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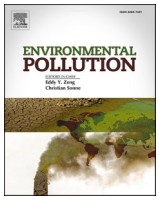

Fate of chlordecone in soil food webs in a banana agroecosystem in Martinique☆

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ABSTRACT

Large quantities of chlordecone-based insecticides were produced and used throughout the world. One of its most important uses was to control the damage caused by *Cosmopolites sordidus* in banana-growing regions. In the islands of Martinique and Guadeloupe, 18,000 ha of farmland are potentially contaminated. Despite the key role played by soil macrofauna in agroecosystems, there are currently no data on their contamination. The aim of this study was to explore the fate of chlordecone (CLD) and its transfer to different organisms of the soil food web.

Seven species of invertebrates representing different taxonomic groups and trophic levels of the soil communities of Martinique were targeted and collected in six experimental banana fields, with a level of contamination within a range of values classically observed. Soil samples and macrofauna from the study sites were analysed for CLD and chlordecol (CLDOH) its main transformation product. The contamination of the soil fauna were related to $\delta^{15}N$ (trophic level), proportion of soil ingestion (diet) and types of epidermis (mucus or exoskeleton) in order to study the different mechanisms of macrofauna contamination.

Presence of CLD and CLDOH could be quantified in all the soil organisms from contaminated fields. Results showed a significant relationship between the CLD contamination of detritivorous and the ash content of their faeces, suggesting that soil ingestion was the main contamination pathway. In contrast, the exoskeleton-bearing diplopod *Trigoniulus coralinus* and the soft-bodied earthworm *Eudrilus eugeniae,* both detritivores with a comparable diet, had similar contamination levels, suggesting that the type of tegument has little influence on bioaccumulation. At the scale of the entire trophic network, a significant relationship was uncovered between $\delta^{15}N$ values and CLD contamination of the fauna, therefore providing some *in situ* evidence for a bioamplification process along the soil food chain.

1. Introduction

Organochlorine pesticides (OCP) are synthetic pesticides widely used all over the world. Some of these compounds are toxic, and many have the particularity of degrading slowly and being persistent, which led to their inclusion as Persistent Organic Pollutants (POPs) in the 2009 Stockholm Convention (Madaj et al., 2018). Therefore, even many years after their use has ceased, they still raise environmental and human health concerns. This is the case with chlordecone (CLD), large quantities of which were produced and used throughout the world, for a wide variety of purposes: bananas, potatoes, citrus, but also for domestic or ornamental garden use (Luellen et al., 2006; Madaj et al., 2018). Soil pollution in the French Antilles could last several hundred years according to some predictions (Cabidoche et al., 2009). Contamination with CLD in the French Antilles comes from its use as a pesticide in banana fields to control the banana weevil *Cosmopolites sordidus*

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between 1972 and 1993. In Martinique, 12,000 ha of soils were exposed to CLD contamination (Cabidoche and Jannoyer-Lesueur, 2011). Environmental factors such as soil types and agricultural practices can influence its persistence, and according to certain predictions the pollution could last for several decades in nitisols, centuries in ferralsols and half a millennium in andosols (Cabidoche et al., 2009). Even though recent debated findings suggest that the pollution could last less than previously thought (Comte et al., 2022), soil pollution by chlordecone remains a major problem in the French Antilles, where all the compartments of the ecosystem are contaminated and more than 90 % of the population have detectable concentrations (i.e. above 0.02 $\mu g.L^{-1}$) of CLD in the blood (ANSES, 2017). Because of its massive production and use around the world, studying the fate of this molecule in this rather unique situation of massive pollution of an entire Caribbean island ecosystem could serve as a model for understanding similar cases discovered elsewhere in the future.

Chlordecone contamination of aquatic organisms has been studied in the Antilles. Given its low aqueous solubility and its strong affinity for organic matter, chlordecone tends to be found in the sediments rather than in the water column, and therefore mainly accumulates in detritivores and carnivores. A study assessing chlordecone contamination of the marine fauna in Guadeloupe and Martinique revealed significant variations from one trophic group to the next (Dromard et al., 2016). Detritivorous fish were the most contaminated, with a mean CLD concentration (±SE) of 156.4 ± 7.4 $\mu g\ kg^{-1}$. Plankton-feeders, second-level carnivores (eating invertebrates and small fishes) and piscivorous fish species made up another group exhibiting intermediate contamination levels (57.5 ± 4.2; 67.4 ± 14.9 and 55.9 ± 3.7 $\mu g\ kg^{-1}$ respectively). The last group, composed of herbivores and first-level carnivores (invertebrate-feeders), was the least contaminated trophic group, with mean concentrations of 10.4 ± 4.6 for herbivores and 33.2 ± 11.2 $\mu g\ kg^{-1}$ for first-level carnivores. In addition, the positive relationship between biocontamination and $\delta^{15}N$ concentration found by Dromard et al. (2022) confirms that the chlordecone bioaccumulation model is diet-dependent in marine food chains and that the bioamplification process is at stake concerning the CLD in marine environment.

Until now, much attention was devoted to characterising the contamination of aquatic trophic networks because aquatic organisms are an important source of contamination for the human population. In terrestrial habitats, research focused on crops, another important source of human contamination, but there are currently no data available on the contamination of soil food webs. Given the key role played by soil organisms in agroecosystems, and their high risk of exposure, it seems essential to study their level of contamination and the fate of CLD and transformation product in the food webs. Soil invertebrates are involved in a number of ecological processes that are part of critical ecosystem services, such as the maintenance of soil structure, nutrient cycles, carbon fixation in soils and the biological control of pathogenic organisms and pests (Barrios, 2007; Lavelle et al., 2006). Despite their importance, there is a gap in the literature about CLD and soil fauna that is likely due to the significant amount of animal tissue required for analysis, which would have prevented dosage on soil organisms which are relatively small.

Because of their habitat, soil invertebrates are particularly exposed to soil pollutants, however, depending on their morphology, physiology and behaviour, soil organisms are not all similarly exposed (Peijnenburg et al., 2012). The type of epidermis is an important morphological parameter, as soft-bodied organisms living in the soil are exposed to the penetration of pollutants through their permeable mucosa, whereas arthropods have a cuticle that gives them some protection against this particular type of contamination (Hedde et al., 2012). Diet of organisms is also an important physiological parameter modulating exposure to pollutants, and is directly linked to trophic level and the ingestion of soil particles. Since CLD contamination is in the soil, detritivores, which actually feed on soil particles, are of particular concern regarding risk of ingesting contaminated elements. In addition, trophic position could be an important factor explaining CLD contamination, since the bioamplification process may be at play and induce significant contamination of predators and organisms with a high trophic position, as it was previously shown for aquatic organisms.

The aim of this study was to shed light on the mechanisms of chlordecone transfer to soil invertebrates and thence to the soil food web. We hypothesised that contamination by bioaccumulation depends on how the organism is exposed to the contaminant (by ingestion and/or by skin contact). More precisely, we expected that organisms with permeable skin (mucosa), such as earthworms and gastropods, would be more contaminated than macroarthropods possessing an exoskeleton (H1). Building on the principle that detritivores consuming soil would be contaminated by ingestion, we also hypothesised (H2) that organisms of the 'brown' food web (saprophagous and more particularly geophagous) would be more contaminated than organisms belonging to the 'green' food web (herbivores). And finally, given the bioamplification process, we hypothesised (H3) that CLD contamination would be greater in predators than in primary consumers.

In order to test these hypotheses, we measured chlordecone concentration in soil invertebrates belonging to different trophic levels in banana fields with a well-documented history of chlordecone contamination. The data were then crossed with $\delta^{15}N$ and amount of soil ingestion in the diet to study in detail the modes of contamination and transfer of chlordecone in the trophic network.

2. Materials and methods

2.1. Description of the sites sampled

Collections were carried out on 6 fields located in the CIRAD research station of Rivière Lézarde (14.66° N, 60.00° W, 46 m asl), each having the same surface area (*ca.* 500 m^2). Soil is classified among the Nitisols (WRB classification). Mean granulometry of this soil type is dominated by clays (59 % clay, 20 % silt, 21 % sand) (Venkatapen, 2012). Climate is moist tropical, with a mean temperature of 26.4 ± 0.1 °C and annual precipitation of 2413 ± 252 mm (mean over the 1997–2017 period). Seasonality is weak, with February as the driest month (95 mm) and October the wettest (313 mm).

The fields studied have been treated with chlordecone in the past and their contamination is therefore historical. Available data on chlordecone in these soils prior to this study indicate concentrations between 0.1 and 1 $mg\ kg^{-1}$ (DEAL, 2023), due to Kepone applications as insecticide on banana plantations before 1993. From the end of the 1990s ~~decade~~ and for 25 years, pineapple was grown on the fields, with no rotation. Since 2018, banana is grown there as part of the BANABIO project, and conducted following a middle-of-the-road approach relative to the current practices applied in Martinique for this crop. The soil, climate and history of contamination of these fields are thus representative of many agroecosystems in the French Antilles. More information on the agricultural practices, the experimental setup and the physical and chemical characteristics of the soil can be found in Coulis et al. (2023). In addition to these 6 banana fields, the soil from a vegetable garden with no history of chlordecone contamination and where soil CLD concentration was shown to be below the limit of quantification (LOQ) was used as control (14.62° N, 61.08° W, 105 m asl). All the samples were collected over a period of 4 months, between April and August 2022.

2.2. Soil sampling and analysis of chlordecone and chlordecol

For each field, three banana trees were selected 5–10 m apart and at least 10 m from the edge of the field. Three soil samples forming a triangle were taken at a distance of 1 m around each banana plant, from soil depth 0–30 cm. Subsequently, all the three soil sub-samples of a given banana tree were pooled and manually homogenized to make a single composite soil sample from which an aliquot was extracted. All

the composite soil aliquots were cooled to 4 °C prior to being sent for analyses within one month maximum. In all, 3 samples were taken from each of the 6 plots, making a total of 18 independent soil analyses. Soil samples were sent out for analyses to the LDA26, accredited by Cofrac, the French Accreditation Committee for pesticide analyses, thereby guaranteeing the technical skills, reliability and good management practices. LDA26 complies with ISO 17025 standards for testing and calibration. The CLD and CLDOH analyses were thoroughly described by Comte et al. (2022).

2.3. Selection of the sampled organisms and sampling method

Soil macrofauna communities are very diverse in tropical agro-ecosystems and it is therefore not feasible to study all the species present on a site (Dupont et al., 2023). Prior research enabled us to list the species representative of the macrofauna communities in the soil of Martinique agroecosystems (Coulis, 2021; El Jaouhari et al., 2023; El Jaouhari et al., 2022; Gabriac et al., 2024; Schileyko et al., 2024). This list was established so as to: 1) maximise taxonomical diversity, 2) represent the different trophic groups present in the soils, 3) include species from the detritivorous ('brown') food web and the herbivorous ('green') food web, and 4) include both soft-bodied species and species with an exoskeleton (cuticle) to study the effect of contamination by direct contact *versus* contamination by ingestion, while remaining logistically feasible. A total of 7 species were thus selected as target species before starting the collections (Table 1, Fig. 1). The minimum quantity required for CLD analysis in tissues was set at 1g of dry mass, which meant collecting and grouping a varying number of individuals depending on the individual mass of the species studied. This ranged from 3 individuals for the lizard species to over 250 individuals for the ant species (Table 2). Due to the low mass of some samples, quantities were adapted to use all the mass available. Thus, the mass of the samples analysed varied between 0.056 and 1.019 g in dry mass, and when converted to fresh raw mass it varied between 0.187 and 6.732 g (full data are available in the public repository at the doi given later in the text). In cases where, after an intensive search for the target species, the number of individuals required for analysis could not be reached because the species was too rare or even absent from a plot, it was replaced by another locally present species with characteristics as close as possible to those of the original species. In addition to the target species, less abundant species were occasionally collected and analysed in order to document their level of contamination (thereafter referred to as 'non-target species').

Sampling was carried out by active collection, searching the soil, leaf litter and other microhabitats. After initial identification in the field, the organisms collected on each plot were placed in airtight boxes and brought back to the laboratory, where identification was confirmed under a binocular microscope. Detritivores and snails were left to fast for 48 h at 25 °C in a box lined with paper towels to allow as much soil as possible to exit the digestive tract. Faeces were collected and pooled per species. Regarding lizards, the digestive tract was extracted post mortem and the contents of the stomach were preserved in 70 % ethanol for posterior analysis. Other species could not be left to fast because gut transit was too slow, or they were too small to be dissected. After this

Table 1
Species studied, indicating scientific name, trophic level and other information with relevance to their mode of exposure.

Common names	Species names	Soft body	Food web	Trophic group
Target species				
Lizard	*Dactyloa roquet*	no	Green	predator
Ant	*Camponotus sexguttatus*	no	Green	omnivore
Weevil	*Cosmopolites sordidus*	no	Green	herbivore
Snail	*Lissachatina fulica*	yes (but shell)	Green	herbivore
Millipede	*Trigoniulus corallinus*	no	Brown	detritivore
Epigeic earthworm	*Eudrilus eugeniae*	yes	Brown	detritivore
Endogeic earthworm	*Pontoscolex corethrurus*	yes	Brown	detritivore
Non-target species				
Leech	*Hirudinea* sp	yes	Brown	predator
Flatworm	Geoplanidae spp. (3 species: *Bipalium kewense*, *Amaga expatria*, *Dolichoplana* cf. feildeni)	yes	Brown	predator
Centipede	*Scolopendra subspinipes*	no	Brown	predator
Centipede	*Otostigmus salticus*	no	Brown	predator
Centipede	*Scolopocryptops ferrugineus*	no	Brown	predator
Millipede	*Anadenobolus monilicornis*	no	Brown	detritivore
Epigeic earthworm	*Perionyx excavatus*	yes	Brown	detritivore
Epigeic earthworm	*Amynthas rodericensis*	yes	Brown	detritivore

Fig. 1. Illustration of four of the species studied, representative of the soil food web of banana agroecosystems of Martinique. A. *Pontoscolex corethrurus* B. *Dactyloa roquet* C. *Trigonius coralinus* D. *Camponotus sexguttatus*. Pictures by Baptiste Bentameur ©.

phase, all the specimens were euthanised by exposure to −20 °C in a freezer, freeze-dried and ground to a fine powder with a ball mill (Retsch, MM400). The dehydrated powder thus obtained was thereafter conserved in the dark at ambient temperature.

2.4. Chlordecone and chlordecol analyses on animal tissues

Chemical substances, equipment and apparatus were previously described (Devriendt-Renault et al., 2023) and are summarized in supplementary information (Text SI-1 and SI-2).

2.4.1. QuEChERS extraction procedure

Sample extraction procedure was adapted from a previous study (Devriendt-Renault et al., 2024, 2023). Briefly, 1.0 ± 0.1 g dry mass of freeze-dried animal tissue was placed in a 50 mL centrifuge tube before adding 50 μL of internal standard ($^{13}C_8$-CLD and $^{13}C_8$-CLDOH) concentrated to 5 μg mL^{-1} to the sample along with 9 mL of ultrapure water. The sample was then vortexed using Genie 2 for 1 min, homogenized with the IKA Ultra-turrax at 5000 rpm for 3 min before adding 10 mL of ACN. Each sample was then mixed for 1 min before and after adding the QuEChERS extraction kit and centrifuged at 4000 rpm for 5 min using the Eppendorf 5810 centrifuge. The same procedure was realized using procedural blank sample to ensure the extraction quality. The organic phase was removed and 6 mL was placed in a Supel QuE PSA/C18 ″Fatty samples" purification tube. The purification tube was then vortexed for 1 min and centrifuged at 4000 rpm for 5 min. Finally, 1.5 mL of the supernatant from each tube was removed, filtered through a 0.20 μm PTFE filter and transferred to a 2 mL amber vial prior to HPLC-MS/MS analysis.

2.4.2. HPLC-MS/MS conditions

The current HPLC-MS/MS analysis method is described by Saint-Hilaire et al. (2018). Liquid chromatography conditions and mass spectrometry parameters are presented in Tables SI–1a and SI-1b. Analyst 1.5.1 software (AB Sciex, Les Ulis, France) was used for equipment control and data acquisition. Following the SANTE guidelines (Sante/11312/2021), two consecutive single reaction monitoring (SRM) runs were realized for both CLD (506.7/426.7 and 508.7/428.7) and CLDOH (490.7/35.0 and 492.7/37.0). Selected identification criteria were retention time deviation and difference between the two SRM ratios of less than 0.1 min and 30 %, respectively. Instrument response linearity was considered between 0.09 and 180 ng mL^{-1} of CLD or CLDOH in ACN using a constant concentration of $^{13}C_8$-CLD and $^{13}C_8$-CLDOH (25 ng mL^{-1}). The limit of quantification (LOQ) was set to 2 ng g^{-1} for CLD and CLDOH, corresponding to the lowest spiked level during the QuEChERS extraction optimization. The LOD of 0.7 ng g^{-1} for CLD and CLDOH corresponded to the third of the LOQ (Devriendt-Renault et al., 2023).

2.5. Analysis of the soil macrofauna trophic network

2.5.1. Isotope analyses

All the specimens of macrofauna collected were analysed in the isotope laboratory of the B&PMP research unit in Montpellier, France. The specimens were analysed using isotope-ratio mass spectrometry using a Vario-PYROcube elemental analyser coupled to an IsoPrime Precision mass spectrometer (Elementar, UK). The method is detailed in Tixier et al. (2022). All the stable isotope values were reported as delta values, with δ13C or $\delta^{15}N$ calculated as follows: [(Rsample/Rstandard) - 1] × 1000. where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Standards used were the PeeDee Belemnite (Peterson and Fry, 1987) for C and atmospheric air (Mariotti, 1983) for N.

2.5.2. Diet analysis

Two methods were used to characterise in greater detail the diet of the species studied. For lizards (*Dactyloa roquet*), the contents of the digestive tract were examined with a binocular microscope (optical magnification x50). Remains of arthropod cuticles were sorted and identified to the highest possible taxonomical resolution, depending on their state of conservation. For other species whose faeces could be collected, the organisms were left to fast after their collection in the field. Faeces were collected after 24 h and dried at 60 °C. They were pooled for each species when the quantity collected was insufficient to analyse a sample individually. The faeces were then calcined at 450 °C

Table 2
Chlordecone (CLD) and chlordecol (CLDOH) contamination levels of soil and macrofauna living in contaminated plots and in an uncontaminated plot. The average number of individuals used for one analysis is indicated in brackets next to the species or plot name (n). The total number of analyses carried out per species is indicated in the next column (N). All concentrations are given per dry mass of soil or animal tissue, the dry matter content is given from our own measurements in the right-hand column to allow conversion to fresh mass if required.

	N	CLD concentration (ng.g^{-1} dw)	CLDOH concentration (ng.g^{-1} dw)	Dry matter content
		mean (min-max)	mean (min-max)	(%)
Soil of contaminated banana plots (3):				
Fauna living on contaminated banana plots:	6	253 (113–474)	23 (10–80)	NA
Target species				
D. roquet (3)	6	2352 (1450–3490)	15 (8–34)	26
C. sexguttatus (160)	3	72 (35–118)	13 (5–19)	30
C. sordidus (14)	5	1304 (1170–1430)	13 (11–17)	50
A. fulica (3)	6	17 (7–49)	1 (0–6)	17
T. coralinus (15)	6	1182 (808–1520)	29 (18–39)	35
E. eugeniae (7)	5	1313 (783–1890)	162 (56–231)	18
P. corethrurus (13)	6	5072 (2770–8820)	224 (127–332)	15
Non-target species				
hirudinea sp.	1	8720	86	NA
Geoplanidae spp (3)	1	8360	20	NA
O. salticus (1)	1	9280	489	30
S. ferrugineus (7)	1	11500	540	27
S. subspinipes (1)	1	1500	<LOQ	23
A. rodericensis (3)	3	1119 (898–1260)	196 (170–225)	15
P. excavatus (3)	1	392	40	17
Soil of uncontaminated plots (10):				
Fauna living on the uncontaminated garden plot:	1	<LOQ	<LOQ	NA
D. roquet (3)	1	12.4	<LOQ	30
C. sexguttatus (250)	1	10.7	<LOQ	50
A. fulica (3)	1	<LOQ	<LOQ	17
A. monillicornis (19)	1	42.6	0.498	30
E. eugeniae (10)	1	161	5	18
P. corethrurus (12)	1	26.5	<LOQ	15

to measure ash content. Ash content was also measured in parallel on soil and vegetation (weeds) samples collected from the fields studied. Given that the ash content is generally much higher in soils (>80 %) than in plants (<20 %), the results give an indication of the proportion of soil in the diet of the species studied.

3. Statistical analyses

Differences in CLD and CLDOH concentrations in the soil of the six plots and differences in CLD and CLDOH concentrations in the seven target species were tested using one-way ANOVAs. Both ANOVAs were followed by a pairwise comparison test (Tukey's HSD test).

Relationships between pollutant concentrations in the soil and in the soil macrofauna were first tested for each target species separately using OLS regressions. To compare species, elevation tests and multiple slope comparisons tests were then performed with the R software.

Data on ash content in faeces (ash content were averaged at the species level) were related to pollutant contamination values for each individual; for both CLD and CLDOH, a power model was compared to a simple linear model using AIC criteria, with the model with the lowest AIC retained at the end.

To investigate the relationship between $\delta^{15}N$ and contamination of the soil macrofauna, Major Axis (MA) regressions using all macrofauna species (including the non-target species) were used. For this test, a global relationship including all the species was first tested, then a relationship for each food web (species of the brown food web and species of the green food web separately) was tested followed by elevation tests. The analysed data are available in CIRAD's institutional repository at the following link: https://doi.org/10.18167/DVN1/VAZLK7. All statistical analyses were performed using the R software (version 4.1.0). The following functions and packages were used: aov , HSD.test in the agricolae package (Mendiburu, 2023), lm, sma in the smart package (Warton et al., 2018) and nls).

4. Results

4.1. Chlordecone contamination of the different compartments (soil and macrofauna)

In soil, the mean concentrations of chlordecone (CLD) differed significantly among the 6 sampled banana fields ($p = 0.0217$). The least contaminated field had a chlordecone concentration of 147.4 ± 47.9 ng g^{-1} dw, *versus* 336.3 ± 39.4 ng g^{-1} dw for the most heavily contaminated. These values are in line with contamination data reported earlier by DEAL (2023) in this area (between 0.1 and 1 ng g^{-1} dw). Mean chlordecol (CLDOH) concentrations in the soil of the 6 fields were also highly variable, but not significantly so ($p = 0.0843$). High intra-field variability was moreover observed for both molecules over the 3 measurements obtained from each field, as reflected in the minimum and maximum values (Table 2). CLD and CLDOH concentrations in the soil of the uncontaminated control site were below the quantification limit (Table 2).

In fauna, the mean CLD concentrations varied significantly from one species to the next ($p < 0.001$) (Table 2). The most heavily contaminated species was *Pontoscolex corethrurus* (5072 ± 2000 ng g^{-1} dw), followed by *Dactyloa roquet* (2352 ± 688 ng g^{-1} dw) and *Cosmopolites sordidus* (1304 ± 126 ng g^{-1} dw). The least contaminated species was *Achatina fulica* (17 ± 16 ng g^{-1} dw). Mean CLDOH concentrations also varied significantly across species ($p < 0.001$) and the most contaminated species was again *Pontoscolex corethrurus* (224 ± 65 ng g^{-1} dw). However, in contrast, the second and third most contaminated species were *Eudrilus eugeniae* (161 ± 76 ng g^{-1} dw) and *Trigoniulus coralinus* (29 ± 8 ng g^{-1} dw). The least contaminated with CLDOH was, as for CLD, *Achatina fulica* (0.97 ± 2.39 ng g^{-1} dw). Mean contamination values revealed a great variability, as reflected in the considerable range of values (Table 2). Among the 7 non-target species, 2 species of epigeic earthworm, 3 species of chilopod, and 1 species of predator leech were analysed as 6 separate samples (Tables 1 and 2). Three species of geoplanid predator flatworm (*Amarga expatriata, Bipalium kewense* and

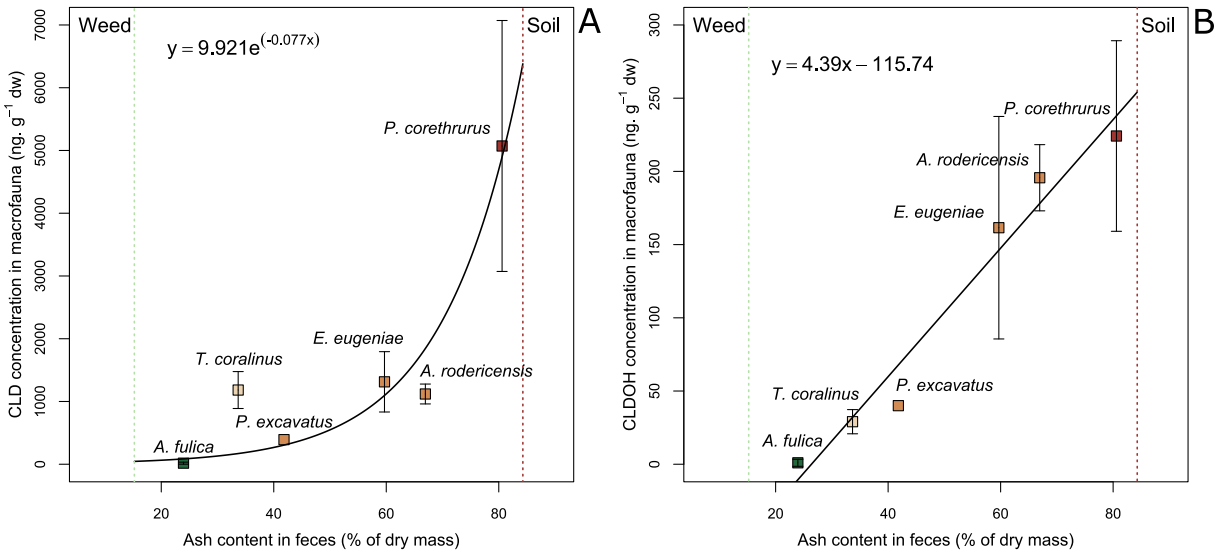

Fig. 2. Relationships between ash content of detritivore faeces and CLD (A) and CLDOH (B) concentrations in their body. Dashed lines refer to mean ash content of weed and soil measured on composite samples from the same experimental fields. The symbols correspond to those shown in Table 1.

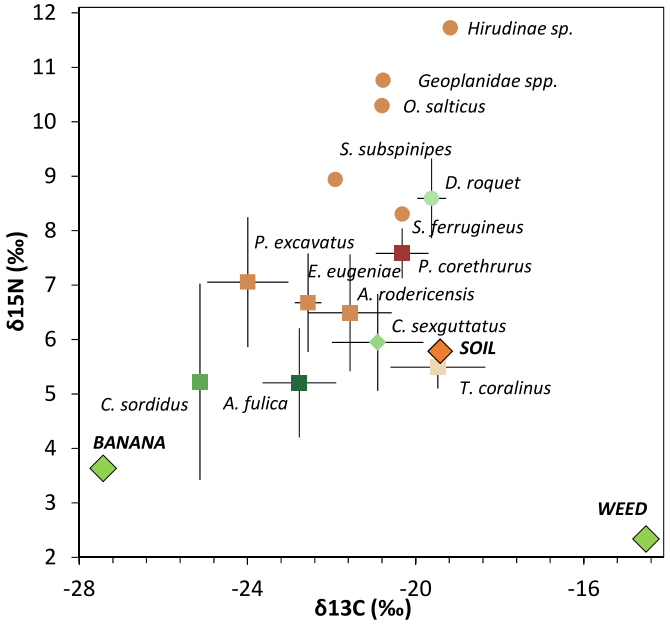

Fig. 3. δ15N and δ13C of the organisms studied and their food sources. Diamonds represent the primary resources and the symbols correspond to those shown in Table 1. Colours indicate the food web to which the organisms studied were attributed (green for the herbivores' food web and orange for the detritivores' food web). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Dolichoplana sp.) were pooled into a seventh sample analysed as a whole (Tables 1 and 2). The fauna of the control plot had detectable concentrations of CLD (Table 2). The most contaminated species was *Eudrilus eugeniae*, followed by *Trigoniulus coralinus* and *Pontoscolex corethrurus*. No detectable contamination was found in *Achatina fulica*. CLDOH concentrations were either extremely low or undetectable in most species except *Eudrilus eugeniae*, which had a CLDOH contamination of 5 ng g^{-1} dw. We found no significant relationship between CLD concentration in the soil and CLD concentration in the organisms studied (Figs. SI–2). A significant relationship between CLDOH concentration in the soil and CLDOH concentration in the animal tissues was detected in the endogeic earthworm *Pontoscolex corethrurus* only ($R^2 = 0.81$, $p = 0.015$). A similar trend was apparent for *Eudrilus eugeniae*, but the relationship was not significant ($p = 0.110$).

4.2. Transfer of contamination to primary consumers (bioaccumulation)

Ash content, measured in the faeces of 6 primary consumers species collected in the contaminated plots, gave an indication of the proportion of soil in the diet. The average ash content in the potential food sources was 15.21 ± 1.91 % for the vegetation cover and 84.28 ± 0.86 % for the soil (Fig. 2). Mean ash content in faeces varied between 23.95 ± 1.93 % in *Achatina fulica* and 80.59 ± 0.40 % in *Pontoscolex corethrurus;* reflecting a broad diet gradient for the species sampled, from rather strict herbivory to rather strict geophagy. These feeding parameters were good predictors of their contamination, since CLD contamination of the macrofauna was positively correlated with the ash content of their faeces, the exponential relationship was chosen because it had a lower AIC (98) than the linear relationship (106) (Fig. 2 A). The CLDOH contamination of the fauna was also positively correlated with the ash content of their faeces, in this case, the linear relationship ($R^2 = 0.96$, F = 108, p > 0.001) was chosen because it had a lower AIC (57) than the exponential relationship (65) (Fig. 2 B).

4.3. Transfer of contamination through the soil food web to higher trophic levels (bioamplification)

The contents of the digestive tract of 21 *Dactyloa roquet* specimens were examined under the binocular microscope. Remains of insects only were recorded, but it is possible that soft-bodied organisms were ingested without leaving any identifiable remains due to quick degradation. Six orders of insects were identified: Hymenoptera (4 observations), Coleoptera (4), Hemiptera (1), Orthoptera (1), Blattodea (1), and Dermaptera (1). Hymenoptera were all ants, among 3 species could be identified (*Wasmania auropunctata*, *Odontomachus rugigondis and Camponotus sexguttatus*), a beetle was also identified - the weevil *Polytus mellerborgii*. This result confirmed that the lizard was indeed a predator of soil food webs being investigated, since a species of ant studied as a target species was identified in its tract.

Values of $\delta^{15}N$ varied considerably according to the trophic level of each taxon (Fig. 3). The $\delta^{15}N$ value of the three primary resources tested were 3.33 ± 1.56 for banana, 2.04 ± 1.13 for weeds and 5.48 ± 0.25 for soil. Trophic levels in the herbivore food web were clearly differentiated by their $\delta^{15}N$ (Fig. 3). The 2 phytophagous species had $\delta^{15}N$ values of

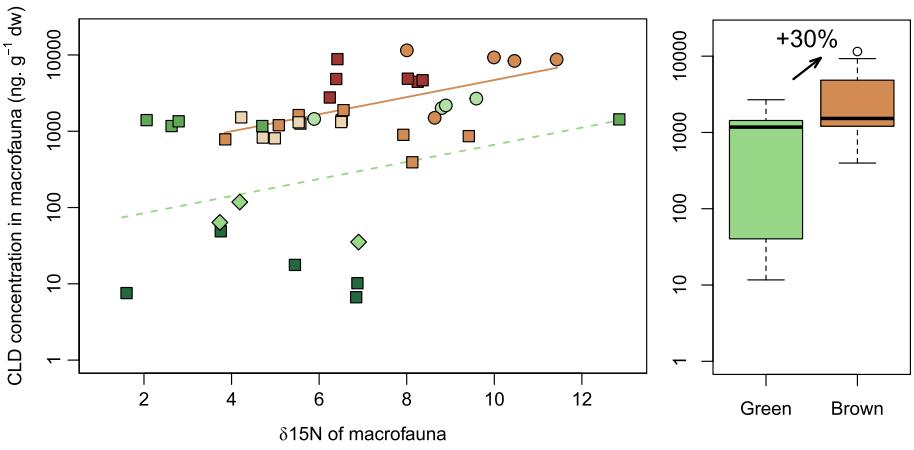

Fig. 4. Relationship between chlordecone contamination and δ15N of the soil macrofauna of the 'green' and 'brown' food webs. Non-significant relationship is indicated by dashed lines whereas significant relationship is indicated by a solid line. Difference in chlordecone contamination between the 'brown' and 'green' webs is significant, and the percentage shown in the boxplot refers to the difference between the medians. The symbols correspond to those shown in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

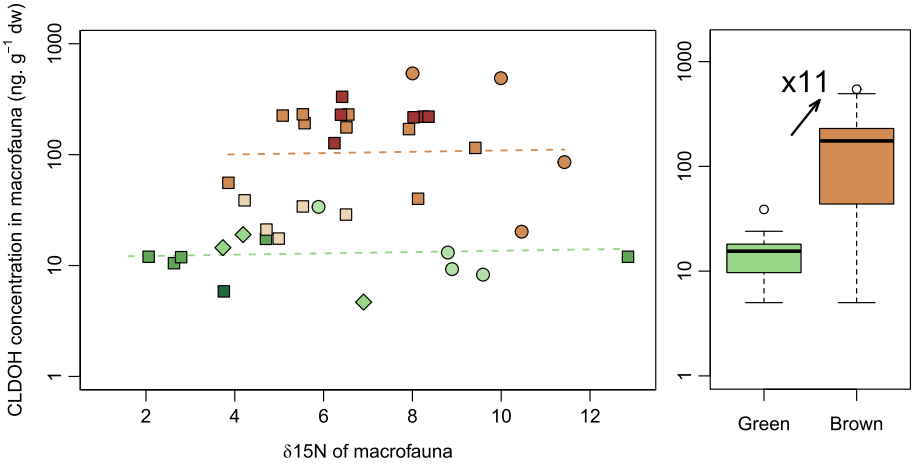

Fig. 5. Relationship between chlordecol contamination and δ15N of the soil macrofauna of the 'green' and 'brown' food webs. Neither relationship is statistically significant. Difference in chlordecol contamination is significant, and the order of magnitude shown in the boxplot refers to the difference between the medians.1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.90 ± 2.24 and 4.92 ± 4.02 (Fig. 3), the predator *Camponotus sexguttatus* had a $\delta^{15}N$ value of 5.64 ± 1.99 and the generalist predator *Dactyloa roquet* a $\delta^{15}N$ value of 8.29 ± 1.64. Species of the detritivorous food web had greater $\delta^{15}N$ values on average than species of the herbivorous food web, but differences between trophic levels were less marked (Fig. 3). Detritivorous primary consumers had mean $\delta^{15}N$ values comprised between 5.19 ± 0.87 for *Trigoniulus coralinus* and 7.28 ± 1.03 for *Pontoscolex corethrurus*. Predators of the detritivore food web had $\delta^{15}N$ values that varied between 8.00 for the centipede *Solopocryptops ferrugineus* and 11.42 for the unidentified predator leech (*Hirudinae* sp.).

At the scale of the entire food web ('brown' and 'green' webs pooled), a significant relationship was found between $\delta^{15}N$ values and CLD contamination of the fauna ($R^2 = 0.17$, $p = 0.006$). Comparing the two types of food web ('brown' and 'green'), the slopes of the two lines were not significantly different ($p = 0.845$), but CLD contamination was significantly higher ($p < 0.001$) in the fauna of the 'brown' trophic web (+30%) than in that of the 'green' web (Fig. 4). No significant relationship could be shown between $\delta^{15}N$ values and CLDOH contamination of the fauna (Fig. 5), whether at the scale of the entire food web ($p = 0.175$) or at that of the 'brown' or 'green' food webs considered separately ($p = 0.258$), but CLDOH contamination was significantly greater ($p < 0.001$) in the fauna of the 'brown' food web fauna than in that of the 'green' web (11 times) (Fig. 5).

5. Discussion

By studying for the first time the contamination of soil fauna by CLD, we have shown that the entirety of the trophic network was contaminated with chlordecone (CLD) and one of its main transformation product chlordecol (CLDOH). All the fauna samples (100 %) from the contaminated fields displayed detectable levels of CLD and even more remarkably, CLD was detected in fauna taken from a control field whose soil CLD concentration was below the LOQ (Table 2). Moreover, most species tested displayed higher CLD concentrations than the soil, the two exceptions being the herbivorous snail species and one omnivorous ant species, which both belonged to the 'green' food web. This suggests that most species are subject to bioaccumulation and/or bioamplification. The trend is different for CLDOH, which is found at greater concentrations than the soil in the 'brown' food web species and below soil concentration level in the 4 species of the 'green' food web tested.

The previous absence of data on soil fauna may seem surprising, given that the soils of these agroecosystems were the ones on which chlordecone was applied until 1993, and which constitute the pesticide's entryway into the other compartments of the ecosystems.

However, this can be explained by the difficulties of analysing the molecule, as until recently its dosage required large quantities of material to be analysed, and therefore prevented the study of small organisms. Another reason was that efforts were concentrated on edible foodstuffs for obvious human health reasons. Recent improvements in extraction methods have enabled the study of small soil organisms with extremely low individual biomass (Devriendt-Renault et al., 2023). Our data show a very broad contamination of the soil fauna, ranging between 17 and 11,500 ng g^{-1} dw in soils with a mean concentration of 253 ng g^{-1} dw. The average contamination of the species studied here lies in the upper range of what was previously known for lagoon fishes, freshwater invertebrates and ruminant meat (Dromard et al., 2022, 2016; Lavison-Bompard et al., 2021). However, our results cannot be directly compared with these, as most publications express CLD concentrations in fresh weight (fw) rather than dry weight (dw). In our data, the dry matter content varied by a factor of 3.3 between the organism with the lowest dry matter content 15 % (earthworms) and the one with the highest 50 % (beetles), a discrepancy that could potentially distort the final results. Additionally, water content could also vary according to other factors not studied here, such as the relative humidity of the air, the length of time the sample was left in the open air before analysis, etc. For future studies, we suggest expressing concentrations in dry matter or, if drying the sample before analysis is not possible, systematically providing moisture content data. Our results are still above the maxima reported in coastal organisms in Guadeloupe and Martinique, which peaked at 733 ng g^{-1} fw in crustaceans (Dromard et al., 2016). However, more recent findings obtained on mangrove organisms display a maximum mean concentration of 3689 ng g^{-1} fw in crabs (Dromard et al., 2022), which is of a similar level as the concentrations we found in soil invertebrates. The values obtained in our study are still very high for terrestrial animals. In comparison, maximum contamination levels of cattle reported by Lavison-Bompard et al. (2021) ranged between 70 and 400 ng g^{-1} fw.

The species included in the present study were chosen in particular to test whether fauna was contaminated by skin contact (H1) or by pollutant ingestion (H2). Our results highlight a stronger contamination of the brown food web and more precisely the predominance of ingestion as a contamination factor, confirming the H2 hypothesis. Indeed, the two species with the most contrasted diets were the giant African land snail (*Achatina fulica*), a herbivore that has a very limited soil intake (13 %) and whose CLD contamination was low (17 ng.g^{-1}dw), and the geophagous earthworm *Pontoscolex corethrurus*, whose diet is mainly made up of soil (95 %) and whose level of contamination was the highest among the target species (5072 ng.g^{-1}dw). More generally, the gradient of geophagy created by the different species investigated explains very well the level of contamination of each species. The importance of contamination *via* soil ingestion is also suggested by the generally greater contamination level of organisms of the 'brown' food web (Figs. 4 and 5), which live off dead organic matter and soil as their primary resource, in contrast to members of the 'green' food web, which depend on live plants, which are usually less contaminated. Diet-related traits, such as the percentage of soil intake in the diet we used here, thus appear to be good predictors of the CLD contamination level of soil organisms. This result confirms the findings showing that detritus feeders are more contaminated in the aquatic environments of the Lesser Antilles (Dromard et al., 2016). It is also in line with a community-scale study conducted in heavy metal-polluted soils in France (Hedde et al., 2012). In this study, the proportion of geophagous organisms decreased as pollution increased, indicating that geophagy was a response trait to heavy metal soil pollution. Our results also highlight the extent to which earthworm species differ from one another in terms of soil intake. This last fact is interesting not only for predicting earthworms level of contamination, but also as an eye-opener onto the ecology of earthworms and the possibility to build on this functional trait to establish finer ecological categories of earthworms (Bottinelli and Capowiez, 2021; Coq et al., 2022), in particular in moist tropical habitats, where many epigeic species live entirely above-ground, such as in epiphytic micro-habitats (Dupont et al., 2023).

The nature of the skin epidermis is another potential factor of bioaccumulation, this one depending on the organism's morphology since soft-bodied organisms could be more exposed to the contaminant *via* direct penetration through the mucosa than organisms protected by a cuticle (Peijnenburg et al., 2012). However, we found similar contamination levels in the soft-bodied earthworm *Eudrilus eugeniae* and the cuticle-bearing diplopod *Trigoniulus coralinus*, which are both detritivores living in the leaf-litter and the uppermost centimetres of the soil. This suggests that the type of skin epidermis had little or no influence on the contamination of these soil organisms, thus invalidating our H1 hypothesis. This is further confirmed by the low contamination level of the snail *Achatina fulica* despite the fact that it crawls along the surface of the soil on a soft part of its body (its foot).

Our findings clearly show the existence of a bioamplification process from one soil trophic level to the next, confirming our H3 hypothesis. The regression lines highlighting the relationship between the $\delta^{15}N$ values and the CLD contamination level of soil organisms was significant at the scale of the entire food web indicating a general bioamplification process. However, when the green and brown food webs were considered separately, only the brown food web showed a significant relationship, indicating a clear trend towards bioamplification for the detritivore-based soil food web. Conversely, the occurrence of bioamplification in the herbivore food web is challenged and would require further data to be clarified. It is moreover interesting to note record-high contamination levels (>10,000 ng g^{-1} dw, Table 2) in predators of earthworms (flatworms and leeches), explained by the fact that their preys are already very heavily contaminated. These small predators, in which CLD is bioamplified, can in turn be preyed upon by birds (Wunderle, 1981), reptiles (Silva-Soares et al., 2022), amphibians or mammals (Vilella, 1998), which are generally larger, more mobile animals, raising the question of CLD transfer to other ecosystem compartments. In addition, some bird species are hunted for their meat in the French West Indies, contamination of soil food webs could be considered an indirect source of exposure for the human population ().

We also documented for the first time the CLDOH concentration, one of the major products of transformation (PT) of CLD in a wide range of soil organisms. The general trend is that CLDOH concentrations are 5–150 times lower than CLD concentrations, with large interspecific differences. More than 30 years after CLD applications were stopped, concentration in the original molecule (CLD) is still much higher than concentration in one of its degradation products (CLDOH), confirming the very slow speed of the degradation process of CLD in the environment. While most species showed CLD concentrations higher than those in the soil, the trend is different for CLDOH, revealing a less pronounced bioaccumulation process. This result could be explained by a more rapid turnover of this molecule in organisms, as suggested by a study carried out on ewes showing that CLDOH is eliminated in just 3 days, compared with over 86 days for CLD (Saint-Hilaire et al., 2021). This could be the result of a less marked hydrophobic characteristic of the molecule. Furthermore, the absence of any significant relationship between $\delta^{15}N$ and CLDOH (Fig. 5) suggests that bioamplification is little, if at all, involved in CLDOH contamination of soil macrofauna. Beyond the particular interspecific variations observed, the overall trend is that organisms of the 'brown' food web have proportionally greater concentrations in CLDOH (11 times more) than organisms of the 'green' food web, regardless of the trophic level (Fig. 5). The CLDOH could be derived from the degradation of CLD in organisms, or from the accumulation of CLDOH from the environment. Although this question remains open, our interpretation would be that the degradation of CLD to CLDOH probably does not occur in macrofauna, but microbiologically in the soil, and that organisms of the "brown" food web are contaminated by soil ingestion. The relationship between ash content and CLDOH concentration in soil macrofauna points in this direction (Fig. 2B). Fernández-Bayo et al. (2013) confirmed the existence of CLD degrading

microorganisms in a tropical andosol. Further research is still needed on this molecule and on the other biodegradation products of CLD to reach a finer understanding of the contamination and mobility of these molecules in the various ecosystem compartments.

6. Conclusion

We showed that the entire food web was contaminated by CLD and CLDOH, one of its main transformation products. The endogenous earthworm studied was among the most contaminated organisms, with a concentration more than 20 times higher than in the soil. We also demonstrated *in situ* the main mechanism of contamination, which appears to be soil ingestion. We propose an easy-to-measure trait (ash content of faeces) to estimate the potential contamination of detritivorous fauna by soil pollutants. We have also shown that pollutants can be detected in earthworms harvested from soils below the LOQ for CLD, suggesting that earthworms, especially the endogeic species, could be used to increase the molecule's detection power in low-pollution contexts. This first study of CLD contamination of soil food webs has therefore proved fruitful, with the prospect of studying earthworm contamination over a broad gradient of CLD soil contamination.

CRediT authorship contribution statement

Mathieu Coulis: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Julie Senecal:** Writing – original draft, Project administration, Methodology, Investigation, Data curation. **Yoann Devriendt-Renault:** Writing – review & editing, Validation, Methodology. **Thierry Guerin:** Writing – review & editing, Supervision. **Julien Parinet:** Writing – review & editing, Supervision. **Lai Ting Pak:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mathieu coulis reports financial support was provided by the BANABIO project funding obtained under DEPHY EXPE program of the Ecophyto II plan. Julien PARINET reports financial support was provided by French National Research Agency (ANR-19-CE21-0002). Lai Ting pak reports financial support was provided by The French Ministry of the Overseas for this study that was part of the Territoires Durables project carried out in Martinique. If there are other authors, they 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

The data will be publicly available in CIRAD's institutional repository at the following link: https://doi.org/10.18167/DVN1/VAZLK7.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124874.

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