) according to soil dieldrin concentration. For each organ, different lowercase letters indicate significant difference in regards to soil dieldrin concentration. For each soil dieldrin concentration, different uppercase letters indicate significant difference in regards to the different organ. Error bars correspond to the standard error of the mean ($N = 5$)

Figs. 2-3. The dieldrin concentration in plant organs increased significantly with soil concentration and followed the order stems > roots > leaves. The corresponding concentrations in stems, roots and leaves ranged from 114 ± 9 to 11,345 ± 598 μg kg^{-1} (DW), from 163 ± 15 to 7017 ± 459 μg kg^{-1} (DW) and from 175 ± 20 to 2329 ± 291 μg kg^{-1} (DW), respectively, for the uncontaminated and the fully contaminated soil, respectively.

The Fig. 2-4 presents the amount of dieldrin accumulated in each vegetative organ according to soil concentration. It shows that the amount of dieldrin followed the same pattern in the different organs, increasing with soil contamination. However, due to organ biomass distribution, the amount of dieldrin is higher in stems > leaves > root, with averaged amount of dieldrin per plant increasing with soil concentration from 0.4 ± 0.1 to 30.6 ± 2.7 μg for stems, from 0.3 ± 0.1 to 10.2 ± 1.0 μg for roots, and from 1.5 ± 0.2 to 10.8 ± 1.6 μg for leaves.

Finally, the Fig. 2-5 shows the distribution of dieldrin in the vegetative organs as a function of soil concentration. First of all, the dieldrin was mainly (80%) located in the aerial vegetative organs (stems, leaves) of plants, independently of soil concentration. The mass distribution of dieldrin had similar pattern as dieldrin amount with stems > leaves > roots for plants growing on contaminated soil mixtures. In contrast, plants growing on the uncontaminated soil showed another distribution pattern, with leaves being the organ with the highest proportion of dieldrin (which remains low, cf. Fig. 2-4). The leaves accounted for 68 ± 3% of the dieldrin contained in the vegetative organs for uncontaminated soil while this proportion significantly decreased to 27 ± 2% for contaminated soils. The opposite behavior is observed for stems, with dieldrin proportion increasing from 20 ± 2% for uncontaminated soil to 59 ± 3% for most contaminated soil mixture. The roots showed less variation and accounted for 12 ± 1% to 19 ± 2% for uncontaminated and most contaminated soil, respectively.

Reproductive organs

The Fig. 3 presents the biomass, the dieldrin concentration and amount for the reproductive organs of plants harvested at 90 d in function of dieldrin concentration in soil. During this period, 3 to 6 fruits were harvested for each level of soil contamination, leading to a total of 24 fruits. The Fig. 3-1 shows that biomasses were not significantly different ($p = 0.62$) for fruits according to soil concentration with an average biomass of 11 ± 1 g (DW) per fruit. This is directly related to the fruit harvest criteria. Concerning flowers, the biomasses harvested were between 0.1 and 0.3 g (DW).

The concentration of dieldrin in fruits and flowers increased with dieldrin contamination and then stabilised or decreased, respectively, when the soil dieldrin concentration was equal or greater than 54 μg kg^{-1} (Fig. 3-2). For the fruits, the dieldrin concentration ranged from 198 ± 50 to 789 ± 199 μg kg^{-1} (DW), which was below the EU MRL for zucchini (1000 μg kg^{-1} DW). Concerning flowers, the dieldrin concentration ranged from 218 to 1085 μg kg^{-1} (DW). To the author's knowledge, there is no MRL for the flower even if it is an edible organ.

Consequently, the amount of dieldrin in fruits increased and then stabilized according to soil concentration (Fig. 3-3), with 2.4 ± 0.6 μg of dieldrin per fruits for uncontaminated soil and up to 7.5 ± 1.3 μg per fruits growing on soil with higher dieldrin concentration. The amount of dieldrin in flowers were very low, between 0.1 and 0.2 μg.

BCF of C. pepo L. 'Allegria'

The BCFs of the different organs of *C. pepo* L. 'Allegria' according to soil concentration are presented in Fig. 4. Concerning the vegetative organs, the two-way ANOVA (testing the effect of organ type, soil contamination level and their interaction) showed that BCF variation was first explained by the organ type ($\eta^2 = 0.85$, $F = 214.8$; $p < 0.001$) with a mean of 70 ± 3, 47 ± 2 and 19 ± 1 for stems, roots and leaves, respectively.

Concerning the BCFs of the reproductive organs, they decreased with soil contamination, reflecting the stabilisation of the concentration in these organs when dieldrin concentration in soil exceeded 54 μg kg^{-1}. Thus, no averaged BCF was calculated for these organs. Nonetheless, a significant linear relationship was established between the fruits and flowers BCFs. This was not the case for the other organs. However, few data were collected here and many more would be required to confirm these trends.

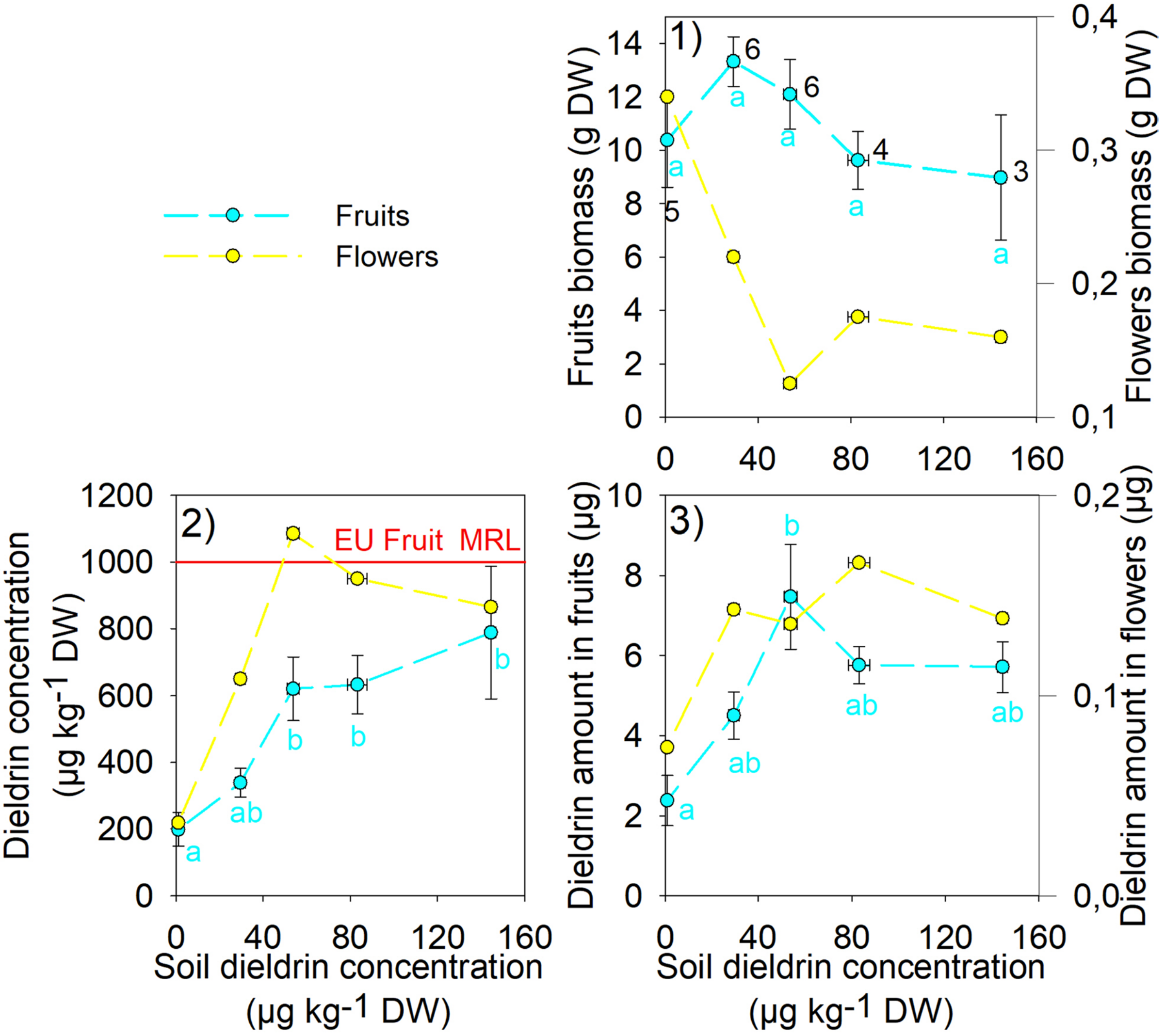

Fig. 3 Biomass (1), dieldrin concentration (2) and dieldrin amount per fruit (3) in the reproductive organs (fruits and flowers) according to soil dieldrin concentration. Different lowercase letters indicate significant difference in regards to soil dieldrin concentration. Error bars correspond to the standard error of the mean for fruits ($N=1$ for flowers). The number of harvested fruits per soil mixture is indicated in (1) and the EU dieldrin MRL for zucchini is indicated in (2)

Influence of plant development on dieldrin distribution

The Fig. 5 presents the results obtained on vegetative organs for plants grown in pots with soil presenting the highest dieldrin concentration level (145±2 µg kg^{-1} DW) and harvested at 30, 90 and 140 d. As expected, the biomasses (DW) of vegetative organs of *C. pepo* L. ‘Allegria’ increased significantly with plant development (Fig. 5-1). These biomasses were between 2.8±0.5 and 11.6±0.7 g for leaves, 0.4±0.1 and 3.7±0.1 g for stems, and 0.7±0.1 and 2.1±0.1 g for roots.

The Fig. 5-2 shows that vegetative organ mass fractions were similar throughout the plant’s life with the following order: leaves > stems > roots, except for small plants (30 d) for which root fractions were higher than stem fractions. The leaves mass fraction showed a minimum (52±2%) after 90 d of growth and reversely, stem mass fractions showed a maximum (31±1%) after 90 d of growth. The proportion

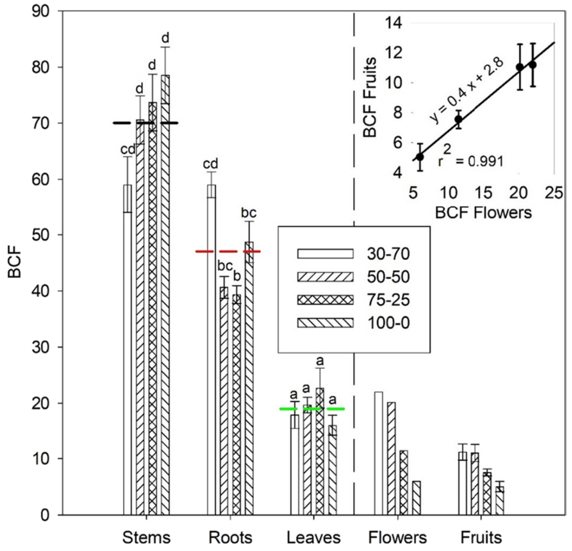

Fig. 4 Bio-concentration factor (BCF) of vegetative (left) and reproductive (right) organs of C. pepo "Allegria" and BCF relationship for reproductive organ (top right). Means ± standard error of the mean are shown ($N=5$ for roots, stems and leaves, $N=1$ for flower and $3 \leq N \leq 6$ for fruits). Different lowercase letters indicate significant differences between different groups of BCF values. Long-dashed lines indicate average BCF value for each vegetative organ

of roots significantly decreased with plant development from 18 ± 1 to 12 ± 1%.

The dieldrin concentration (µg kg^{-1}, DW) in organs in function of plant development is presented in Fig. 5-3. The concentration of dieldrin was always higher in stems (10,614 ± 596 µg kg^{-1}) followed by roots (4455 ± 326 µg kg^{-1}) and finally leaves (2671 ± 228 µg kg^{-1}). The dieldrin concentration in leaves did not present any significant evolution ($p=0.50$) with plant development. In stems, dieldrin concentration significantly increased from 5970 ± 765 µg kg^{-1} to a stabilised concentration of 11,233 ± 478 µg kg^{-1} after 90 d. The concentration of dieldrin in roots was significantly higher after 90 d (7017 ± 459 µg kg^{-1}) than after 30 and 140 d (3743 ± 166 µg kg^{-1}).

The amount of dieldrin in each organ (product of Fig. 5-1 and 5-3) is shown in Fig. 5-4. It increased significantly for leaves and stems during plant development from 7.8 ± 0.9 to 29.6 ± 5.1 and 2.3 ± 0.4 to 41.2 ± 2.5 µg, respectively. For roots, dieldrin amount increased significantly from 2.8 ± 0.4 to 10.2 ± 1.0 µg for plants harvested at 30 and 90 d, and then stabilized between 90 and 140 d.

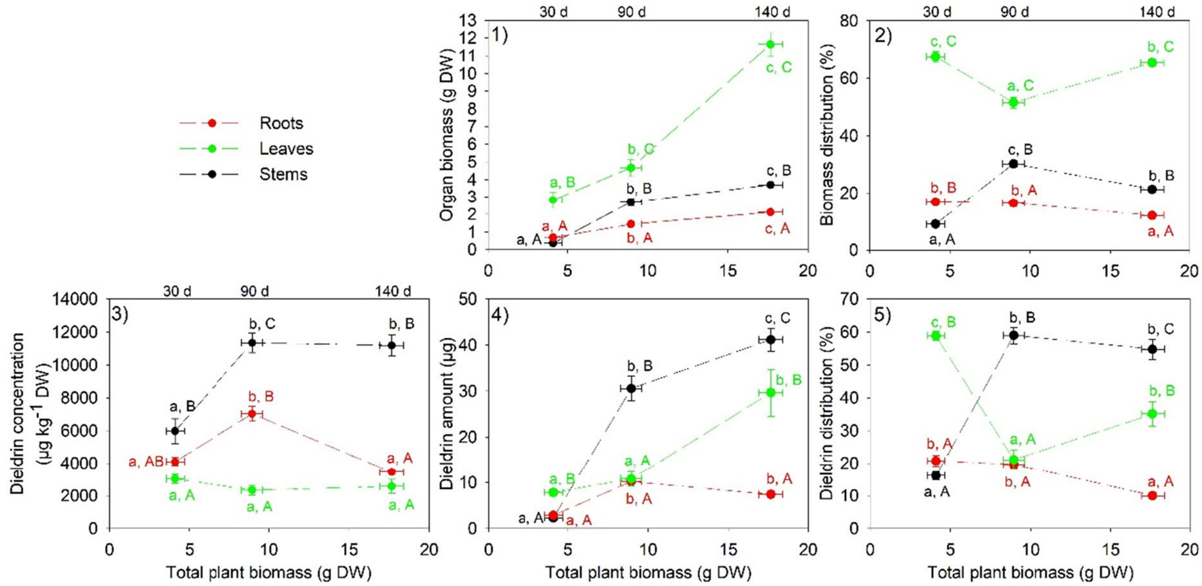

Fig. 5 Biomass (1), biomass distribution (2), dieldrin concentration (3), dieldrin amount (4) and dieldrin distribution (5) in the vegetative organs (roots, stems, leaves) according to total plant biomass. For each organ, different lowercase letters indicate significant difference in regards to soil dieldrin concentration. For each soil dieldrin concentration, different uppercase letters indicate significant difference in regards to the different organ. Error bars correspond to the standard error of the mean ($N=5$)

Finally, the dieldrin distribution in the vegetative organs with plant development is presented in Fig. 5-5. At 30 d, the leaves were the highest contributor (62 ± 2%) of dieldrin compared to roots (22 ± 2%) and stems (17 ± 1%). However, at 90 and 140 d of growth, stems became the most dieldrin-accumulating vegetative organ (59 ± 3 and 55 ± 3%, respectively), followed by leaves (21 ± 3 and 35 ± 4%, respectively) and finally roots (22 ± 2 and 10 ± 1%, respectively). Overall, during all the experiments, dieldrin was mainly (80%) accumulated in the above ground vegetative organs of the plants, with the leaves being the most accumulating organ for small plants (30 d) and the stems for bigger plants (90 and 140 d).

Discussion

Transfer path of dieldrin to *C. pepo* L.

Even when grown on the uncontaminated soil, all organs of *C. pepo* L. 'Allegria' showed low but quantifiable dieldrin concentrations (Figs. 2-3 and 3-2), with approximately the same values for the five organs (114 to 218 µg kg^{-1}). This is in accordance with studies conducted on *C. pepo* L. 'Black Beauty' grown on uncontaminated soil (< LOD ≈ 5 µg kg^{-1}), with leaves, stems, fruits and roots containing 40, 105, 140 and 380 µg kg^{-1} (DW) of chlordanes, respectively (Lee et al. 2003; Mattina et al. 2004). This was explained by aerial transfer which could be involved here too since the pots were disposed on the contaminated field. However, as the pots were buried here, a slight absorption of dieldrin from the surrounding soil by roots cannot be totally excluded even if high precautions were taken. Nonetheless, considering the difference of dieldrin concentration measured in the organs of the plants grown on contaminated soil, the dieldrin impregnation of *C. pepo* through root is clearly predominant in comparison to the aerial path, even if it cannot be totally excluded as observed for dichlorodiphenyltrichloroethan (DDT) (Whitfield Åslund et al. 2010).

Besides, the concentration of dieldrin in the roots was twice higher after 90 d than after 30 and 140 d (Fig. 5-3), while other studies showed a constant decrease as a function of plant growth with other OCs and *C. pepo* cultivars (Kelsey et al. 2006; Low et al. 2010; White et al. 2006a). This could be related to (i) a peak in root exudate production which could occurred between 30 and 90 d, increasing dieldrin absorption as presented by (White et al. 2006a), and/or (ii) the quality and quantity of roots which evolve with thick (older) roots of *C. pepo* L. being less contaminated than fine (younger) ones (Wang et al. 2004). Since it can be assumed that dieldrin is not degraded in *C. pepo* roots, the latter could be linked to (i) dilution phenomena occurring while roots become thicker and/or (ii) the transfer of dieldrin absorbed by roots to shoots while (iii) local soil source depletion regarding contaminant (Tester and Leigh 2001; Trapp 2002; Zhang et al. 2013). On the other hand, the modification with ageing of root water and nutrient uptake and/or the degeneration of individual cell layers inducing the decrease of contaminant uptake and/or its release back to surrounding soil cannot completely be excluded (Bingham 2007; Eissenstat et al. 2000; Liu et al. 2019; Schneider and Lynch 2018). More studies are necessary to elucidate this.

Allocation of dieldrin in vegetative and reproductive organs of *C. pepo*

The results of the study clearly highlighted differences of behavior between the reproductive and vegetative organs of *C. pepo* in response to dieldrin exposition. Indeed, while the vegetative organs showed an increase of contamination with soil dieldrin concentration, indicating a passive mechanism of dieldrin uptake (Briggs et al. 1982), flowers and fruits revealed a stabilisation of dieldrin concentration for soil dieldrin concentration equal or higher than 54 µg kg^{-1} (Figs. 2 and 3). These results are in accordance with the ones of Affholder et al. (2023) highlighting the low effect of soil dieldrin concentration on fruit contamination for soil concentrations ranging between 60 and 180 µg kg^{-1} DW. Besides, it appears a high relationship between flowers and fruits accumulation for which much lower dieldrin concentration than in vegetative organs were observed, except for leaves (Fig. 4). This suggested the same dieldrin impregnation path for both reproductive organs and might be related to their alimentation path. Indeed, reproductive organs are mainly fed by phloem sap (Kvesitadze et al. 2015; White 2011) which contains less contaminant than xylem sap, except in case of aerial contamination which may induce contaminant transfer to the phloem (Kvesitadze et al. 2015; Lee et al. 2003; Trapp and Legind 2011). On the other hand, since flowers and fruits are the furthest from the roots, dieldrin may have been sequestrated in vegetative organs before reaching the reproductive ones. Similarly, a decrease in OCP concentration with distance from the roots was observed for vegetative organs of *C. pepo* but barely for fruits (Affholder et al. 2023; Whitfield Åslund et al. 2010). This points out the need for more data to tackle the subject.

Concerning vegetative organs, the BCFs calculated here were much higher than those found in the literature for *C. pepo*. Indeed, the highest BCFs identified for *C. pepo* concerned Dichlorodiphenyldichloroethylene (DDE) with BCFs of 23 and 17 for root and stem, respectively, for *C. pepo* L. "Raven" cross "Zephyr" hybrid cultivar (Eevers et al. 2018; White 2010), in comparison to 47 and 70 for roots and stems, respectively, in this study. Furthermore, the BCF ranking of different OCPs and *C. pepo* cultivars found in the literature showed the following order: roots > stems > leaves > fruits (Chhikara et al. 2010; Isleyen et al. 2013; Kelsey et al. 2006; Mattina et al. 2000; White 2010; White et al. 2003b, 2006a, c) while they were ordered here as follows: stems > roots > leaves > flowers > fruit. A single higher BCF for stems than for roots was reported for Chlordecone in *C. pepo* L. 'F1 Darky' by (Clostre et al. 2014).

These differences can have several explanations. First of all, neither the molecule nor the extraction method used for its quantification in the different matrices were the same in the different studies. This has prime influence on BCF estimation because of contaminant bioavailability consideration, especially for the soil matrix. However, contrary to inorganic compounds, there is yet no standard extraction protocol established for organic contaminant bioavailability estimation in soils. Secondly, as for fruits, the uptake and translocation of OCPs in vegetative organs seem to depend on the Cucurbitaceous cultivar and OCPs properties (Affholder et al. 2023; Fujita et al. 2020; Mattina et al. 2006; White 2010; Zhang et al. 2017). Thirdly, the growth period considered in the other studies were different. However, even if the distribution of dieldrin in vegetative organs varied with plant development (Fig. 5-5), these variations were mainly due to the greater fluctuation in organ biomass during growth (e.g. 10-fold for stems between 30 and 140 d) than to the variation in concentrations (only 2-fold). Thus, higher BCF could be related to (i) the root exudates quantity and/or quality of the 'Allegria' cultivar which could be more efficient in desorbing dieldrin from soil particles and/or solubilising it in the pore water solution, leading to a greater absorption capacity (no studies have been identified); (ii) the production of different quantity and/or quality of MLPs, inducing different capacity of dieldrin transfer to shoots and/or (iii) the higher affinity of root exudates and MLPs to dieldrin, inducing higher accumulation in the different plant organs for this molecule.

The mechanism explaining the difference of dieldrin allocation require deeper investigations but some hypotheses can already be proposed: (i) since the contamination mainly transits from the roots to the stems through xylem vessels (Fujita and Inui 2021a; Garvin et al. 2015; Murano et al. 2010a), a difference in chemical composition in roots and stems cell walls (e.g. suberin, (Chen and Schnoor 2009) which have main transport and support function may involve a higher affinity for dieldrin; (ii) MLPs can be different between organs (Goto et al. 2019) and thus may not bind and/or unload dieldrin in the same way; (iii) MLPs binding dieldrin may have specific function in stems and/or be more present in this organ, involving higher accumulation there and leading thus to a residual amount translocated elsewhere (Goto et al. 2019) and/or; (iv) as the binding of MLP to hydrophobic contaminant seems to be regulated by pH (Fujita et al. 2023), variations of the latter could also participate to the allocation pattern. As the role of MLPs is largely suggested by the literature, further research quantifying MLPs and OCP in the different organs and cultivars of *C. pepo* L. are required to precise their influence on OCP allocation in Cucurbits.

Insights for dieldrin contaminated agricultural soil management

Nowadays, since it is common worldwide to randomly identify agricultural fields contaminated by dieldrin (Colin et al. 2022; Donnarumma et al. 2009; Fujita et al. 2023; Getenga et al. 2004; Hashimoto 2005; Meijer et al. 2001; Tsiantas et al. 2021), it is of main importance to identify the contaminated plots for eventual remediation. However, punctual or composite soil samplings may lead to undetected contaminated areas because of representativeness or dilution consideration, respectively. For this purpose, the analyses of *C. pepo* L. leaves and/or stems could be an efficient proxy since they would permit: (i) to increase the sensitivity of the detection, as their BCF is way higher than one (Fig. 4); (ii) to improve the relevancy of the measurement since it would correspond to the effective area investigated by the plant roots and provide a results taking the soil dieldrin bioavailability into account. Moreover, it would permit to decrease analyzing costs. Indeed, phytoscreening or other phytoforensics tools are known to provide rapid, cheap and ecologically friendly results because the sampling is fast, inexpensive and causes little or no discernible damage (Algreen et al. 2014; Burken et al. 2011; Limmer and Burken 2014). This could be helpful for regional or national assessment of agricultural soil contamination by dieldrin. Besides, considering the relationship between fruits and flowers contamination, the analyses of the latter might allow anticipating the production of contaminated fruits with dieldrin concentration above MRL. However, it is necessary to comfort the presented results taking into account other cultivars.

Being able to predict the amount of OCPs in plants by knowing their BCF and biomass would be an asset for phytoextraction purposes. Indeed, the results presented here highlight the need of conditions that favor the development of shoot biomass (Low et al. 2010) to extract a maximum of dieldrin in aerial parts. This is required in order to avoid the recovery of roots since it is much more complicated and they represent only a small quantity of dieldrin in the plant (< 20%), decreasing wih time (Figs. 2-5 and 5-5). Even if other studies found higher concentration of OCPs in roots than in others organs, the roots biomasses were very low and conducted to a higher or equal amount of OCPs in aerial part (Namiki et al. 2013; Wang et al. 2004; White 2002). Thus, the plant growth period could be optimized in order to enhance the capacity of *C. pepo* L. for dieldrin phytoextraction from soil. However, since these results come from pots experiment limiting plant development, they must be completed with field trials in order to check potential dilution phenomena. Moreover, testing other varieties would allow the selection of the most accumulative cultivars presenting the best concentration x biomass alternative. Nonetheless, the fate of contaminated harvested parts also need investigation to provide full answer for dieldrin contaminated soil management.

Conclusion

This study showed that the *C. pepo* L. variety 'Allegria' was able to absorb, translocate and accumulate dieldrin from historically contaminated agricultural soil. The results permitted to conclude about the predominance of root impregnation path in comparison to air contamination which could not be completely excluded. They also showed that dieldrin concentration in vegetative organs (stems, roots and leaves) increased almost linearly with the soil contamination level, indicating a passive mechanism of dieldrin uptake, while it plateaued in fruits and flowers for soil dieldrin concentration equal or higher than 54 µg kg^{-1} DW. This demonstrated the different behavior of dieldrin allocation for reproductive and vegetative organ. Besides, the results highlighted that the dieldrin amount in the vegetative organs depended more on their biomass than dieldrin concentration variation. The dieldrin BCF calculated for the vegetative organs were classified as follows: stems (70) > roots (47) > leaves (19), showing that *C. pepo* L. variety 'Allegria' could be a good proxy for the phytoscreening of agricultural plots historically contaminated with dieldrin as well as a good candidate for phytomanagement purposes. To this end, favoring the production of stems and leaves biomass would permit to maximize the extraction of dieldrin contamination from soil. Other studies are however required to clarify the influence of cultivar and OCP type on soil to plant transfer and allocation.

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The conceptualization, methodology, writing, editing and visualisation of the study were performed by F. COLIN and G. J. V. COHEN.

Material preparation, data collection and analysis were performed by F. COLIN, M-C. AFFHOLDER and G. J. V. COHEN.

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Declarations

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