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RESEARCH ARTICLE

Activated carbon, a useful medium to bind chlordecone in soil and limit its transfer to growing goat kids

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Abstract

Chlordecone (Kepone) (CLD) is a highly persistent pesticide which was extensively used in the French West Indies; high levels of CLD can still currently be found in large agricultural areas. As CLD transfers from soil to animals mainly via involuntary ingestion, the consumption of foodstuffs derived from animals raised in contaminated areas may significantly contribute to exposure of humans to CLD. The present study was designed to test the efficacy of two different activated carbons (ACs) sources in limiting CLD transfer from soil to animal. Three soils (ASs) were prepared according to the OECD guideline 207. One standard soil (SS) lacking AC, and two modified preparations of SS supplemented with 2% coconut-based activated carbon (ORBO), SSO or with 2% lignite-based one (DARCO), SSD. All three soils were spiked with 10 µg of kepone per g of dry matter and aged for three weeks. This study involved 15 goat kids randomly assigned to the 3 experimental groups (n = 5/ group), which were fed the experimental matrices at an exposure dose of 10 µg CLD per kg of body weight per day. After 21 d of oral exposure, CLD in adipose tissue and liver were analysed by LC-MS-MS. A significant decrease of 63.7% and 74.7% of CLD concentrations in adipose tissue and liver, respectively, were obtained from animals exposed using SS containing DARCO as compared to those receiving only SS. Decreases in CLD levels of 98.2% (adipose tissue) and 98.7% (liver) were obtained for animals exposed using SS containing ORBO. This study leads us to conclude that (i) the presence of AC in CLD-contaminated soil strongly reduces CLD bioavailability, and (ii) the efficacy depends on the nature and characteristics of the AC used.

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1. Introduction

Chlordecone (CLD) is a chlorinated polycyclic ketone pesticide recognized as a Persistent Organic Pollutant (POP). The use of this pesticide against the banana black weevil (*Cosmopolites sordidus*) in Martinique and Guadeloupe (French West Indies) resulted in the spread of large amounts of this pesticide before its formal ban in 1993 in France. Since this pollutant is highly retained and persistent in soil, superficial layers of large areas of agricultural soils are

extremely contaminated (>1mg/kg) [1]. This contamination is believed to last even up to several centuries [2]. In addition, its lipophilic properties (log Kow = 4.5 [3]) and high affinity for animal tissues leads to significant accumulation of this pesticide in animals [4–7]. As a consequence, a major human health concern related to CLD transfer from soil to animals has been raised, particularly in regards to local animal food production. Strategies to avoid contamination of outdoor reared animals are therefore urgently needed.

CLD-transfer to lambs [8], piglets [9] and laying hens [10] have been proven to not be limited by the type and properties of Antillean soils (nitisol or andosol). The specific efficiency of this transfer is related to the availability of this pesticide for outdoor reared animals. A means to reduce the soil bound CLD availability and its further potential absorption could be by the sequestration of this pollutant into a non-absorbable shape. Activated carbon (AC) is known as a potential medium for sequestration of many organic pollutants [11–14], and is already used to decontaminate water in the French West Indies [15]. Thus, *in-situ* sequestration using AC could be a promising means to limit the CLD-transfer from soil to animal.

The present study was conducted to assess the potential of two different ACs to efficiently limit CLD transfer from soil to young ruminants.

2. Material and method

2.1. Experimental design

The experimental design was aimed towards comparing CLD bioavailability in CLD-contaminated soils supplemented or not with two different AC sources derived from lignite (DARCO) or from coconut shell (ORBO 32).

Three groups of randomly assigned goat kids (n = 5 per group) were fed either one of these 3 soils. During the exposure period (day1 to day 21), kids received daily a moistened dough ball with one of the experimental soils for an exposure dose of 10 µg of CLD per kg of body weight. At the end of the contamination period (day 21), the animals were euthanized in order to collect the perirenal adipose tissue and the liver. Concentrations of CLD in those tissues were determined using LC-MS/MS. The Relative BioAvailability (RBA) factors of soil bound CLD were estimated using concentrations of CLD in the adipose tissue and in liver [16,17].

The experimental design of the present study is depicted in Fig 1.

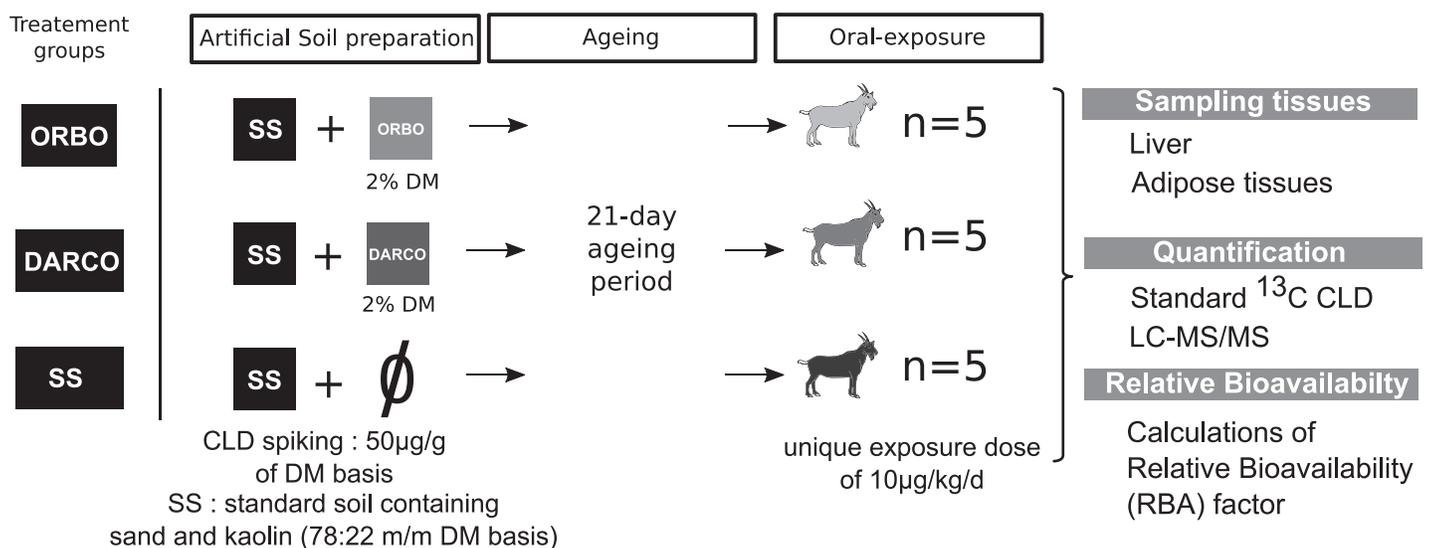


Fig 1. Experimental design of the present study.

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2.2. Animals, ethics and housing

Fifteen 4-month-old Alpine male kids (*Capra hircus*, from Laneuvelotte, France) were used. Animals were allowed a 21-day acclimation period before starting the exposure period. Each experimental group (n = 5) was kept in a cage covered with straw and equipped with individual feeders in the URAFPA animal facilities (Université de Lorraine, Vandœuvre-les-Nancy, France). Temperature was maintained between 20–21°C. All animals were individually weighed every 2 days. Daily ingestion was estimated by subtracting leftovers from provided rations. Water was provided *ad libitum* by nipple waterers throughout the entire study. This project was approved by the institutional committee for ethical use of animal experimentation (Authorization number: 00270.02, authorized by the French Ministry of Higher Education and Research) and was carried out in strict accordance with the principles established in the Guide for the Care and Use of Laboratory Animals of the French Ministry of Agriculture, Agrifood, and Forestry and European Council Directive (European directive 2010/63/EU).

2.3. Dosing material and exposure methodology

2.3.1. Activated carbon characteristics. Surfaces Specific Areas (SSA) were measured at the LIEC laboratory (Vandoeuvre-lès-Nancy, France) using the 77K Nitrogen adsorption method and performed on a Belsorp-mini II device (BELJAPAN, Inc Chemicals, JAPAN). The BET method was then used to estimate the SSA, using a 16.3 Å cross-sectional area of nitrogen molecules [18]. The De Boer method was carried out to determine external surface area.

DARCO displays an SSA of $793.8 \pm 14.5 \text{ m}^2 \cdot \text{g}^{-1}$ and ORBO an SSA of $1126.3 \pm 11.9 \text{ m}^2 \cdot \text{g}^{-1}$. Microporous surfaces represented 56% and 80% of the overall porosity for DARCO and ORBO respectively. Chemicals used and preparation of soils are described in Table 1.

2.3.2. Soil preparation and spiking technique. Three artificial soils were prepared as described in Table 1 according to the OECD guideline [19]. First, the standard soil (SS) contained sand and kaolin only (Sigma-Aldrich, St Louis, USA). This soil fulfilled composition and pH of an OECD artificial soil except for the *Sphagnum* peat portion, which was not included to avoid any sorption competition with CLD. The second soil (DARCO) was based on SS and supplemented with DARCO (Sigma-Aldrich, St Louis, USA) and the third one (ORBO) was based on SS and contained ORBO 32 (Sigma-Aldrich, St Louis, USA). In ORBO and DARCO soils, ACs were added to achieve 2% of the soil mass dry matter.

All three soils were spiked to attain 100 µg of CLD (Kepone, Supelco, Sigma Aldrich, Saint-Louis) per g of soil dry matter. CLD was spread over soil using an aqueous mixture of CLD (20:80; vol:vol; methanol:water). Then, all samples were thoroughly hand-mixed with a spatula

Table 1. Composition of the different artificial soils and treatment of the experiment.

	Sand	Kaolin	Activated carbon	Activated carbon	Kepone	Time of maturation
	See Sand (Carl Roth GmbH, Karlsruhe, Germany)	(Sigma-aldrich, St Louis, USA)	DARCO® (Sigma-aldrich, St Louis, USA)	ORBO 32® (Sigma-aldrich, St Louis, USA)	Concentration (µg.g ⁻¹ of DM) (Sigma-aldrich, Supelco)	in days
Standard soil (SS)	77.8%	22.2%	-	-	100	21
SS with DARCO (AC)	76.2%	21.8%	2%	-	100	21
SS with ORBO (AC)	76.2%	21.8%	-	2%	100	21

Percentages are DM basis of artificial soil.

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during 10 minutes. Solvent traces were evaporated under an extractor hood overnight. Then, milliQ water was added to reach a 17.5% moisture content in each vial. Soil-samples were hand-mix thoroughly using a spatula during an additional 10-minute period. All the three soils were stored at 20°C in amber glass vials for a 3-week maturation period until the first day of exposure (day 1).

2.3.3. Exposure of animals. All goat kids were exposed daily to one soil fraction according to their individual body weight. The day before exposure, the corresponding amount of soil (1 g of soil dry matter per 10 kg of body weight of kid) was incorporated in the centre of a moistened feed dough ball. The dough balls were fed individually to the kids daily after an overnight fasting period.

2.4. Sampling and analysis of biological samples

After 21 days of exposure, kids were anaesthetized by electronarcosis followed by immediate exsanguination. Perirenal adipose tissue was collected as the reference tissue usually collected in slaughterhouses to control CLD residues according to the French national surveillance plan [20]. The whole liver was also collected in view of the high concentrations of CLD in this tissue. Samples were stored at -20°C and freeze-dried.

The CLD Quantification was performed on both of these biological matrices using Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) according to the method ANSES PBM Pest LSA-INS-0164 v5 [21], in the Departmental Analytical Laboratory of Morbihan (LDA 56, Saint-Ave, France). The limit of quantification (LOQ) was 2.0 µg CLD kg⁻¹ in those matrices, and the limits of detection were less than 0.7 µg CLD per kg of the tested matrix.

The analytical method was initially developed by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES, Maisons-Alfort Laboratory for food safety) which is the national reference laboratory for this molecule in France. This methodology is the current regulatory analytical reference used in France [22] to control CLD in food-stuffs according to the maximum residue levels established in European regulation [23].

Prior to the extraction step, a ¹³C (¹³C₈C₂Cl₁₀O, Azur-isotope, Marseille, France, 98% of purity) internal standard of CLD was added to subsamples. Briefly, biological matrices were extracted sequentially. (i) For adipose tissue, 0.5 g of sample were added to 3 ml of a mix of acetonitrile and dichloromethane 75:25 (v:v). After centrifugation (1 200 x g, 20 min at -20°C) the supernatant was extracted. This extraction was performed 2 times. Then, the solvent was evaporated at 40°C under a nitrogen flux until dry. A mix of 15ml of hexane/acetone 85:15 (v:v) was then added. (ii) For liver, a sample of 2 g was used. Ten mL of hexane:acetone 85:15 (v:v) was added to the sample, which was then grinded using Ultraturax® (10 000 rpm, 1 min, S25N-10G). After mixing (Vortex® apparatus) and centrifugation (750 x g, 3 min) the supernatant was collected.

An alkalisation step followed by an acidification step were performed to obtain CLD hydrate and to reform CLD, as described elsewhere [24]. Analytical grade sodium hydroxide solution (addition of 5 mL of 0.5 M NaOH aqueous solution to supernatant). After a mixing procedure (Vortex® apparatus) and centrifugation (750 x g, 3 min), the supernatant was collected. This step was repeated two times.

The resulting aqueous phase was washed with 5 mL hexane to eliminate fat. The supernatant was collected after centrifugation (750 x g, 3 min). CLD was reformed through acidification of the solution by means of sulfuric acid (5mL of 60% solution). A second extraction phase was carried out with hexane:acetone (5mL of 85:15 v/v), followed by mixing (Vortex® apparatus) and centrifugation (750 x g, 3 min). This extraction step was repeated 3 times. The organic phase was then rinsed with 2mL of water, before being subsequently evaporated until dry and 1mL of methanol was then added.

Separation was achieved using a Phenomenex Aqua C18 column (150x2.0 mm 3 μm) and a precolumn Phenomenex Aqua C18 (4x3.0 mm). Two phases were used to perform the separation step: water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B). Five μL of sample was injected per run and the flow was set to 200 μl/min. After separation, a rinse sequence using acetonitrile was performed. Quantitation by isotope-dilution was performed on API 5500. Parent ions (PI) and child ones (CI) were used to respectively quantify (507/427 Da; PI/CI) and quantify (509/429 Da; PI/CI) ¹²C CLD and quantify (517/436 Da; PI/CI) ¹³C CLD.

2.5. Data analyses

2.5.1. Quality control of data acquisition and data set. LDA 56 works under the international standard ISO/IEC 17025:2005 (General requirements for the competence of testing and calibration laboratories). In addition, analyses were carried out in strict accordance with the COFRAC (the French Accreditation Committee) quality accreditation of the LDA 56 when applying the analytical methodology for animal foodstuff ANSES PEST LSA-INS-0164 [21]. This quality accreditation involves frequent interlaboratory tests. Uncertainty of this methodology was proven to be less than 20% for adipose tissue and fish [21].

Some values below LOQ were replaced by LOQ value (2.0 μg CLD kg⁻¹ in tested matrices) in the data set.

2.5.2. Tissues concentrations of CLD. In order to assess the impact of ACs on bioavailability of the contaminant an analysis of variances was performed. The experimental unit was the kid. Concentrations of CLD in adipose tissue and liver were compared between the three treatment groups (SS, ORBO, DARCO; n = 5 for each group) using the ANOVA procedure and the Tukey–Kramer post-hoc test of R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria). Differences were considered significant at P<0.05.

2.5.3. Relative bioavailability (RBA) factor calculation. Statistical analyses were carried out using R (version 3.2.3, R Foundation for Statistical Computing) on CLD concentration. Each kid was considered as an experimental unit. A 95% confidence interval of concentrations was calculated as described elsewhere [25].

The RBA was calculated by dividing “CLD-concentrations in one tissue (liver or adipose tissue) after one treatment (DARCO or ORBO)” by “CLD concentrations obtained in the same tissue after SS treatment (100% reference)”, adapted from a method previously described [16,17]. Linearity between the CLD-dose of exposure and CLD-concentrations in the organ was a prerequisite of this method [26]. This linearity was demonstrated previously for CLD in lambs [8]. Since lambs and goats have a similar physiology, the dose-response of CLD was not assessed in the present study.

The corresponding equation to calculate RBA of CLD after one AC-treatment in the liver is provided below:

$$RBA_{AC\ treatment;liver} = \frac{C_{AC\ treatment;liver}^{\circ}}{C_{SS\ treatment;liver}^{\circ}}$$

$RBA_{AC\ treatment;liver}$: Relative bioavailability of CLD after one AC – treatment in the liver

$C_{AC\ treatment;liver}^{\circ}$: CLD concentration in liver after one AC treatment

$C_{SS\ treatment;liver}^{\circ}$: CLD concentration in liver after SS treatment :

As each part of the division comprises uncertainties, a 95% confidence interval of the RBA factor was calculated via the propagation of uncertainty rule [27]. The corresponding equation

is provided above:

$$\frac{\Delta_{RBA}}{RBA} = \frac{\Delta_{C_{AC}^{o} \text{ treatment}}}{C_{AC}^{o} \text{ treatment}} + \frac{\Delta_{C_{SS}^{o} \text{ treatment}}}{C_{SS}^{o} \text{ treatment}}$$

Δ_{RBA} : Relative uncertainty of relative bioavailability after one AC – treatment

RBA : Relative bioavailability

$\Delta_{C_{AC}^{o} \text{ treatment}}$: Relative uncertainty of concentration found in one tissue after one AC – treatment

$C_{AC}^{o} \text{ treatment}$: CLD concentration after one AC treatment

$\Delta_{C_{SS}^{o} \text{ treatment}}$: Relative uncertainty of CLD concentration found in one tissue after SS treatment

$C_{SS}^{o} \text{ treatment}$: CLD concentration found in one tissue after SS treatment

2.5.4. Calculation of CLD retention by ACs during the digestive process. A factor of CLD retention by AC was determined as the ratio between concentrations obtained after SS treatment and concentrations obtained using ORBO and DARCO in the same biological matrix. In order to be conservative, this factor was minimized using the lowest and highest values of the 95th confidence interval of concentrations of CLD. Details of the calculations are provided above for AC DARCO using concentrations of CLD in liver:

$$R_{DARCO;liver} = \frac{Min_{95} C_{liver}^{o} DARCO}{Max_{95} C_{liver}^{o} SS}$$

$R_{DARCO;liver}$: factor of CLD retention by AC DARCO using concentrations of CLD in liver

$Min_{95} C_{liver}^{o} DARCO$: lowest value of 95th confidence interval of CLD concentration

in liver (statistical model explained in 2.5.2) for SS with DARCO treatment

$Max_{95} C_{liver}^{o} SS$: highest value of 95th confidence interval of CLD concentration

in liver (statistical model explained in 2.5.2) for SS treatment

3. Results & discussion

Daily ingestion of feed was unaffected by treatment during the exposure period ($16 \pm 3 \text{ g.kg}^{-1} \text{ day}^{-1}$, $P > 0.10$). Moreover, no significant effect on daily weight gain ($160 \pm 50 \text{ g.day}^{-1}$, mean \pm SD, $P > 0.10$) could be discerned at the end of the exposure period.

3.1. Concentrations of CLD in biological matrices

Concentrations of CLD in biological matrices showed important differences between the treatment groups (cf Fig 2). As expected, the highest CLD concentrations were obtained in the SS group (without AC) for both matrices: $155 \pm 22 \text{ ng/g}$ of DM (adipose tissue; mean \pm SD) and $2110 \pm 180 \text{ ng/g}$ of DM (liver; mean \pm SD). Intermediate ones were obtained in DARCO treated animals: $41.3 \pm 5.2 \text{ ng/g}$ of DM (adipose tissue; mean \pm SD); $449 \pm 71 \text{ ng/g}$ of DM (liver; mean \pm SD), and the lowest CLD concentrations in ORBO treated animals with non-quantifiable levels for adipose tissue ($< 2 \text{ ng/g}$ DM) and $24.0 \pm 2.4 \text{ ng/g}$ DM (liver; mean \pm SD).

The ANOVA analyses revealed a major treatment effect (cf Fig 2) for both biological matrices ($p < 0.0001$ for both tissues). Further investigation using a post-hoc Tukey test demonstrated that all treatment groups were distinct from each other ($p < 0.01$).

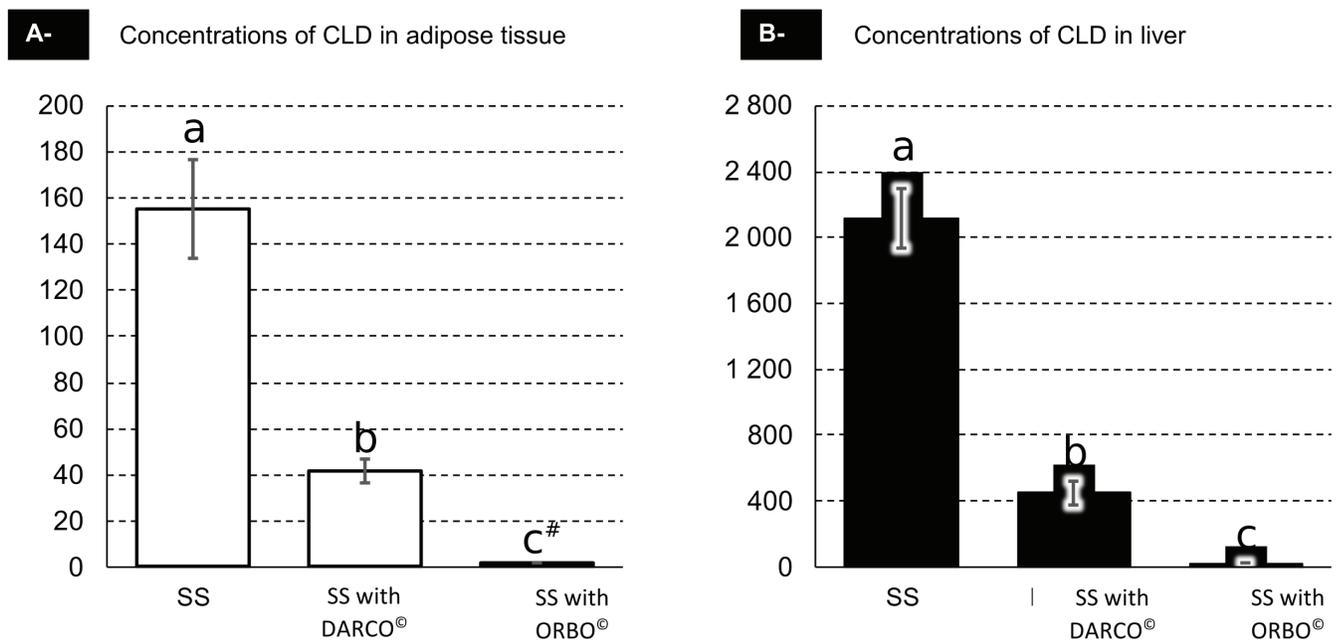


Fig 2. Concentrations of CLD in biological matrices (ng of CLD per g of DM). Concentrations of CLD are expressed in ng.g^{-1} of DM. Values correspond to the mean \pm SD. Mean values with different superscript letters (a, b, c) were statistically different ($P < 0.05$). Statistical analysis was performed using the two-way ANOVA procedure of R software and a Tukey post-hoc test. Two effects were used in the model: Organ and Treatment. Both effects were significant ($p < 0.0001$) and RMSE = 12.84. ($n = 5$). RMSE: Root means square error. #: values are below limit of quantification (LOQ).

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Within each treatment group, CLD concentrations in liver were > 10 -fold higher than those of adipose tissue. These differences between both organs are consistent with previous observations in rodent [28], lambs [8], pigs [9] and humans [29]. This particular affinity of CLD to liver could be explained by non-clearly identified proteins which readily bound CLD [30].

3.2. Effects of activated carbon on the CLD RBA

The RBA factors were calculated in order to estimate the CLD bioavailable fraction after the following treatments: ORBO or DARCO. Compared to SS, the DARCO treatment showed RBAs of 26.6% and 21.2% (respectively from adipose tissue and liver) and the ORBO treatment revealed RBAs of 1.3% (maximum value from adipose tissue) and 1.1% (liver). Similar RBA factors were obtained from adipose tissue and liver with the exception of ORBO for adipose tissue, where just a maximum value could be assessed (as all concentrations were below LOQ). Thus, SS with DARCO.

Statistical analyses were performed to obtain 95% confidence interval as presented in Table 2. To assess the efficiency of sequestration by ACs during the digestive processes, a factor of sequestration was calculated. DARCO exhibited a reduction of 53.4% (adipose tissue) and 67.2% (liver tissue). ORBO displayed a greater reduction of 98.2% (adipose tissue; base on LOQ) and 98.4% (liver) (Table 3).

In the French West Indies contaminated areas, CLD concentrations in soil vary between 0.011 – 52.1 mg.kg^{-1} of dry soil, as reported by Le Villain *et al* (2012) using the database of the Crop Protection Service (French Department of Agriculture) [31]. Mean and median values of CLD concentrations are respectively 2.1 and 1.1 mg.kg^{-1} [31]. Thus, the level of CLD in soil

Table 2. Relative bioavailability factors of CLD in biological matrices (% and 95% confidence interval).

	Adipose Tissue	Liver
SS with DARCO	26,6% [12.0% -41.2%]	21.2 [11.5%–30.9%]
SS with ORBO	1,3% [NA]	1.1% [0.7%–1.5%]

Values in brackets indicates 95% confidence interval (2,7xSE). SE were calculated via propagation of errors formula. (n = 5)

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Table 3. Reduction factor (% , based on 95th confidence interval).

	Adipose Tissue	Liver
SS with DARCO	53.4%	67.2%
SS with ORBO	98.2%	98.4%

Reduction factor are calculated as described in material and method section.

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(50 µg.g⁻¹) used in this study was similar to the maximal value within this database (52.1 mg.kg⁻¹). Thus, the saturation of AC medium by CLD should not be a limiting process if in field conditions.

The surface properties and textural characteristics of ACs drive the extent of adsorption of CLD [15,32]. In particular, the surface specific activity (SSA) is believed to be positively correlated to its binding potentiality [33]. ACs used in the present study are microporous media, even if ORBO presents a greater part of microporosity (80%) than DARCO (56%). The differences of the precursor materials of those ACs (coconut shell and lignite for ORBO and DARCO ACs, respectively) lead to different structures of porosity. Upon supplementation, this nanoporous material binds tightly halogenated compounds present in soil [33–35]. In addition, this structure could explain the limitation of the CLD desorption in the digestive chyme [36]. This result is in line with previous observations on polychlorinated biphenyls [16,36]. This process referred to as “physical trapping” of organic pollutants is a very limiting step towards uptake of CLD by animal [36]. The effectiveness of this trapping appears greater for ORBO than DARCO as shown by the RBA factors. This difference can be explained by the higher SSA displayed by ORBO (1126 versus 794 m² g⁻¹) leading to an increase of its CLD-binding capacity. Such a high level of microporosity could explain its capacity to physically trap CLD.

4. Conclusion

Both ACs efficiently retained CLD during the digestive processes of goat kids. When added to artificial soil, these ACs allowed the significant limitation of the bioavailable fraction, ranging from 64% to 98% for DARCO and ORBO respectively. These differences were linked to the specific SSA of the two ACs. This study performed on artificial soils is a very promising step before investigating the effects of ACs in on-field contaminated soils. Further investigations will be developed in order to assess the sequestration-efficiency of different levels of ACs or CLD- historically contaminated soils in the French West Indies.

Supporting information

S1 Table. Concentrations of CLD in biological matrices in each individual (ng of CLD per g of DM).

Concentrations of CLD are expressed in $\text{ng}\cdot\text{g}^{-1}$ of DM.
#: values are below limit of quantification (LOQ).
(DOCX)

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Author Contributions

Conceptualization: MD CF SY.

Data curation: MD.

Formal analysis: MD.

Funding acquisition: CF GR.

Investigation: SY MD.

Methodology: MD.

Project administration: CF MB.

Resources: CF GR.

Supervision: MD AF CF.

Validation: MD CF.

Visualization: SY.

Writing – original draft: MD SY.

Writing – review & editing: MD.

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