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Dieldrin accumulation, distribution in plant parts and phytoextraction potential for several plant species and *Cucurbita pepo* varieties

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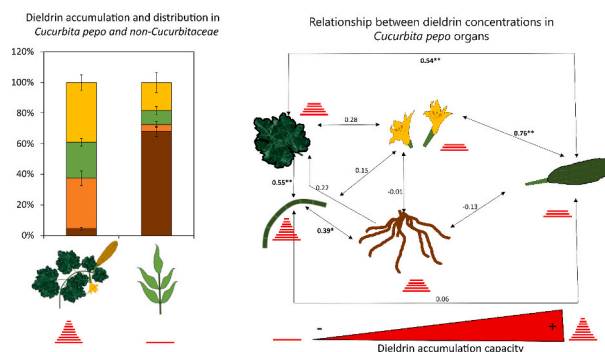
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HIGHLIGHTS

- *Cucurbita pepo* varieties accumulate high dieldrin amounts in their shoots.
- Productive *C. pepo* with low OCP in fruits ensure food safety and phytoextraction.
- Intraspecific variability in dieldrin allocation in *C. pepo* mainly concerns reproductive organs.
- Non-*Cucurbitaceae* barely translocate dieldrin to the shoots.
- Vetiver roots accumulate high dieldrin amounts.

GRAPHICAL ABSTRACT



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ABSTRACT

Dieldrin, an organochlorine pesticide (OCP) widely used for crop protection in the second half of the 20th century till the 70's, is worldwide still present in arable soils. It can be transferred to crops, notably cucurbits, depending on plant species and cultivars. Finding strategies to decrease OCP bioavailability in soil is therefore a main concern. Phytomanagement strategies could provide (i) ready-to-use short term solution for maintaining the production of edible plant parts with dieldrin concentrations below the Maximum Residue Limits (MRL) and (ii) long-term solution for dieldrin phytoextraction reducing progressively its bioavailability in the soil. This field study aimed at determining dieldrin accumulation capacities and allocation pattern in 17 non-*Cucurbitaceae* species and 10 *Cucurbita pepo* varieties, and assessing the dieldrin phytoextraction potential of these plant species when grown to maturity in a historically dieldrin-contaminated soil. Out of the non-*Cucurbitaceae* species, vetiver was the only one able to accumulate significant amounts of dieldrin, which mainly remained in its roots. All *C. pepo* varieties were able to uptake and translocate high dieldrin amounts into the shoots, leading to the highest phytoextraction potential. Despite the intraspecific variability in dieldrin concentration in zucchini plant parts, mainly in the reproductive organs, the phytoextraction capacity for shoots and fruits was high for all tested varieties (147 to 275 μg dieldrin plant^{-1} , corresponding to 5.6 % of the n-heptane extractable soil dieldrin), even

Abbreviations: DDT, dichlorodiphenyltrichloroethane; DW, dry weight; EU MRL, European Union's Maximum Residue Limit; LOQ, limit of quantification; NA, not applicable; OCP, organochlorine pesticide; POP, persistent organic pollutant; p,p'-DDE, p,p'-dichlorodiphenyl-2,2-dichloroethylene; SOM, soil organic matter.

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for the one with low fruit dieldrin concentration. Both food safety and phytoextraction could be achieved by selecting productive zucchini varieties displaying low dieldrin concentration in fruits and high one in shoots.

1. Introduction

Dieldrin is an organochlorine pesticide (OCP) belonging to the “dirty dozen”, a list of Persistent Organic Pollutants (POP) particularly harmful and remaining intact in the environment for long periods. It is widely distributed throughout the environment, accumulates in the fatty tissue of humans and wildlife, and is potentially toxic to humans and non-target wildlife (EFSA, 2007; Lallas, 2001; Matsumoto et al., 2009; Padayachee et al., 2023; Pang et al., 2022; Antignac et al., 2023). It was widely used to protect crops between the 50's and the 70's, replacing DDT, before being banned for agricultural use in France in 1972 (EFSA, 2007). Today, dieldrin is still present worldwide in arable soils and can be transferred to crops depending on plant species and cultivars (Fujita et al., 2023; Namiki et al., 2018; Otani et al., 2007; Singh and Singh, 2017). Dieldrin has low phytotoxicity, plants being generally affected only by application rates much higher than suggested use rates (Honeycutt and Shirley, 2014). The particularly high ability of cucurbits to uptake, accumulate and translocate OCPs, including dieldrin, can lead to the production of vegetables with concentration exceeding the European Union's Maximum Residue Limits (EU MRL) (Affholder et al., 2023; Mierzejewska-Sinner et al., 2024; Namiki et al., 2018; Official Journal of the European Union, 2008; Saito et al., 2012). As a result, such production must be destroyed and market gardeners face significant production constraints. Finding strategies to decrease OCP bioavailability in soil therefore matters.

Effective physico-chemical solutions for remediating OCP-contaminated soils have been reported on a laboratory scale (Castelo-Grande et al., 2010; Colin, 2022; Morillo and Villaverde, 2017). However, they are often difficult to implement at field scale, especially in the case of arable soils whose agronomic quality must be maintained. Besides, excavation followed by soil disposal is not a sustainable option given the dynamic decline in European farmland (Lyuri, 2008). In this case, phytomanagement strategies would be more appropriate. Indeed, exploiting intraspecific variation in OCP accumulation in edible parts of cucurbits could provide an interesting ready-to-use short term solution for maintaining the production of edible plant parts with dieldrin concentrations below the EU MRL (Affholder et al., 2023; Clostre et al., 2014; Donnarumma et al., 2009). However, this is not enough, as it may not be possible to do that for all plant species in the *Cucurbitaceae* family, which could moreover lead to reduce variety and species diversity. Thus, reducing OCP bioavailability in soil is essential to offer a long-term solution. Yearly phytoextraction of the bioavailable OCP fraction could be one way of achieving this. Indeed, the ability of *Cucurbitaceae* to uptake OCPs could be used for this purpose, as OCPs are significantly accumulated in their vegetative plant parts (Otani et al., 2007; Colin et al., 2024). Previous studies have also highlighted promising non-*Cucurbitaceae* species for OCP phytoextraction such as sunflower, castor bean, and chrysanthemum (Garba and Dauda, 2017; Mitton et al., 2016; Namiki et al., 2018; Rissato et al., 2015). However, the experiments were mainly carried out on a limited number of molecules, given the large number of OCPs and their specific behavior, with plants grown over short periods (Garba and Dauda, 2017; Namiki et al., 2018) and/or on spiked soils (Mitton et al., 2016; Namiki et al., 2018; Rissato et al., 2015), which is irrelevant given the ageing of OCPs in soil matrices. The frontiers in science indicate a lack of data on the potential for dieldrin phytoextraction by plants grown to maturity on historically OCP-contaminated farmland. Two major hypotheses require greater attention, namely (1) the existence of intraspecific variability in zucchini cultivars, and (2) the possibility of having other plant species that are equally or more effective in terms of dieldrin phytoextraction, particularly with a view to crop rotation and intercropping.

This field study, using large pots buried in the surface layer of the plot from which the soil was taken, aimed at (i) determining dieldrin accumulation capacities and allocation patterns in 17 non-*Cucurbitaceae* species and in 10 *Cucurbita pepo* varieties, and (ii) assessing the dieldrin phytoextraction potential of these plant species when grown to maturity in a historically dieldrin-contaminated market garden soil.

2. Material and methods

2.1. Plant selection and cultivation

2.1.1. Selection of plant species and *Cucurbita pepo* varieties

Non-*Cucurbitaceae* species were selected based on several criteria: i) their presumed ability to stimulate the dissipation or to accumulate OCPs and/or POPs according to the literature, ii) their agronomic interest (nitrogen fixation, natural nematicide effects, etc.), and iii) their capacity to release surfactants likely to solubilize dieldrin. The list was then submitted to local market gardeners to assess acceptance of the selected plant species. More details on the selection criteria for the non-*Cucurbitaceae* species are presented in the supplementary material (SI 1).

Concerning *Cucurbitaceae*, selection focused especially on *C. pepo* (10 varieties), as this crop is one of the most important for local market gardeners, enabling a wide range of phenotypic traits to be investigated.

Table 1 listed the 17 non-*Cucurbitaceae* species and 10 *C. pepo* varieties tested. Seeds were obtained from local suppliers and market gardeners.

2.1.2. Seedlings, transplantation in field and crop maintenance

Detailed information on cultivation practices, climate and soil

Table 1
Plant species and *C. pepo* varieties used for the experiment.

Botanical family	Plant species	Common name	Variety
<i>Cucurbitaceae</i>	<i>Cucurbita pepo</i>	Zucchini	Anissa
	<i>Cucurbita pepo</i>	Zucchini	Bélor
	<i>Cucurbita pepo</i>	Zucchini	Gold rush
	<i>Cucurbita pepo</i>	Zucchini	Kopana
	<i>Cucurbita pepo</i>	Zucchini	Mirza
	<i>Cucurbita pepo</i>	Zucchini	Noire maraîchère
	<i>Cucurbita pepo</i>	Zucchini	Ola escaladora
	<i>Cucurbita pepo</i>	Zucchini	Orélia
	<i>Cucurbita pepo</i>	Zucchini	Floridor
	<i>Cucurbita pepo</i>	Zucchini	Verte d'Italie
<i>Lamiaceae</i>	<i>Ocimum basilicum</i>	Basil	
<i>Apiaceae</i>	<i>Coriandrum sativum</i>	Coriander	
<i>Asteraceae</i>	<i>Chrysanthemum coronarium</i>	Chrysanthemum	
	<i>Tagetes patula</i>	French marigold	Red favourite
	<i>Helianthus annuus</i>	Sunflower	
<i>Malvaceae</i>	<i>Corchorus olitorius</i>	Jute mallow	
	<i>Abelmoschus esculentus</i>	Okra	
	<i>Lupinus albus</i>	Lupine	
<i>Fabaceae</i>	<i>Medicago sativa</i>	Alfalfa	
<i>Tropaeolaceae</i>	<i>Tropaeolum majus</i>	Nasturtium	
<i>Cannabaceae</i>	<i>Cannabis sativa</i>	Hemp	Dioïca 88
<i>Linaceae</i>	<i>Linum usitatissimum</i>	Flax	
<i>Poaceae</i>	<i>Zea mays</i>	Maize	Golden Bentam
	<i>Chrysopogon zizanioides</i>	Vetiver	
	<i>Chenopodium quinoa</i>	Quinoa	
<i>Euphorbiaceae</i>	<i>Ricinus communis</i>	Castor bean	Impala
<i>Solanaceae</i>	<i>Nicotiana tabacum</i>	Tobacco	Badischer Geudertheimer BaG

conditions were reported in Affholder et al. (2023). Briefly, crops were grown during spring/summer 2021 in a field located in the Bordeaux conurbation (France) with historical dieldrin contamination. The plot has a sandy soil classified as Umbrisol (Food and Agriculture Organization of the United Nations, 2015) with a neutral soil pH (6.8), high organic matter content (9.7 %), high cation exchange capacity for a sandy texture ($27 \text{ cmol}^+ \text{ kg}^{-1}$), and an average dieldrin concentration of $123 \pm 32 \mu\text{g kg}^{-1}$ (0–30 cm depth).

Sowing was carried out in an uncontaminated substrate (Motte Bio 20, Proveen Substrates) for all tested species except for vetiver. For the latter, 4-month-old plantlets were prepared by vegetative propagation using tillers from tufts. The plantlets were grown in a greenhouse for one month before being transplanted to the field as follows. For each plant, the soil was sampled using a stainless steel spade in the 0–30 cm soil layer, sieved (1 cm), and homogenized on site before being introduced into a 25 L plastic pot. This was to determine exactly how much dieldrin each plant's roots were exposed to, and to account for its potential spatial variability in the soil. For each pot, soil samples (about 90 g) were collected after homogenization for dieldrin quantification (results for each pot in SI 2). Four plant replicates were set up in individual pots for the 27 tested species/cultivars. The pots were randomly placed 1 m apart in the field from which the topsoil was taken. They were buried in the topsoil at two-third of their height to quench the potential heating of the below-ground plant parts and were regularly inspected to cut any roots that might emerge from the drainage holes. Depending on climatic conditions, particularly rainfall, crops were watered two or three times a week with local groundwater (the dieldrin concentration in the well water was below $0.1 \mu\text{g L}^{-1}$). Once a week, the crops were fertilized with an organic fertilizer (Bio-grow, Biobizz®), with NPK values of 4–3–6 (%), diluted at 1:1000 (v:v) with local groundwater.

2.1.3. Crop harvest and sample preparation

Cucurbita pepo fruits (zucchini) produced throughout the cultivation period were harvested when they reached commercial size (15–20 cm in length). Plants were harvested 3 to 3.5 months after transplanting, at maturity (seed production) or at the first signs of senescence. Back in the laboratory, stems, leaves, flowers, and roots were separated, thoroughly washed with tap water to remove all the soil particles and air-dried at 30°C for a week in an oven.

2.2. Dieldrin analysis in soil, plants and fruits

For soil samples, dieldrin was analyzed after extraction with n-heptane (n-heptane, 99+ %, residues analysis, ACROS Organic, UK) according to the protocol described in Colin et al. (2022). Dieldrin quantification in individual fruits was performed with n-heptane according to the protocol developed by Affholder et al. (2023). The other plant organs were analyzed following the protocol described in Colin et al. (2024). Shortly, dried plant samples were ground to a homogeneous powder using a knife mill (Pulverisette 11, Fritsch), and 48 h solid-liquid extractions were performed with n-heptane. Supernatant was recovered after centrifugation and placed in vial. Prior analysis, 50 ng of phenanthrene d-10 (Restek, France), used as internal standard, were added to the sample. Analyses were carried out by gas chromatography-mass spectrophotometer (GC-MS) according to Colin et al. (2022). The full protocols for determining the dieldrin concentration in each matrix, sample conditioning, the GC-MS analytical method and their validation for determining OCP concentrations are presented in SI 3, 4 and 5.

2.3. Calculations and statistical analysis

To compare results from different plant parts, fruit weight and dieldrin concentration were expressed as Dry Weight (DW). As *C. pepo* fruits contain 95 % of water (Rana, 2018), fresh biomass and dieldrin concentration were multiplied and divided by 0.05, respectively.

For establishing a relationship between the dieldrin concentration in the soil and that in the various plant parts, the Bio-Concentration Factor (BCF) was computed for each plant part of each harvested plant. This parameter allows (i) a rapid overview of contaminant accumulation in the plant parts and (ii) a data comparison with the literature, both independently of changes in soil concentration. They were calculated as follows (Mattina et al., 2003):

$$BCF_{Organ} = C_{Organ} / C_{Soil} \quad (1)$$

with C_{Organ} and C_{Soil} being the dieldrin concentrations measured in the plant organ and in the soil, respectively.

Statistical analyses were performed using the R open source software environment (R Core Team, 2021) and functions available in the 'rstatix' and 'agricolae' packages (Alboukadel, 2021; De Mandiburu, 2021). As the data distributions were not normal, mean value comparisons were made with the non-parametric Kruskal-Wallis test. For multiple comparisons, the post hoc test (Fisher's least significant difference) was performed and *p*-value was adjusted with the Benjamini & Hochberg correction. Relationships between dieldrin concentrations in the plant parts and other parameters such as soil dieldrin concentration, were determined using Spearman's rank correlation tests, assessing monotonic relationships, which can be linear or not.

The dieldrin amount extracted by each plant organ and the whole plant were calculated by multiplying the dieldrin concentration in each plant organ by its biomass. The results were compared to the extractable amount of dieldrin contained in the soil for each pot, given the n-heptane extraction method carried out on the soil mentioned above.

3. Results and discussion

3.1. Biomasses

High phytoextraction capacities require plant species able to produce significant harvestable biomass with relatively high contaminant concentration, and being tolerant to other stress. Shoot, root and total DW yields of the plants tested are presented in Fig. 1 and details for all organs are presented in SI 6. No visible symptoms were detected on the above-ground parts of the plants. For *C. pepo*, mean values for the biomass of vegetative shoots, reproductive parts (fruits and flowers), and roots, and for total plant biomass were respectively 46.1 ± 28.8 , 102.9 ± 59.9 , 3.2 ± 1.7 and $150.9 \pm 64.7 \text{ g DW plant}^{-1}$. Such mean values reached 48.4 ± 39.5 , 22.9 ± 27.9 , 20.9 ± 27.9 and $87.8 \pm 69.9 \text{ g DW plant}^{-1}$ for non-*Cucurbitaceae* plant species.

Biomasses significantly differed between *C. pepo* and non-*Cucurbitaceae* plants with higher root biomass and lower shoot biomass on average, as well as greater variability for the latter. However, some non-*Cucurbitaceae* species have produced a shoot biomass equivalent to that of *C. pepo*, i.e. sunflower, vetiver, chrysanthemum, maize, hemp, and French marigold (Fig. 1). In average, 69 % of *C. pepo* shoot biomass was constituted by the fruit biomass, while for non-*Cucurbitaceae*, 68 % of the total shoot biomass consisted of above-ground vegetative biomass. For all the plant species, except alfalfa, shoot DW yields were higher than the root DW yields.

3.2. Dieldrin concentration and distribution in the plant parts

Dieldrin concentrations measured in the plant parts of *C. pepo* and non-*Cucurbitaceae* species are presented in Fig. 2. Mean values for dieldrin concentrations in *C. pepo* were significantly higher than those in non-*Cucurbitaceae* species for all organs (roots, stems, leaves, flower/grains, and fruits), particularly for the shoots.

For *C. pepo*, dieldrin concentration in the organs followed the decreasing order: stems > roots >> leaves > fruits > flowers, which agreed with data previously reported for the Velvia and Allegría varieties of *C. pepo* harvested when the edible plant parts have reached a

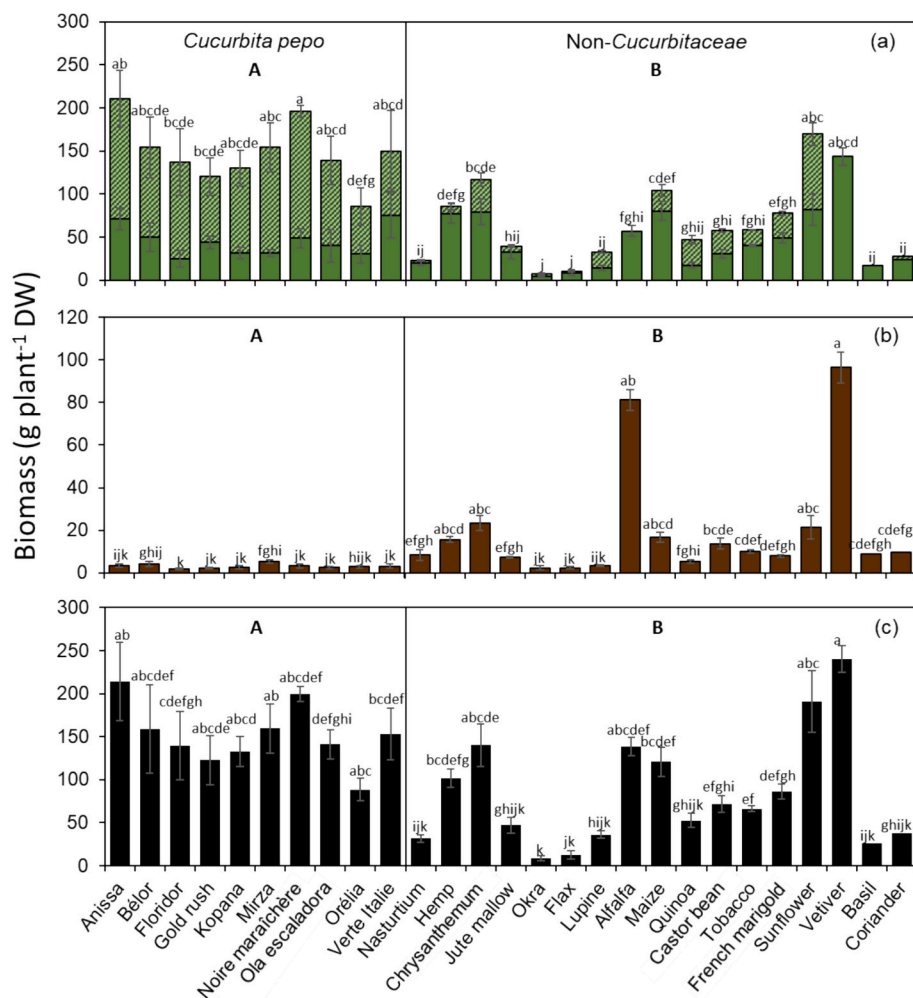


Fig. 1. (a) shoot, with reproductive biomass (fruits and flowers) hatched, (b) root and (c) total biomass for the *Cucurbita pepo* varieties (left) and non-Cucurbitaceae species (right). Mean value \pm SE (n = 4) in g DW plant⁻¹. For a same panel, different letters on the bars show significant differences (Kruskal-Wallis, $p < 0.05$).

marketable size (Donnarumma et al., 2009; Colin et al., 2024). The mean dieldrin concentrations (in $\mu\text{g kg}^{-1}$ DW) ranged between 5583 ± 1080 and 9680 ± 1087 for the stems, 2420 ± 437 and 5132 ± 497 for the roots, 960 ± 181 and 2368 ± 167 for the leaves, 107 ± 33 and 1537 ± 145 for the fruits, and 162 ± 16 and 779 ± 73 for the flowers. These results highlighted lower dieldrin concentration in reproductive plant parts than in the vegetative ones as shown in Colin et al. (2024). In addition, intraspecific variability in dieldrin concentration for *C. pepo* mainly concerned fruits and flowers. Depending on *C. pepo* varieties, there was a factor of five to 15 for dieldrin concentrations in flowers and fruits, respectively, while only a factor of two to three occurred for the vegetative organs. Consequently, low dieldrin concentrations in fruits and flowers would be more likely due to a specific dieldrin allocation in the reproductive organs than to an ability to uptake or translocate dieldrin to the shoots.

Dieldrin concentration in non-Cucurbitaceae species followed the decreasing order: roots \gg leaves $>$ flowers/seeds $>$ stems, with mean concentrations (in $\mu\text{g kg}^{-1}$ DW) ranging between 313 ± 118 and 3037 ± 248 in the roots, $<$ Limit of Quantification (LOQ) and $429 \pm \text{NA}$ in the leaves, $<$ LOQ and $561 \pm \text{NA}$ in flowers/seeds, and $<$ LOQ and 415 ± 62 in the stems. Except for Coriander, all the non-Cucurbitaceae species had significantly lower dieldrin concentrations in their leaves and flowers/seeds than in those of *C. pepo*. However, several non-Cucurbitaceae species (i.e. chrysanthemum, vetiver, jute mallow, French marigold, basil, and coriander) presented dieldrin concentrations in roots similar

to the ones measured in *C. pepo*'s roots, which was in line with previous results (Murano et al., 2010; Otani et al., 2007). These results confirmed that some non-Cucurbitaceae species were able to uptake dieldrin. Here, only *C. pepo* out of the tested plant species displayed the ability to highly translocate dieldrin into the above-ground organs (Translocation factors in SI 7). However, it cannot be ruled out that other Cucurbitaceae species, such as the ones tested by Affholder et al. (2023) (i.e. *C. sativus*, *C. maxima*, *C. moschata*, etc.), would show the same ability.

Dieldrin distribution in roots, stems, leaves, and flowers/seeds for non-Cucurbitaceae species and in fruits for *C. pepo*, in percentage of total dieldrin amount in the whole plant are presented in Fig. 3. In *C. pepo*, dieldrin was mostly stored in the above-ground organs. Indeed, in average 95.3 ± 0.8 % of dieldrin was accumulated in the above-ground plant parts, with in average 38.9 ± 5.2 % contained in the fruits, 33.7 ± 4.9 % in the stems, and 24.0 ± 2.5 % in the leaves. This matched with previous results showing that in *C. pepo* p,p'-DDE was mainly stored in the shoots (99 % and 86 % in Wang et al. (2004) and White (2002), respectively) and a similar distribution pattern with 17 %, 31 % and 51 % in the stems, leaves and fruits, respectively, was reported by Wang et al. (2004). However, due to intraspecific variability, the latter can significantly vary depending on *C. pepo* cultivar (Affholder et al., 2023). Moreover, as our trials were carried out with outdoor pots placed in the field (to better determine the exposure of each individual plant in an identical soil volume), field-grown plants may be able to explore a larger soil volume, modifying these proportions. For the non-Cucurbitaceae

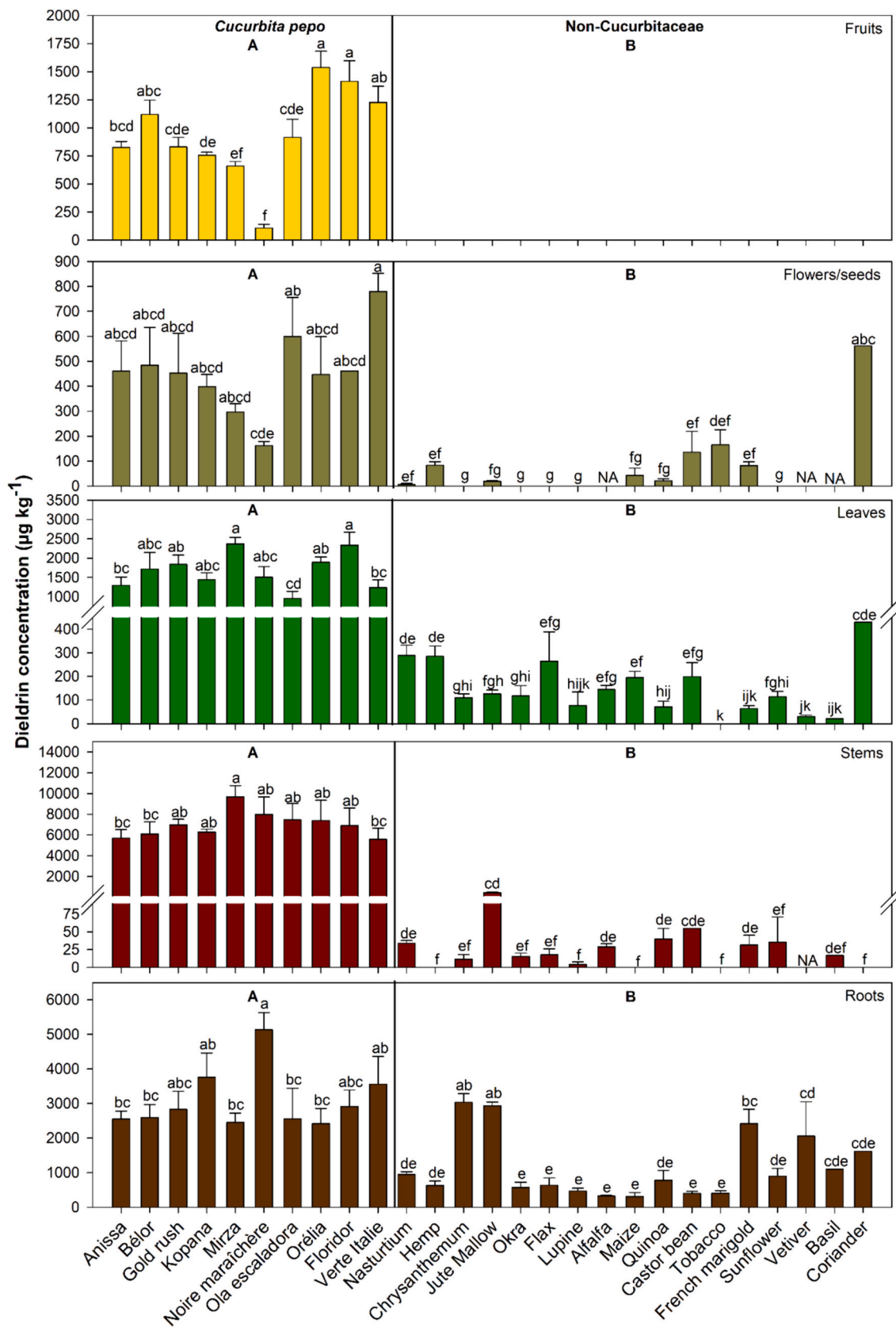


Fig. 2. dieldrin concentration ($\mu\text{g kg}^{-1}$ DW) in zucchinis (fruits), in other reproductive parts (flowers/seeds), leaves, stems and roots of all the *C. pepo* varieties and non-Cucurbitaceae species. Mean value \pm SE ($n = 4$). For a same panel, different letters on the bars indicated significant differences (Kruskal–Wallis, $p < 0.05$).

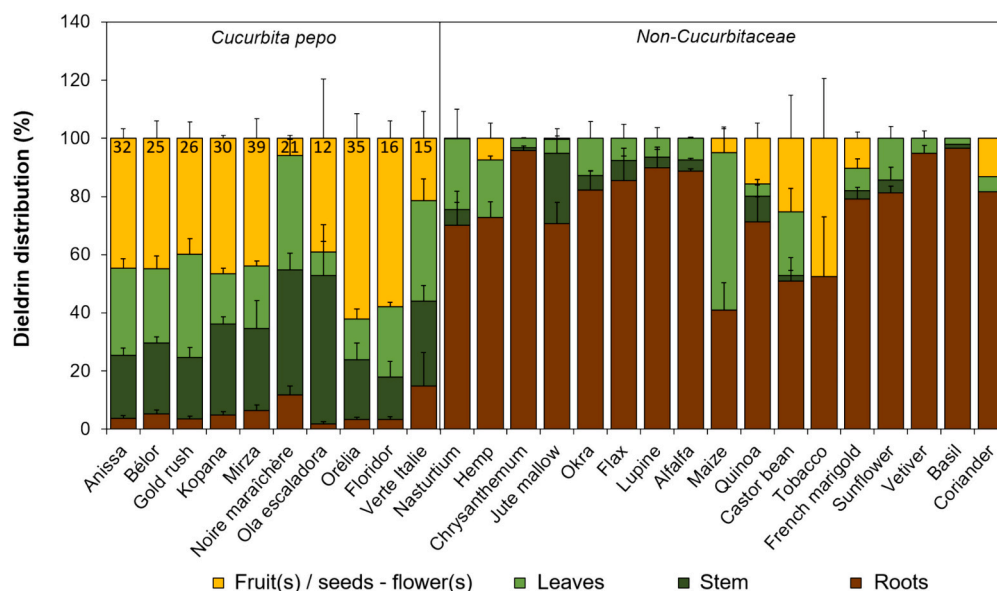


Fig. 3. Dieldrin distribution in the different organs of *C. pepo* varieties and non-Cucurbitaceae species in percent of total dieldrin taken up. Mean value \pm SE ($n = 4$, except for *C. pepo* fruits for which n is indicated in the bars).

species, the opposite was found, with over 70 % of dieldrin stored in the roots, except for maize, castor bean and tobacco, where around 50 % of dieldrin was stored in the shoots. This partly agreed with previous results showing that *Chrysanthemum coronarium* grown in dieldrin-spiked soils accumulated 99 % of dieldrin in the roots (Namiki et al., 2018). However, maize in this latest study stored >90 % of dieldrin in the shoots, which was not the case here. Differences in dieldrin distribution in maize between both studies could be related to the varieties tested (Gold dent in Namiki et al. (2018) and Golden bantam here), the exposure time, and dieldrin bioavailability due to the use of spiked soil versus aged soil contamination with chronic applications of insecticides. Some plant species transferred a relatively higher percentage of dieldrin into their fruits and/or seeds (Fig. 3): tobacco, castor beans > coriander, quinoa and French marigold > hemp and maize.

Cucurbitaceae are known to produce proteins, called major latex like proteins (MLPs) transporting OCPs toward above-ground plant parts (Fujita and Inui, 2021), which could partly explain the differences for dieldrin distribution between *C. pepo* and non-*Cucurbitaceae* plants tested. Although MLPs have been identified in dicots and monocots as they have several functions (resistance to diseases, stress tolerance, etc., Fujita and Inui, 2021), the MLPs produced by *Cucurbitaceae* have a specific structure with a hydrophobic cavity, allowing lipophilic molecule transportation (Fujita et al., 2022). By suppressing these protein expression (Fujita et al., 2020a) or inhibiting the dieldrin-MLP binding through competition with other molecules (Fujita et al., 2020b), uptake and translocation of dieldrin in the xylem of *C. pepo* were reduced. Moreover, expression of zucchini MLP genes in tobacco enhanced the translocation of hydrophobic pollutants in the transgenic plants: for instance, an increasing concentration of one polychlorinated biphenyl was determined in the xylem sap (Stoykova and Inui, 2021).

To highlight the existence of a ubiquitous dieldrin distribution pattern among *C. pepo* varieties, bioconcentration factors (BCF) for organs were compared (BCF values for all plant species in SI 8). By using the BCF value instead of dieldrin concentrations in plant parts, small differences that might result from small changes in soil dieldrin concentrations across the pots can be quenched. No common trends were identified in the relationships between dieldrin concentrations in soil and organs, which could be explained by the narrow soil concentration range (61 to 82 $\mu\text{g kg}^{-1}$). Indeed, soil dieldrin concentration can influence the dieldrin concentration for some organs and zucchini varieties (SI 9) as highlighted by Mattina et al. (2004) and Colin et al. (2024) who

evidenced a linear relationship between roots, stems and leaves and soil concentrations ranging between <5 and 4600 $\mu\text{g kg}^{-1}$ of chlordane, and between 0 and 160 $\mu\text{g kg}^{-1}$ of dieldrin, respectively (gradient obtained using the fading method for both studies). Significant correlations occurred between dieldrin BCF in several *C. pepo* organs (Fig. 4, SI 10). A positive correlation ($r = 0.75$, $p < 0.001$) was found between flowers and fruits, suggesting that dieldrin supply to reproductive organs remained more or less consistent over time (no dieldrin dilution in the fruit despite a longer growth period and greater biomass than flowers). For vegetative organs, positive correlations were evidenced between root and stem BCF values ($r = 0.39$, $p < 0.05$) and between stem and leaf ones ($r = 0.55$, $p < 0.001$). A significant correlation between the BCF values of leaves and fruits was also determined ($r = 0.54$, $p < 0.01$). This could suggest that dieldrin supply to fruits does not come directly from the roots, but from dieldrin mainly located in the leaves, or that dieldrin supply to leaves and fruits follows the same path. Isotopic tracing methods would be useful to further assess that assumption. Correlations obtained with the *C. pepo* dataset (10 varieties) showed a common trend in dieldrin distribution patterns for this plant species, even though fruit accumulation capacities differed. The number of replicates ($n = 4$) per variety was too low to conclude on organ distribution patterns per variety. However, some trends were evidenced (SI 11) and dieldrin distribution slightly varied from one variety to another.

3.3. Phytoextraction potential

The dieldrin amounts extracted by the whole plant and the percentage of dieldrin extracted from the potted soils are presented in Fig. 5. *Cucurbita pepo* and vetiver accumulated the highest dieldrin amount with mean values ranging between 147.0 ± 33.6 and $275.0 \pm 86.4 \mu\text{g plant}^{-1}$, corresponding to between 4.1 ± 0.9 and 8.3 ± 2.7 % of the *n*-heptane-extractable soil dieldrin. For *C. pepo*, dieldrin was mainly located in the shoots while it was mainly (>90 %) present in the vetiver roots. As the essential oil made from vetiver roots is nowadays a key ingredient in the perfume and cosmetic industry (Burger et al., 2017), the potential presence of OCPs in extracted essential oils may matter (if the cultivated soil contains dieldrin and the roots are used for their essential oil). The other non-*Cucurbitaceae* species accumulated, on average, between two and 200 times less dieldrin than *C. pepo* and vetiver, with average dieldrin amounts ranging between 1.3 ± 0.5 and $73.4 \pm 10.8 \mu\text{g plant}^{-1}$ for Okra and Chrysanthemum, respectively. This

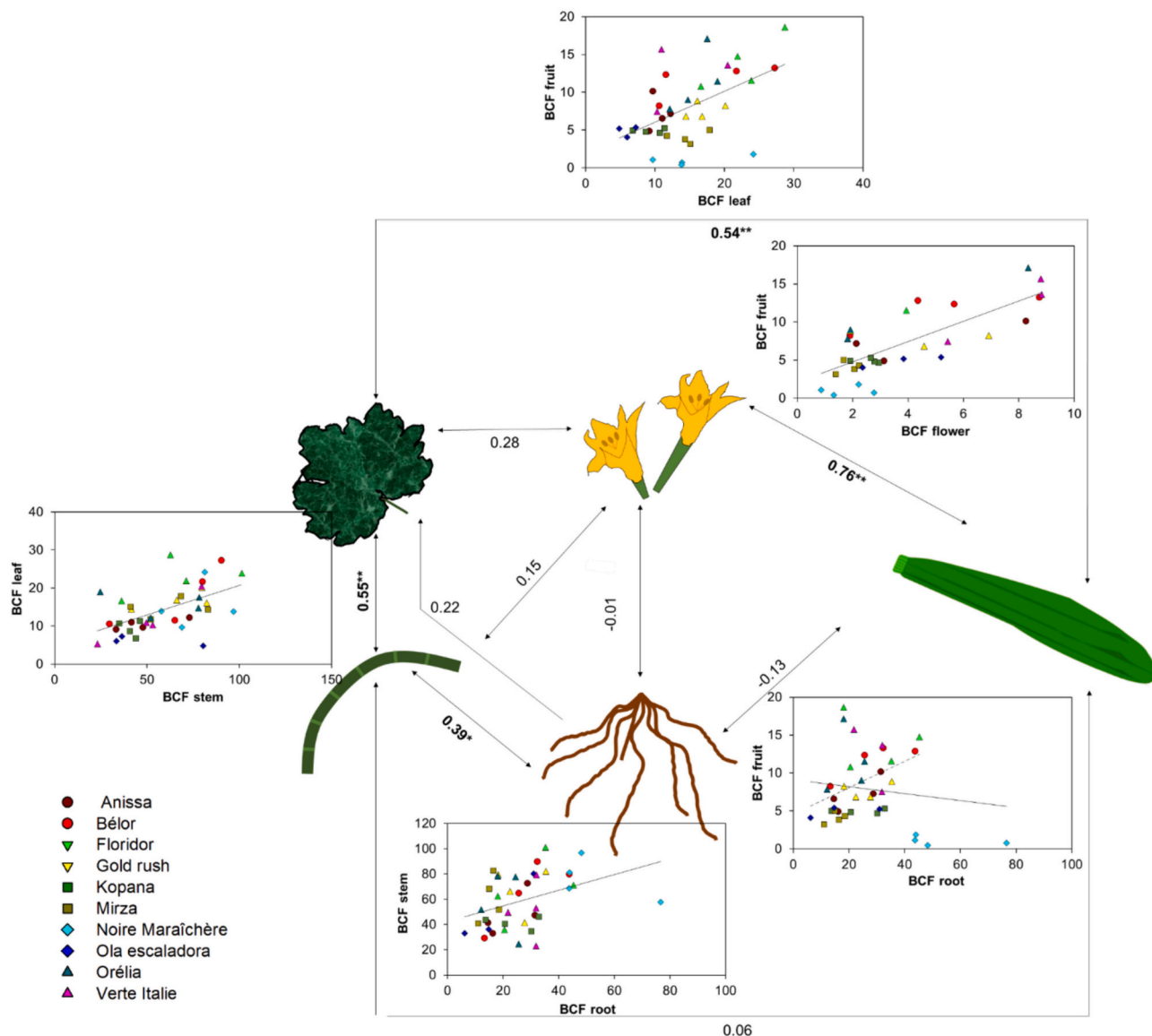


Fig. 4. Correlations coefficient (Spearman's rank correlation coefficient) for the dieldrin BCF values between *C. pepo* plant organs. Significant correlations are written in bold and corresponding plots are displayed. Significance levels: $p < 0.01$: **; $p < 0.05$: *; and not significant: ns.

represented between 0.04 ± 0.01 and 2.1 ± 0.4 % of the n-heptane-extractable dieldrin in the potted soil. In average, given the entire plant (shoots, including fruits, and roots) was harvested, 5.8 ± 0.6 % ($221 \pm 20 \mu\text{g plant}^{-1}$) of the extractable dieldrin contained in the potted soil was phytoextracted by *C. pepo*. With 5.6 ± 0.6 % ($211 \pm 20 \mu\text{g plant}^{-1}$) of the extractable soil dieldrin occurring in the shoots, most of the dieldrin amount phytoextracted (95 %) was recovered in the above-ground plant parts and distributed as follow: 60 % in the vegetative parts ($123 \pm 12 \mu\text{g plant}^{-1}$) and 40 % in the fruits ($89 \pm 11 \mu\text{g plant}^{-1}$) (details in SI 12). This agreed with previous studies on p,p'-DDE, showing that 90 % of this contaminant was accumulated in the shoots of *C. pepo* cv. Black Beauty (Wang et al., 2004). In practice, it is essentially the above-ground parts of zucchinis that should be exported by market gardeners to avoid dieldrin returning to the soil. Concerning the fruits, that count for a great part of the dieldrin phytoextracted, those from several zucchini varieties would still be marketable (i.e. mainly Noire maraîchère, Mirza, and Kopana, Affholder et al., 2023), while harvested

fruits from the more accumulative varieties would have to be destroyed. Therefore, it would be preferable for the market gardeners to grow a highly productive variety, such as Mirza, producing fruits with mean dieldrin concentration below the MRL and leading to dieldrin phytoextraction equivalent to the amount phytoextracted by varieties with high dieldrin concentration in fruits, such as Orélia (Fig. 5). Here, after one culture, difference between dieldrin concentrations in the potted soils before and after cultivation remained below the variability of dieldrin concentration in the potted soil (± 20 %, SI 13). Only the soil cultivated with *C. pepo* cv. Mirza showed a significant decrease in dieldrin concentration, despite it was not the zucchini variety extracting the highest dieldrin amount. This underlined the need for investigations over several crop rotations to determine the decrease in extractable soil dieldrin.

These data were consistent with White et al. (2003) reporting a rate of 2.53 % for the phytoextraction efficiency of aged p,p'-DDE by *C. pepo* (cv. Gold Rush) vegetative parts (against 3.60 ± 0.38 % here). In other

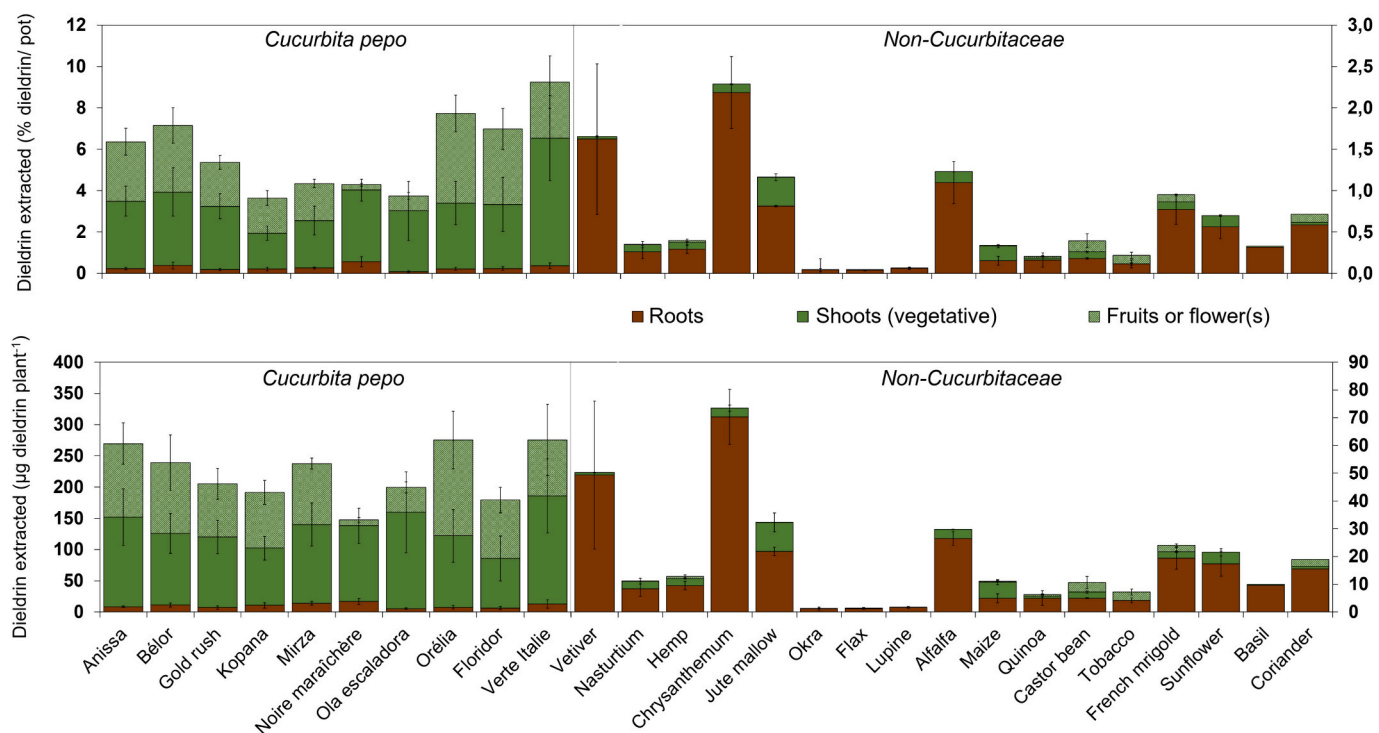


Fig. 5. Dieldrin extracted in % of dieldrin present in the potted soil (top) and dieldrin amount extracted (bottom) by the fruits or flowers (green striped bar graph), vegetative shoots (uniform green bar graph), and roots (brown bar graph) for the *C. pepo* varieties and the non-Cucurbitaceae species. Left scale for all the *C. pepo* varieties and vetiver, right scale for all the other non-Cucurbitaceae species. Mean value \pm SE ($n = 4$, except for *C. pepo* fruits for which n is indicated in Fig. 3).

studies, the percentage of OCP extracted by *C. pepo* was lower: the whole plant extracted between 0.19 % (cv. Black beauty, pot experiment, 50 to 80 d of cultivation) and 0.4 % (cv. Gold Rush, field trial, 28 d of cultivation) (Wang et al., 2004; White, 2002). For dieldrin phytoextraction by zucchini, one available data was obtained with only a short cultivation period: 20 μg dieldrin plant^{-1} was accumulated in shoots after 21 d of growth in a historically contaminated soil, while 120 μg dieldrin plant^{-1} was extracted when plants were grown in spiked sand (Otani et al., 2007). These differences are significant and require further discussion on several points before extrapolating these results to the field. Firstly, the extraction methods used to quantify OCPs in soil differ from one study to another. Unlike trace metals, there is as yet no standard method for quantifying the labile, bioavailable and (pseudo) total fractions of organic compounds such as OCPs in soil, the latter being irrelevant to risk assessment. This is a key point for assessing the proportion of available OCPs removed by plants. In addition, the labile fraction of organic xenobiotic compounds may coexist with natural organic constituents (e.g. soil organic matter (SOM)), inducing changes in availability expressed as tail and/or rebound effects in the remediation of soils with aged contamination. It is therefore not yet possible to accurately estimate the bioavailable dieldrin fraction in the soil and to assess how much of this fraction has been either removed by the plants or dissipated by the plant-microbiome-mesofauna interactions. However, as mentioned above, repeated planting on the same soil may help to answer this question. If the bioavailable fraction is greatly reduced, the second and/or third crops would have lower dieldrin concentrations. Previous researches on the repeated planting of *Cucurbitaceae* on soils historically contaminated with PCBs (Whitfield Åslund et al., 2008) and p,p'-DDE (Kelsey et al., 2006; White et al., 2003) however have shown the opposite and suggested that rather than being depleted, the bioavailable fraction was enhanced after one crop of *Cucurbitaceae*.

On the other hand, Kelsey et al. (2006) reported a significant decrease in p,p' DDE concentration in stems for the second and third crops of *C. pepo* ssp. *pepo* (pumpkin). It is therefore necessary to carry out long term field trials on historically contaminated soils, and to harvest

and analyze crops for monitoring OCP bioavailability in the soil, as well as the extraction capacity of plants. Plant removal is crucial in contaminated plots to prevent OCPs returning into the soil. Such OCP fraction included in plant residues undergoing biodegradation (including the actions of mesofauna and microbes) may be more easily available for root uptake by the following crops. In parallel, processes must be developed to treat or eliminate plant biomass that has accumulated dieldrin. Microbial degradation is one promising treatment method (Pang et al., 2022) but other techniques such as supercritical fluid extraction and/or thermal treatment could be appropriate (Colin, 2022). Nonetheless, the degradation products and their toxicity should be assessed for these disposal technologies.

Secondly, it would be relevant to test whether the dieldrin phytoextraction would be similar under field conditions with no constraints on root development. Here, *C. pepo* plants were cultivated in 25 L pots for having roots in a homogenized soil volume where the dieldrin concentration has been determined, but this may limit the plant growth. Fully field-grown zucchinis presenting a much higher reproductive and vegetative biomass could result in greater dieldrin phytoextraction, if their concentrations remain unchanged with increasing harvested biomass, as suggested by other studies, or in similar or lower phytoextraction efficiency if increasing biomass results in dilution. In both cases, dieldrin phytoextraction would be limited by OCP bioavailability in the soil volume explored by roots. Moving from pot (~100 L) to field conditions, Wang et al. (2004) demonstrated a two to three-fold decrease in p,p'-DDE concentration in zucchini (cv. Black Beauty) roots and leaves and an increase in fruit concentration, while they recorded a 7.5-, 30-, 19- and 140-fold increase in biomass for roots, stems, leaves and fruits, respectively. However, the contaminant accumulation with increasing biomass may depend on the contaminant as well as the plant species and varieties. Indeed, contradictory results are presented in the literature. Colin et al. (2024) and Low et al. (2010) reported no changes in dieldrin and PCB concentrations in roots and shoots of zucchini (cv. Allegría) and pumpkin (cv. Howden) plants, respectively. However, decrease in BCF values of p,p'-DDE for roots, stems and leaves, with an increasing

biomass due to increasing plant age (14, 21, and 28 days) was reported by Kelsey et al. (2006). The different behavior observed above highlight the need of further experiments to unravel the relationship between plant/organ biomasses as well as contaminant allocation and accumulation in regards to OCP bioavailability in soil.

4. Conclusion

Out of the non-*Cucurbitaceae* species cultivated in large pots placed under field conditions, vetiver was the only one able to accumulate significant amounts of dieldrin, which remained mainly in the roots. The production of a dense and extensive root system that can be used for essential oil production makes it an interesting candidate for dieldrin phytoextraction, if dieldrin is not recovered with lipophilic compounds or can be separated. For *C. pepo*, all varieties displayed the ability to uptake and translocate high dieldrin amounts in the shoots, leading to the highest phytoextraction potential whatever the variety considered. Despite intraspecific variability for dieldrin concentrations in the zucchini plant parts, the phytoextraction capacity determined for harvested shoots and fruits was high for all varieties (147 to 275 µg of dieldrin extracted per plant grown in the 25 L-pot, corresponding to 5.6 % of the n-heptane extractable soil dieldrin), even for the one with low dieldrin concentration in fruits. Therefore, by selecting highly productive zucchini varieties with low dieldrin concentration in fruits, both food safety and phytoextraction could be achieved for *C. pepo* cultivated in farmland with dieldrin-contaminated soils. Intraspecific differences in dieldrin concentration for zucchini plant parts mainly concerned reproductive organs and were limited for vegetative ones, meaning that dieldrin allocation in the flowers and fruits would be variety specific, but not related to root uptake capacity. A better understanding of the translocation of dieldrin and other organic contaminants in *C. pepo* fruits, including the search for molecular determinants such as MLPs, could lead to select varieties with low dieldrin concentration in fruits, enabling safe production of these cucurbits even under field conditions with chronic historical soil contamination.

CRedit authorship contribution statement

M.-C. Affholder: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **M. Mench:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **S. Gombert-Courvoisier:** Conceptualization. **G.J.V. Cohen:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.172968>.

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