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Amendment of soil by biochars and activated carbons to reduce chlordecone bioavailability in piglets

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Keywords

Biochars; Activated carbons; Bioavailability; Availability; Amendment; Exposure assessment

Abstract

Chlordecone (Kepone or CLD) is a highly persistent pesticide formerly used in French West Indies. Nowadays high levels of this pesticide are still found in soils which represent a subsequent source of contamination for outdoor-reared animals. In that context, sequestering matrices like biochars or activated carbons (ACs) are believed to efficiently decrease the bioavailability of such compounds when added to contaminated soils. The present study intends to test the respective efficiency of soil amendment strategies using commercial ACs or biochars (obtained by a 500 °C or 700 °C pyrolysis of 4 distinct type of wood). This study involved three experimental steps. The first one characterized specific surface areas of biochars and ACs. The second one assessed CLD-availability of contaminated artificial soils (50 µg g⁻¹ of Dry Matter) amended with 5% of biochar or AC (mass basis). The third one assessed CLD bioavailability of those artificial soils through an *in vivo* assay. To limit ethically the number of animals, selections of the most promising media were performed between each experimental steps. Forty four castrated male 40-day-old piglets were exposed during 10 day by amended artificial soils according to their group (n = 4). Only treatment groups exposed through amended soil with AC presented a significant decrease of concentrations of CLD in liver and adipose tissue in comparison with the control group (p < 0.001). A non-significant decrease was obtained by amending artificial soil with biochars. This decrease was particularly high for a coconut shell activated carbon where relative bioavailability was found lower than 3.2% for both tissues. This study leads to conclude that AC introduced in CLD contaminated soil should strongly reduce CLD bioavailability.

1. Introduction

Chlordecone (CLD) is a chlorinated Persistent Organic Pollutant (POP) particularly resistant to biochemical degradation due to a very stable bishomocubane structure (Cabidoche et al., 2009, Matolcsy et al., 1988). Its large application as an insecticide from 1972 to 1993, especially in the French West Indies to control the proliferation of banana root borers, resulted in high contamination levels of plantation soils (until >1 mg/kg for CLD) (Cabidoche et al., 2009, Le Déaut and Procaccia,

2009, Levillain et al., 2012). Despite CLD formal ban in 1993, soil contamination will last several centuries taking account the low pollutant water solubility in conjunction with a high affinity for organic matter (with KOC in the range of 2.5–20 m³ kg⁻¹) (Cabidoche et al., 2009). Within this context, CLD soil contamination may constitute a major threat to locally produced foodstuff including animal food products (Jurjanž et al., 2016). Indeed involuntary soil ingestion represents a major

pesticide exposure pathway for outdoor reared animals (Fries et al., 1982) especially in the Antillean context (Jurjanz et al., 2017). Furthermore, it has recently been shown that CLD-transfers to animals are not limited by soil type or properties, as noticed in piglets (Bouveret et al., 2013, Delannoy et al., 2014a), lambs (Jurjanz et al., 2014), and laying hens-related studies (Fournier et al., 2012, Jondreville et al., 2013). Overall, for these domestic animal species, relative CLD bioavailability factors were not statistically different from 100% (Bouveret et al., 2013, Jondreville et al., 2013, Jurjanz et al., 2014).

One way to reduce soil-borne organochlorinated compounds availability in the intestinal lumen and their subsequent absorption relies on their sequestration level in the soil matrix in a non-bioavailable form. Activated carbon (AC) has been shown to be very efficient for the long-term sequestration, i.e. several years, and bioavailability limitation of a variety of organic pollutants such as PCBs and PAHs in sediments and water (Choi et al., 2014, Hilber and Bucheli, 2010, Jakob et al., 2012, Perelo, 2010). Biochars, which can be defined as pyrolysed biomass or organic waste materials under very low oxygen conditions (without any activation process), are an eco-friendly alternative for AC, being also considerably cheaper (Ahmad et al., 2014). Nevertheless, biochars are usually characterized by distinctly lower specific surface area and microporosity volume than AC, which may significantly limit the sequestration and subsequent bioavailability reduction factor of organic contaminants (Ahmad et al., 2014). In this perspective, the pyrolysis temperature used for biochar production play a major role in the extent of the resulting pyrogenic

material porosity and its pollutant immobilization capacity (Ahmad et al., 2014, Xie et al., 2015). Despite successful carbon amendment application trials for PCB sequestration in soils (Denyes et al., 2012, Denyes et al., 2013, Denyes et al., 2016), no attempt has been made concerning the immobilization of chlorinated bishomocubane compounds like CLD so far. In the French West Indies, only CLD-contaminated water treatment relying on the use of AC or surface-coated AC have been assessed, despite little data is available for the substance adsorption onto such condensed organic material (Durimel et al., 2013; Rana et al., 2017). Thus, in the context of CLD-contaminated land management, in-situ soil pollutant sequestration methods based on the use of a diverse range of geosorbents, i.e. condensed organic materials such as AC or biochars, may also appear as a valuable strategy worth investigating in order to limit the contaminant transfer from soil to farmed animals.

The present study was conducted to assess the potential of biochars to reduce bioavailability of CLD as soil pollutants. In order to ethically limit the *in vivo* assay, an *in vitro* test was firstly used to select the most relevant biochars. Thus, two different assays were used after a porosity characterization of biochars: one proceeding to a chemical extraction of CLD bound to soil constituent via a cyclodextrin solution and the other one studying the *in vivo* transfer of CLD to animal. Those tests refer respectively to the environmental availability and the relative bioavailability of soil bound CLD.

2. Material and Methods

2.1. Production and characterization of experimental matrices

biochars samples were ground and sieved until <500 μm (Fig. 1).

2.1.1. Production and acquisition of condensed materials

Three distinct sources of wood and two temperatures (500 °C and 700 °C) were used to obtain a set of six biochars (cf Table 2). An industrial scale oven was used (CARBOFRANCE, Montiers-sur-Saulx, France), to ensure the industrial feasibility of the process. In addition, two commercial activated carbons were obtained from Sigma-Aldrich (Saint-Louis, USA) and were used as reference products all along the study according to their particular activity and porosity. Biochars were produced by pyrolysis using the industrial ovens of CARBOFRANCE (Montiers-sur-Saulx, Lorraine, France). After the pyrolysis process,

2.1.2. Part 3

Specific surface areas (SSA) of both commercialized ACs, ORBO™ and DARCO® were determined at the LIEC laboratory (LIEC, Vandœuvre-lès-Nancy, France). The measurements were performed on the basis of nitrogen adsorption-desorption volumetry at liquid N₂ temperature of -196 °C. Nitrogen adsorption-desorption isotherms were recorded on a Belsorp-mini II set-up (BEL Japan, Inc). The device is equipped with pressure sensors in the range 0–133 kPa. After outgassing the samples at 30 °C during 12 h under a residual vacuum of 0.01 Pa, nitrogen adsorption-desorption isotherms (i.e., volume of nitrogen adsorbed at -196 °C vs. relative

pressure P/P_0 , where P is the equilibrium pressure of the adsorbing gas at that temperature and P_0 is the vapour saturation pressure) were performed using a step-by-step method in the interval of relative pressures, P/P_0 , extending from 10 to 5 to 0.98. All experiments were carried out with ultrapure nitrogen (>99.9995%). The Brunauer–Emmet–Teller (BET) method was used to estimate the SSA, using a 16.3 Å cross-sectional area of nitrogen molecules (Brunauer et al., 1938). The De Boer method (or t -plot, Lippens and de Boer, 1965) was carried out to determine microporous volume and external surface area. Micropore filling happens at low and very low relative pressure values, and then includes the domain of the monolayer adsorption on external surface. To be able to distinguish adsorption onto external surface from adsorption into the micropores (pore size <20 Å), the experimental isotherm is compared to a reference curve obtained for a non-porous solid, with chemical features and energetic constant as close as possible to the studied matrix.

2.1.3. Artificial soils fabrication

For both availability and bioavailability assays, artificial soils were prepared as described in Table 1 accordingly the OECD guideline 207. Briefly, the common portion presents in each artificial soil contains sand and kaolin (77:22, m:m dry basis) (Sigma-Aldrich, St Louis, USA). An additional portion was introduced differing from each artificial soil from other as constituted by neither or one of the condensed materials (5% dry mass basis of whole soil) with no or 5% (dry mass basis of whole soil) of sphagnum peat (Tourbe de Sphaigne, Florentaise, Truffaut, Paris). Composition of all soils prepared for each part of the study are presented in Table 1. All artificial soils were spiked with 100 µg of CLD (Kepone, Supelco, Sigma Aldrich, Saint-Louis) per g of DM. Briefly, a CLD mix was spread over artificial soils using an aqueous mixture of CLD (20:80; vol:vol; methanol:water and 10:90 vol:m, aqueous solution:dry soil). After spiking, solvent traces were evaporated under an extractor hood overnight. At last, ultrapure water was added to reach 18% of mass of the wet soil. Then, all artificial soils were aged during 80 days prior the following experimental parts.

2.2. Determination of CLD environmental availability

CLD environmental availability of soil was assessed using ISO/DIS 17 651 protocol (in standardization process when this article was written). The whole procedure is thoroughly described elsewhere in the standard ISO draft 17 651 – Method A (ISO, 2015). Briefly, a 4 g

aliquot of each dry soils was agitated 20 h in 40 mL of a 100 mmol.L⁻¹ β-hydroxycyclodextrin aqueous solution (Kleptose HP oral grade, Roquette - France). Then, samples were centrifuged 15 min at 2 000 g and the whole supernatant was collected. Extraction of a 10 mL aliquot of the supernatant was performed using petroleum ether three times. Then, CLD analyses were performed as described in 1.4 at La Drôme laboratory (Valence, France).

2.3. Determination of CLD relative bioavailability

2.3.1. Animals

The experimental design was approved by the Ethical Committee of Lorraine (Permit Number: EU0387, delivered by French ministry for agriculture and food). Forty four castrated male 40-day-old piglets from the farm “les deux chènes”(Saint-Maurice-aux-Forges, France) were used. An 8-day acclimation period was applied prior to the start of the 10-day exposure one (Day 1 to Day 10). During the exposure period the animals were hand fed prior distribution of their daily ration, one dough ball containing the adequate mass of the exposure matrix according to the body weight (0.2 g of dry artificial soil per Kg of Body weight (BW)). Each experimental group (n = 4) was kept in individual cages in the animal facility of URAFPA (Université de Lorraine, Vandœuvre-les-Nancy France) in order to ensure the correctness of the exposure dose. Temperature was kept to 22–24°C. All animals were individually weighed three times a week. Ration was provided daily at 4.5% of body weight of piglets (Super VE, Lorial, France). Water was provided ad libitum by nipple waterers throughout the entire study. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the French Ministry of Agriculture for Animal Research (MAAR) and European Council Directive (European directive 2010/63/EU).

2.3.2. Sampling

After the 10-day exposure period, the piglets were anaesthetized by electronarcosis followed by immediate exsanguination. Pericaudal adipose tissue and the whole liver were collected, stored at –20 °C and freeze-dried.

2.3.3. Analytical process

Quantification of CLD was performed on extracted phase of the environmental availability test and the two biological matrices using Liquid

Chromatography-tandem Mass Spectrometry (LC-MS/MS).

Values below LOQ were replaced by LOQ value in the data set. CLD.

For the biological matrices the validated method LSA-INS-016 was used in the Departmental Analytical Laboratory of Morbihan (LABOCEA, Brest, France). The same validated method was used by La Drome Departmental Laboratory on supernatants obtained from the environmental availability test. Reported limits of quantification were 2.0 µg CLD kg⁻¹ in those matrices and both laboratories.

Briefly prior the extraction step, a 13C internal standard of CLD was added to subsamples. In short, biological matrices were extracted sequentially. For adipose tissue, 0.5 g of samples were added to 3 mL of a mix of acetonitrile and dichloromethane 75:25 (v:v). After centrifugation (1200 g, 20 min at -20 °C) the supernatant was collected. This extraction was performed 2 times. Then, the solvent was evaporated at 40 °C under nitrogen flux until dry. A mix of 15 ml of hexane:acetone 85:15 (v:v) was then added. For liver a sub sample of 2 g was used. 10 mL of hexane:acetone 85:15 (v:v) was added and grinded using Ultraturrax® (10 000 rpm, 1 min, S25N-10G). After homogenisation (vortex) and centrifugation (750 g, 3 min) the supernatant was collected.

Separation was achieved using Phenomenex Aqua C18 column (150 × 2.0 mm 3 µm) and a precolumn Phenomenex Aqua C18 (4 × 3.0 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). 5 µL of sample were injected per run and the flow was set to 200 µl/min. After separation a rinse sequence using acetonitrile was applied. Quantification was performed on API 5500, parent ions (PI) and child ones (CI) were used to respectively quantify (507/427 Da; PI/CI) and qualify (509/429 Da; PI/CI) 12C CLD and quantify (517/436 Da; PI/CI) 13C CLD.

2.4. Data analyse

2.4.1. Quality control

Belsorp-mini device is regularly controlled against TiO₂ reference material (Community Bureau of reference). Experimental error is estimated to 0.50 m2 g⁻¹.

CLD quantification in animal tissues were carried out at LDA 56 in strict accordance with ISO 17 025 standard controlled by an accreditation delivered by the COFRAC (French committee for accreditation) quality accreditation of LDA 56.

2.4.2. Availability and tissues concentrations of CLD

In order to assess the impact of biochars and ACs on CLD availability and bioavailability a t-test was performed. The t-test procedure and the Tukey–Kramer post-hoc test of R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria) were used. Differences were considered significant at P < 0.05.

2.4.3. Relative bioavailability (RBA) estimates calculation

For each group the CLD Relative bioavailability was calculated as the ratio of CLD concentration in adipose tissue or in liver from each soils compared to S (100% reference), adapted from a method previously described (Delannoy et al., 2014a, Wittsiepe et al., 2007). Linearity between the CLD-dose of exposure and CLD-concentrations in organ was a prerequisite of this method (Littell et al., 1997). This linearity was proven for CLD contaminated piglets elsewhere (Bouveret et al., 2013). Each piglet was considered as an experimental unit. Statistical analyses were carried out using R (version 3.3.1, R Foundation for Statistical Computing) on RBA values.

2.4.3.1. Calculation of CLD retention by ACs and biochars during the digestive process

A factor of CLD retention for each biochar and AC was calculated as the ratio between concentrations obtained after S treatment and those obtained after an amended soil treatment. In order to be conservative, this factor was minimized using the lowest (5th) confidence value for SS and the highest one (95th) for tested group of concentrations of CLD. Details of the calculations are provided above for soil with DARCO® using concentrations of CLD in liver:

$$R_{Soil\ with\ DARCO;liver} = 1 - \frac{p_5 C^{\circ}_{liver\ Soil\ with\ DARCO}}{p_{95} C^{\circ}_{liver\ SS}}$$

$R_{Soil\ with\ DARCO;liver}$: reduction factor of CLD retention by AC DARCO® amending contaminated soil using concentrations of CLD in liver.

$p_5 C^{\circ}_{liver\ soil\ with\ DARCO}$: Lowest value of confidence interval (5th) of CLD concentrations in

liver (statistical model explained in 2.5.2) for contaminated soil with DARCO® treatment.

$p_{95} C^{\circ}_{liver SS}$: highest value of the confidence interval (95th) of CLD concentrations CLD in liver (statistical model explained in 2.5.2) for SS treatment.

3. Results

3.1. ACs and biochars characteristics

All isotherms exhibited a Type IV profile (Sing, 1982) according to the BET classification, which is characteristic of microporous adsorbents.

As expected, ACs surface areas were higher than those of biochars (Table 2). ORBO™ presents the highest surface area (1126.3 m² g⁻¹) followed by DARCO® (793.8 m² g⁻¹). Within the range of the precursors chosen to produce biochars, similar trends in surface areas were observed for biochars originating from a 700 °C pyrolysis. At this temperature, all biochars exhibit a surface area in the range of 332.5–376.7 m² g⁻¹. No such homogeneity were found from biochars originating from a 500 °C pyrolysis which present also the lowest values of surface areas (between 52.5 and 211.6 m² g⁻¹). Such results indicates the predominance of the type of the wood when a 500 °C pyrolysis is applied.

Interestingly, the portion of the porosity due to microporosity distinguished obviously the biochars originating from a 500 °C pyrolysis from 700 °C ones. Indeed, 76%–83% of 700 °C biochars surface area were explained by microporosity whereas only 49%–55% of 500 °C biochars. Those values are close to those of chosen ACs (ie 80% for ORBO™ and 56% for DARCO®). Due to the apparent homogeneity of biochars characteristics, a first selection step was performed on the set of biochars. Biochars from the two pyrolysis processes (ie 500 °C and 700 °C) from oak and resinous trees were chosen. Both ACs were also selected.

3.2. Soil CLD environmental availability

Hence, this set of experiment involved 8 soils comprising either one of selected ACs and biochar with peat (the organic fraction of the OECD 207 artificial soil) or peat or ORBO™ only. This choice will permit to apprehend the impact of organic matter on the potentialities of sequestration offered by ACs and biochars on the CLD availability. CLD-availability of the OECD standard soil was 62.5% indicating that the majority of the CLD in this soil was available. Surprisingly, obtained results show no reduction of CLD availability from amended soils with selected biochar in comparison to OECD

standard soil (59.1–74.4%; $p > 0.05$). If a slight decrease could be discerned when biochar originate from a higher temperature of pyrolysis, no statistical evidence of the impact (1) of the wood species (2) the pyrolysis process was reached through this assay ($p > 0.05$) (Fig. 2).

In contrast, activated carbons amended soils with or without organic matter displayed an important limitation of CLD availability in comparison to OECD standard soil (0.2–5.8%; $p < 0.0001$). ORBO™ appears to be the most availability limiting media. Interestingly, the lowest CLD availability was found from the soil containing ORBO™ and peat (0.2% (ORBO™ and peat) vs 2.2% (ORBO™ only)).

Due to the similarity of CLD-availability obtained from biochars originating from the two wood types, a final selection step was performed and only biochars originating from oak trees were chosen. Both ACs were also selected.

3.3. Soil CLD relative bioavailability

As expected, the highest CLD concentrations were obtained from the SS group (control without organic matter) for both tissues: 94 ± 13 ng g⁻¹ of DM (adipose tissue; mean \pm SD, Fig. 3) and 686 ± 117 ng g⁻¹ of DM (liver; mean \pm SD, Fig. 3). Groups exposed to soils devoid of organic matter and amended with biochar displayed similar concentrations than the OECD soil: 63 ± 16 to 65 ± 10 ng g⁻¹ (adipose tissue; mean \pm SD, Fig. 3) and 505 ± 45 to 586 ± 108 ng g⁻¹ (liver; mean \pm SD, Fig. 3). Groups exposed to soils with both biochar and organic matter displayed slightly lower CLD-concentrations in collected tissues than those exposed to soils containing only biochar or OECD soil: 47 ± 6 to 53 ± 13 ng g⁻¹ (adipose tissue, mean \pm SD) and 505 ± 45 to 523 ± 120 ng g⁻¹ (liver, mean \pm SD). However no statistical evidence allow a clear distinction between all those groups (SP, SPOT500, SPOT700, SOT500, SOT700).

In contrast, significantly lower CLD concentrations were obtained from animals exposed to soils containing AC only: 3.3 ± 0.3 to 28 ± 4.8 ng g⁻¹ of DM (adipose tissue; mean \pm SD); 19.5 ± 4.1 to 213 ± 7 ng g⁻¹ of DM (liver; mean \pm SD). Similarly for the biochars, piglets exposed to soils

comprising both organic matter and AC displayed slight lower concentrations than groups exposed to AC only: 3 (non-quantifiable levels for the 3 repetitions) to $19 \pm 1.2 \text{ ng g}^{-1}$ (adipose tissue; mean \pm SD) and 5.1 ± 0.9 to $185 \pm 23 \text{ ng g}^{-1}$ (liver; mean \pm SD). However, these differences were not found significant. Again, the lowest CLD concentrations were found when piglets were exposed to soils amended with ORBO™.

Then, the relative bioavailability factors were calculated in order to estimate the CLD relative bioavailable fraction for each group using S group as the 100% bioavailable one (cf Table 3). Additionally a reduction factor was also calculated to determine the effectiveness of each sequestering matrix. Overall, three different levels

4. Discussion

4.1. Availability and relative bioavailability assessment

Two different concepts underlie the availability and bioavailability assays. The first one assesses the mobility of the pollutant bound to soil constituents and expresses the maximum fraction of pollutant "available" for biota (ISO, 2008)(Fig. 4). The main assumption at the very basis of this test and its usefulness, is that the mobilization of the pollutant from soil constituents (ie the desorption and the solubilisation of the pollutant in soil interstitial water) is the limiting step toward its uptake by biota (ie the recalcitrant fraction which is still bound to soil will not be transferable). The second one, the relative bioavailability, assesses the in vivo transfer of pollutants from soil to animals or humans. Conceptually, the whole transfer chain of pollutant is realized in vivo: the mobilization of the pollutant from soil constituents (the so call available fraction), and the fraction readily transferred to animals (the bioavailable one) (Delannoy et al., 2014b, ISO, 2008) (Fig. 4). . In the present paper the bioavailability was assessed in a relative way as a reference matrix (standard soil) was used and set to be 100% transferred to animal Bouveret et al., 2013, Delannoy et al., 2014a, Fournier et al., 2012, Jondreville et al., 2013, Littell et al., 1997. This assumption was made 1) to compare soil with and without organic matter and biochars or activated carbon to finely assess the sequestration of each media and 2) such matrix is known to possess the same behaviour than contaminated feed Bouveret et al., 2013, Yehya et al., 2017 which was used to assess the toxicity of those molecules in numerous studies in organic contaminant. By this way, the exposure assessment obtained from the relative bioavailability assay could be compared to the known hazard of those pollutants.

of bioavailability were obtained. (1) Those presenting minor reduction of bioavailability (SP, SOT500, SOT700, SPOT500, SPOT700) presenting the highest levels of bioavailability: 69.7–88% (Liver, mean of bioavailability) and 49.4–70.1% (Adipose tissue). For those groups, reduction factors were below 35%. (2) Groups exposed through amended soils with DARCO™ which present lower relative bioavailability: 26.9–31% (Liver) 20.2–29.7%(adipose tissue). Found reduction factors were higher (53–65%). At last soils amended with ORBO™ present the lowest RBA values (0.8–3.5%) and highest reduction factors: 80.4–86.9%.

Then, it is of particular importance, when a biological exposure assessment is performed from an in vitro assay, than at least a correlation with an in vivo one (targeting the specie studied) was performed (Denys et al., 2009, Wragg et al., 2011). Indeed, this step appears essential to determine the representativeness of the value prior any consideration in terms of risk assessment. In the present results availability data show a significant correlation with relative bioavailability ones ($P < 0.05$ - Fig. 5). This correlation indicates also that the sequestration of CLD by biochars and ACs could be described as a reduction of the mobility of CLD caused by the adsorption of CLD on ACs or biochars surfaces.

Those results appear particularly convincing in the area of oral exposure assessment of CLD through in vitro testing and were obtained from the ISO/DIS protocol (ISO, 2015) call for further investigations to validate the use of this test in order to assess animal exposure of organic contaminants through soil ingestion. But the normalisation, the easiness of the protocol, the ethical consideration and the on-going interlaboratory assays should offer an interesting background on this methodology.

4.2. Sequestration efficiency of biochars vs ACs

ACs and biochars are porous materials obtained from a pyrolysis process of raw material in anoxic condition. ACs material present higher porosity than biochars, as described in literature. This results from the additional activation process known to increase the porosity by unfix incomplete combustion materials on the biochars surface. The 700 °C temperature of pyrolysis was tested in order to increase the combustion rate of those residues materials. If the structural data present a clear

increase of the porosity surface when a higher pyrolysis temperature is applied, poor reduction of availability and relative bioavailability were obtained from those media compared to the 500 °C biochar (Fig. 6). This contrast with relative bioavailability and availability data obtained from ACs which displayed a clear reduction of CLD transfer. This result is in accordance with previous data obtained with the same soil and for different organic pollutants (Delannoy et al., 2014a, Delannoy et al., 2014b) and CLD (Yehya et al., 2017). One main explicative hypothesis should be that the 700 °C pyrolysis process is not enough to achieve the quality of porosity needed to bound CLD and limit its subsequently its availability.

However two distinct relative bioavailability levels were obtained from the two ACs. These two different ACs are originating from two distinct activation processes. Chemical activation used for ORBO™ (supplier information) is known to produce AC of higher microporosity than physical activation such as steam-based methods, used for DARCO® (Durimel et al., 2013). Besides the mode of activation, the intrinsic nature of AC raw materials is well-known to drive the properties of the manufactured carbons. Indeed, these ACs were obtained from different raw materials (peat bog or coconut shell). As an illustration, micropore formation has been shown to be especially favoured during the activation of coconut shells or fibers as precursors (leading to ~ 85–94% microporosity) (Phan et al., 2006, Sobiesiak et al., 2006), whereas wood leads to a lesser extent peat ACs usually possess larger pore (i.e. macro-to mesoporous AC) in relation with the initial structure and composition of the corresponding highly lignified raw materials (Çeçen, 2000, Donati et al., 1994, Pastor-Villegas et al., 2006). Otherwise, all the present results are in line with previous data concerning microporosity and meso-to

macroporosity characterizations of coconut and peat-derived AC (Donati et al., 1994, Hernandez-Ramirez and Holmes, 2008, Phan et al., 2006).

AC application is known to limit organic pollutant transfer to different biota (Ghosh et al., 2011). The same range of reduction factor was also reported from soil studies, but to our knowledge these latter only focused on invertebrates and mostly earthworms as the model organism (Denyes et al., 2013, Denyes et al., 2012, Langlois et al., 2011, Paul and Ghosh, 2011, Wang et al., 2014). As an illustration (Paul and Ghosh, 2011), measured 94% bioaccumulation reduction of PCB from an artificially contaminated soil following earthworm (*Eisenia fetida*) exposure during 28 days to ~ 2.1% (w/w) AC amendment. The effectiveness of carbon amendments in the form of biochars have been also reported but to a lesser extent for organic contaminant immobilization in soils (Chai et al., 2012, Denyes et al., 2016, Denyes et al., 2013, Gu et al., 2016, Jakob et al., 2012, Kołtowski et al., 2017, Kołtowski et al., 2016, Wang et al., 2012). Besides AC application, the use of biochar has been also recently investigated to mitigate PCB bioavailability from soil (Chai et al., 2012, Denyes et al., 2016, Denyes et al., 2013, Denyes et al., 2012, Gu et al., 2016, Jakob et al., 2012, Wang et al., 2012). Best results were recorded at a 11.1% biochar application rate with a 88% PCB biouptake reduction in *E. fetida* (Denyes et al., 2012), which emphasized the potentialities of biochar for efficient pollutant sequestration. A 56% reduction of PCBs was also obtained by (Xu et al., 2012). Noteworthy this reduction was not obtained through our assay whereas the same pyrolysis temperature was used (700 °C). One possible explanation is the very specific characteristic of binding material needed to gain such sequestration (Durimel et al., 2013).

5. Conclusion and Perspectives

Biochars used as an amendment were not effective to reduce availability and bioavailability of CLD. By contrast, soil amendment with a peat- or a coconut shell-derived AC (DARCO® and ORBO™ respectively) resulted in major reductions in CLD availability and relative bioavailability. CLD concentrations either in piglet adipose tissue or in livers were reduced by 58% for DARCO® to 87% for ORBO™ in livers and

65% and 84% respectively in adipose tissue. According to these results, activation appears to be a needed process and further investigation on sustainable ways to produce activated carbon is needed. These results have been obtained with a soil ingestion rate and a CLD contamination level relevant according to rearing conditions in French West Indies.

Table 1. Porosity characteristics of the biochars and ACs

	Sand	Kaolin	<i>Sphagnum</i> peat	Activated carbon	Biochar
Standard soil (S)	77.8%	22.2%		–	
Standard soil with Peat (SP)	77.8%	22.2%	5%	–	
SP with DARCO® (SPD)	70.0%	20.0%	5%	DARCO (5%)	
SP with ORBO® (SPO)	70.0%	20.0%	5%	ORBO (5%)	
SP with oak tree 500 (SPOT₅₀₀)	70.0%	20.0%	5%		Oak Tree 500 °C (5%)
SP with oak tree 700 (SPOT₇₀₀)	70.0%	20.0%	5%		Oak Tree 700 °C (5%)
SP with resinous tree 500 (SPRT₅₀₀)	70.0%	20.0%	5%		Resinous Tree 500 °C (5%)
SP with resinous tree 500 (SPRT₇₀₀)	70.0%	20.0%	5%		Resinous Tree 700 °C (5%)
S with ORBO® (SO)	73.9%	21.1%		ORBO (5%)	
S with oak tree 500 (SOT₅₀₀)	73.9%	21.1%			Oak Tree 500 °C (5%)
S with oak tree 700 (SOT₇₀₀)	73.9%	21.1%			Oak Tree (5%)

Biochar column presents name and massic percentage of each biochar (source and pyrolyse temperature). Percentages are DM basis of artificial soil. Kepone concentration of each soil is 50 µg g⁻¹ of soil DM.

Table 2. Porosity characteristics of the biochars and ACs

	BET surface area m ² .g ⁻¹	Microporosity surface m ² .g ⁻¹	Non Microporous Surface m ² .g ⁻¹	Microporosity surface (% of the sum of the surface)
DARCO® (AC)	793.8±14.5	486.8	382.1	56%
ORBO™ (AC)	1126.3±11.9	971.8	242.4	80%
Oak 500	144.4 ± 7.8	76.6	79.0	49%
Oak 700	357.0 ± 10.1	314.0	74.3	81%
Beech 500	52.5 ± 5.2	31.0	25.0	55%
Beech 700	365.4 ± 6.7	318.0	79.6	80%
Resinous 500	211.6 ± 10.6	119.9	104.3	53%
Resinous 700	376.7 ± 12.0	319.0	86.0	79%
Softwood 500	167.2 ± 9.2	90.5	90.7	50%
Softwood 700	359.5 ± 7.6	293.6	93.5	76%

Table 3. Relative bioavailability factors

1- Relative bioavailability factors of CLD in biological matrices (% and 95% confidence interval)

	Adipose Tissue	Liver
S	100% [90.7-109.2]	100% [90.8-109.1]
SP	67.1% [57.8-76.3]	85.5% [76.4-94.6]
SOT ₅₀₀	69.0% [57.7-80.3]	73.6% [62.4-84.7]
SOT ₇₀₀	70.1% [58.8-81.4]	76.3% [63.4-89.2]
SPOT ₅₀₀	49.4% [38.1-60.7]	88.0% [76.8-99.1]
SPOT ₇₀₀	56.3% [45.0-67.6]	69.7% [63.4-89.2]
SD	29.7% [18.4-41.1]	31.0% [19.8-42.2]
SO	3.5% [0-17.8]	2.8% [0-14.0]
SPD	20.2% [8.8-31.5]	26.9% [15.8-38.1]
SPO	3.2%#	0.8% [0-11.9]

2- Reduction factor (% according 95th value of confidence interval)

	Adipose Tissue	Liver
SP	15.9%	-
SOT ₅₀₀	11.5%	6.7%
SOT ₇₀₀	10.2%	1.8%
SPOT ₅₀₀	33.1%	-
SPOT ₇₀₀	25.47%	1.8%
SD	54.7%	53.5%
SO	80.4%	84.6%
SPD	65.3%	58.0%
SPO	84.0%	86.9%

Values in brackets indicates 95% confidence interval.

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

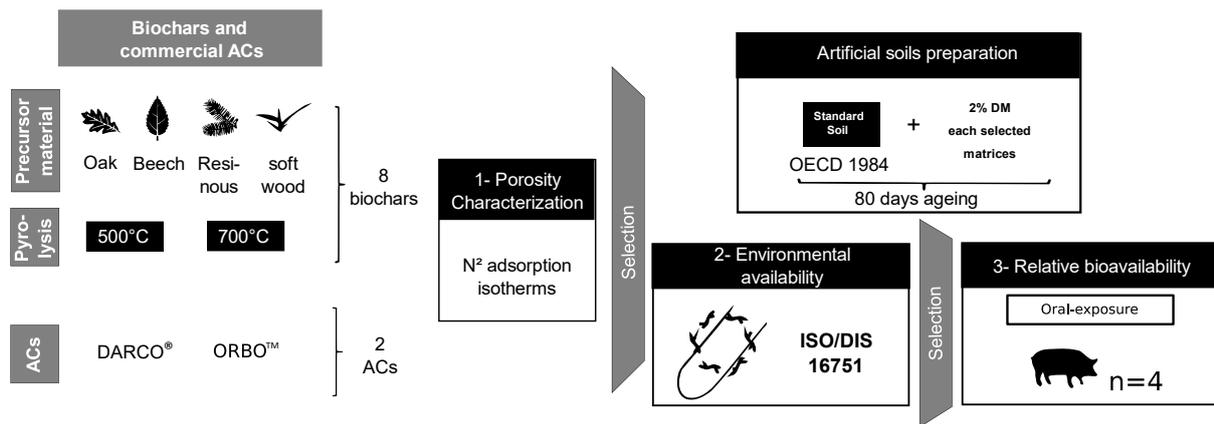


Figure 1. Experimental design.

Environmental availability of CLD

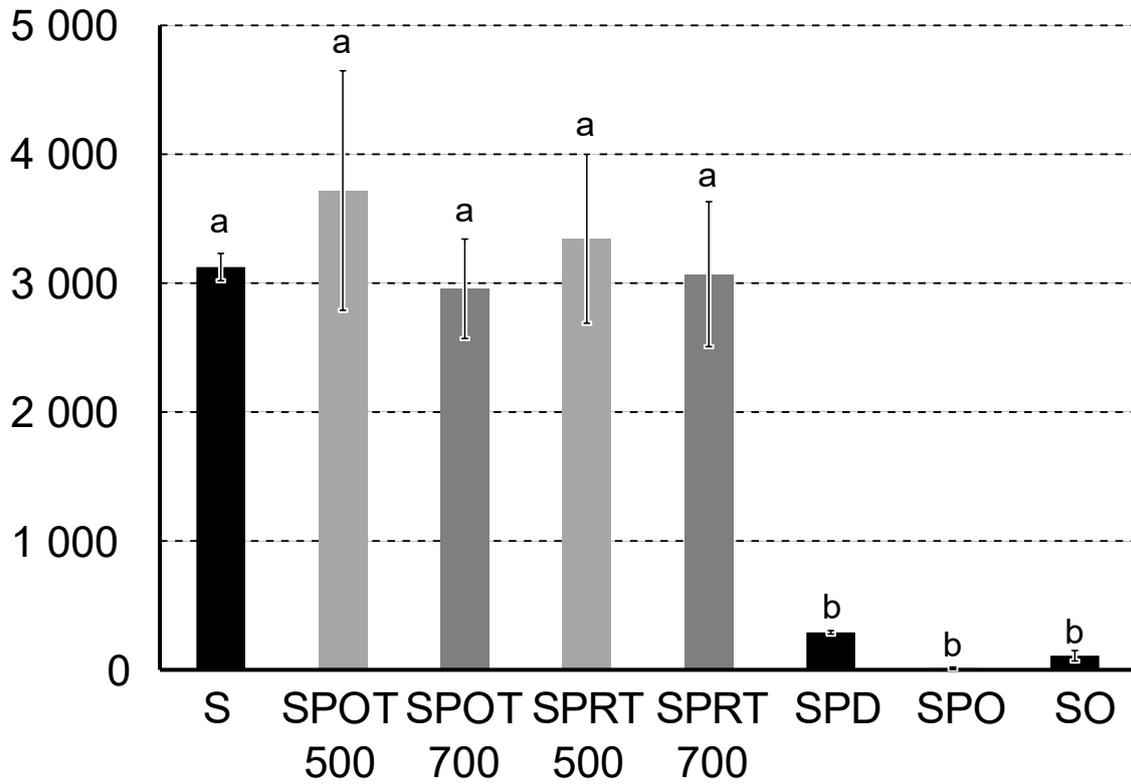
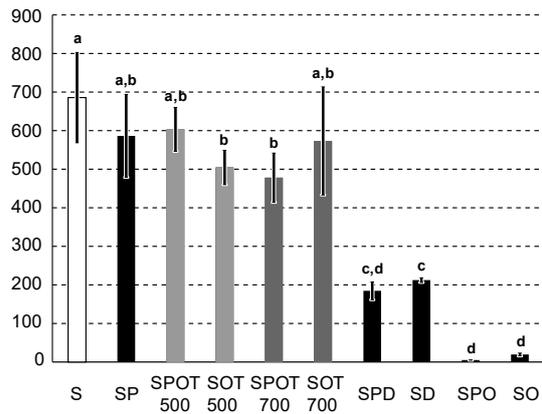


Figure 2. CLD availability in soils.

CLD availability is expressed in %. Values correspond to the mean \pm SD (n = 3). Mean values with different superscript letters (a, b) are statistically different ($P < 0.05$). Statistical analysis was performed using the one-way ANOVA procedure of R software and Tukey post-hoc test.

A- CLD-concentrations in liver



B- CLD-concentrations in adipose tissue

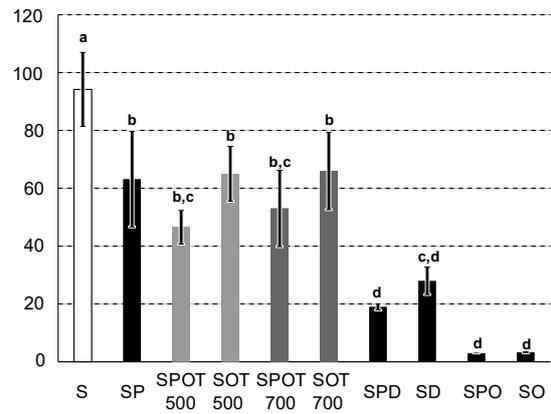


Figure 3. Concentrations of CLD in biological matrices (ng of CLD per g of DM)

Concentrations of CLD are expressed in ng.g⁻¹ of DM. Values correspond to the mean \pm SD (n = 4) except S and SP groups (n = 6). Mean values with different superscript letters (a, b, c, d) are statistically different (P < 0.05). Statistical analysis was performed using the one-way ANOVA procedure of R software and Tukey post-hoc test.

#: values are below limit of quantification.

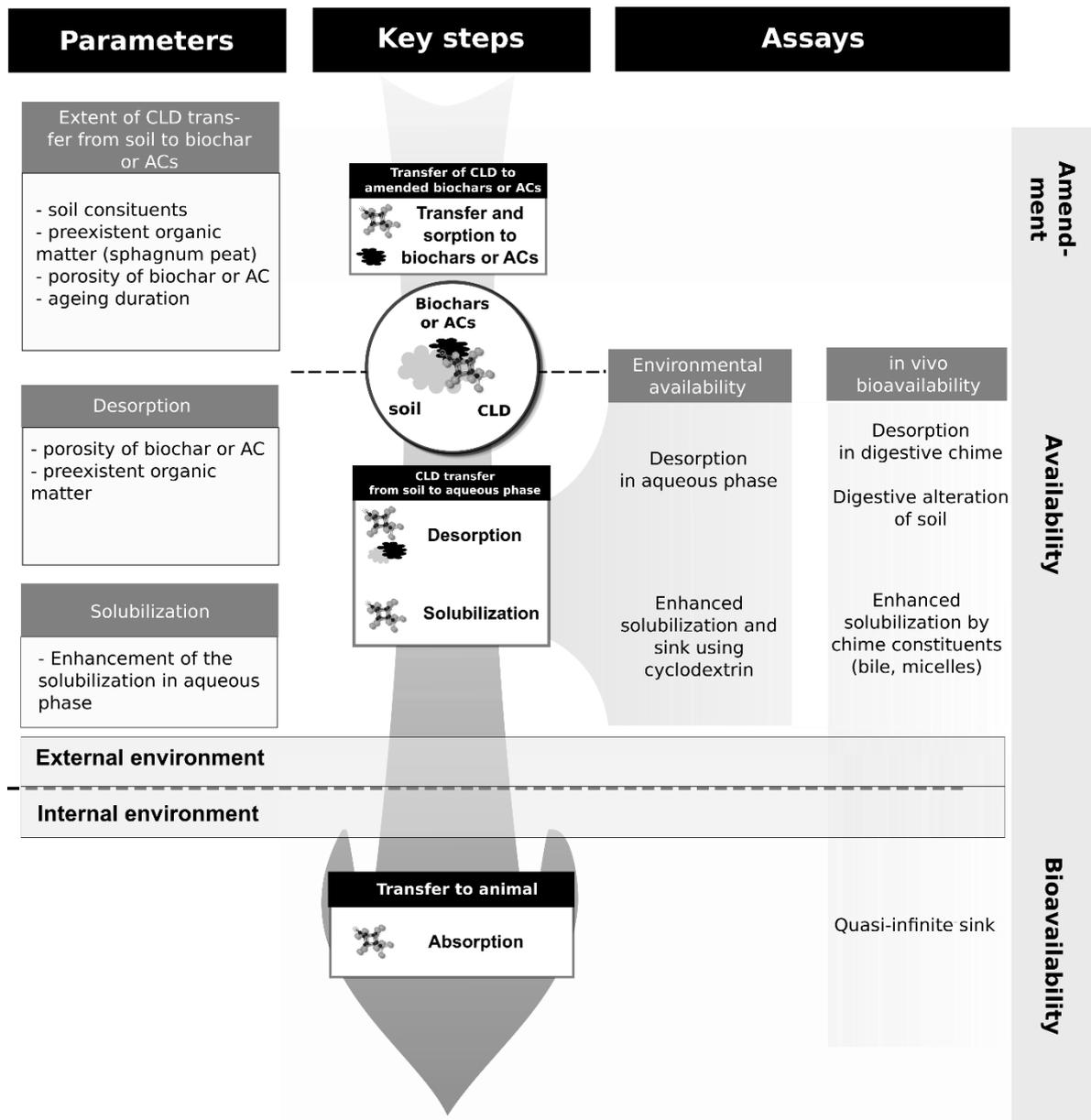


Figure 4. Mechanisms and impact of soil amendment on chlordecone environmental availability and relative bioavailability.

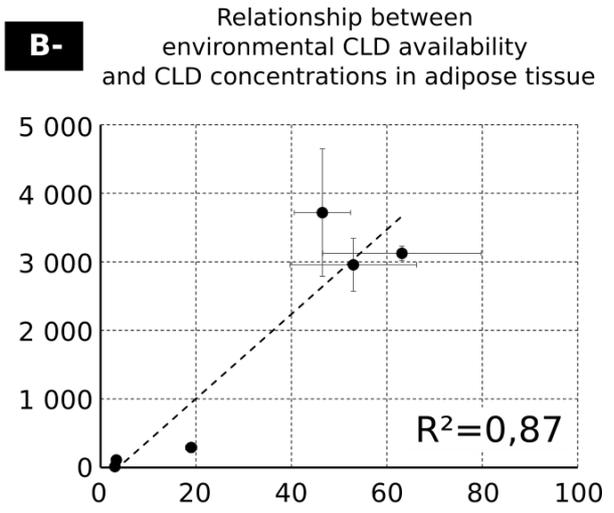
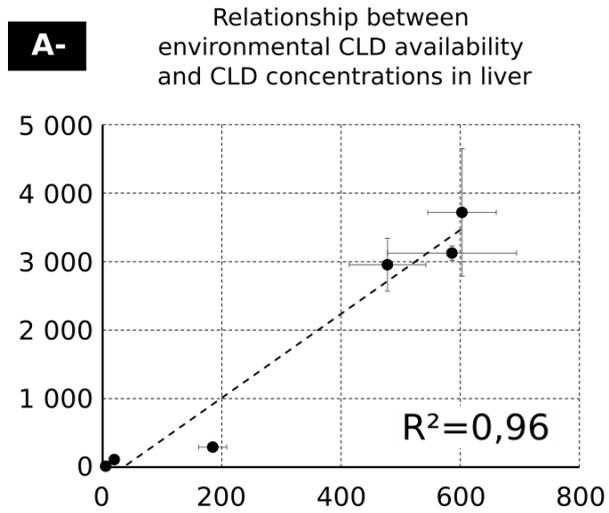


Figure 5. Correlation of environmental bioavailability data and CLD concentration data in liver (A) and adipose tissue (B).

Concentrations of CLD in organs are expressed in ng g⁻¹ of DM. Values correspond to the mean ± SD (n = 4) except S and SP groups (n = 6). Availability data are expressed in ng mL⁻¹ (n = 3).

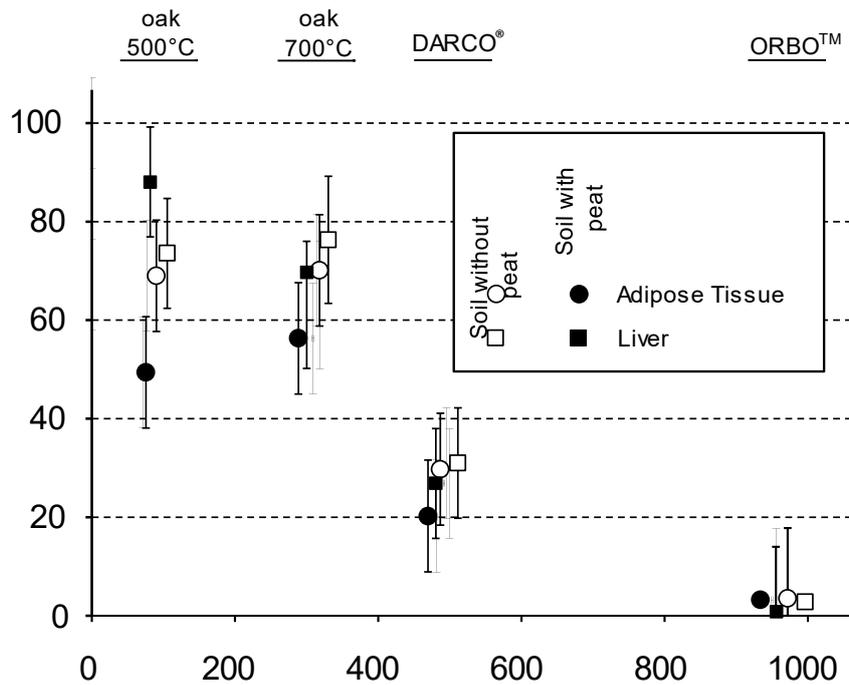


Figure 6. Microporosity surface of biochars and activated carbons and measured bioavailability.

x-axis corresponds to the measured specific surface areas ($\text{m}^2 \cdot \text{g}^{-1}$). y-axis corresponds to the mean of bioavailability and error bar 95th confidence interval ($n = 4$). Each circle.

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