Efficiency assessment of soil amendment with biochars and activated carbons to limit CLD transfer to animal using in vitro and in vivo assays

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1. Introduction

Chlordecone (CLD) is a chlorinated ketone pesticide formerly used to fight the banana black weevil (*Cosmopolites sordidus*) in Martinique and Guadeloupe (French West Indies). Large quantities of this pesticide were spread and superficial layers of large areas of agricultural soils appear to be highly contaminated (>1 mg of CLD /Kg of DM) [1].

In this French West Indies context, an efficient strategy aiming to limit the CLD-transfer to food products is needed. In that purpose, the use of porous media to sequestrate contaminants in a non-bioavailable shape could be a promising strategy. This study aims at assessing the retention of CLD during digestive processes of piglets after amendment of soils by biochars or ACs. The usefulness of two *in vitro* tests was assessed during these investigations.

2. Materials and methods

AC and biochar production, acquisition and characterization

Biochars and ACs were produced from 3 different materials (Coconut, Sargasso, Oak wood). A 700°C slow pyrolysis process was realized. Three different activation processes were tested: without activation, using phosphoric acid, using steam. Then, all matrices were characterized using N2 adsorption.

Contaminated soils preparation

A standard soil (SS) contaminated by CLD (50 µg/g of soil DM). Then, piglets were allocated to distinct treatment groups (n=4). (1) One group were exposed to SS without any biochar or ACs. (2) Other groups were exposed to subsamples of SS amended by one of the ACs or biochar (2% of mass soil basis). A 3-week maturation period was performed on all soils before *in vitro* or *in vivo* assays.

In vitro assays

In order to select the most efficient matrix before *in vivo* assays, two *in vitro* tests were used prior *in vivo* testing. The first one assessed the availability of CLD using the ISO/DIS 16751 Part A methodology [2]. The other one consisted to model physiological processes of piglets using a bioaccessibility test as detailed in Li et *al* (2017) [3]. Quantification of CLD was performed using isotopic dilution and GC-MS.

In vivo assay

After 10 days of exposure to soil, piglets were sacrificed. Pericaudal adipose tissue and liver were collected, stored at -20°C and freeze-dried. Quantification of CLD were performed using Liquid LC-MS/MS according to the method LSA-INS-016 [4], in the Departmental Analytical Laboratory of Morbihan (LDA 56, Saint-Ave, France). Limit of quantification (LOQ) was 2.0 µg CLD kg^{-1} in those matrices.

Data analyses

The ANOVA procedure, the Tukey–Kramer post-hoc test and linear regression of R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria) were used. Differences were considered significant at $P<0.05$.

3. Results and discussion

3.1. *In vitro* assays of CLD availability and bioaccessibility

First results of CLD availability and bioaccessibility showed important differences between the treatment groups as presented in Figure 1. In short, biochars obtained from Oak, Coconut or Sargasso after a 700°C

pyrolysis process show same availability and bioaccessibility value than soil without amendment. However soils amended with activated carbons show low levels of bioaccessibility or availability. Overall, both tests displayed similar values for the same amended soil (data not shown).

3.2. *In vivo* assay of CLD availability and bioaccessibility

Concentrations of CLD in biological matrices showed important differences between the treatment groups as presented in Figure 2. As expected, the highest CLD concentrations was obtained in the SS group (without any strategy of sequestration) for both matrices: Similar levels as SS group were found in both groups where oak biochars was amended in aliments (Figure 2). Intermediate concentrations in organs were obtained when animals were exposed to soil containing DARCO and the lowest CLD concentrations were found when piglets were exposed to soil containing ORBO

3.2. Correlation of *in vivo* assays and *in vitro* ones

Then a correlation between *in vivo* and *in vitro* values was performed. First results show a promising correlation between both test and *in vivo* results.

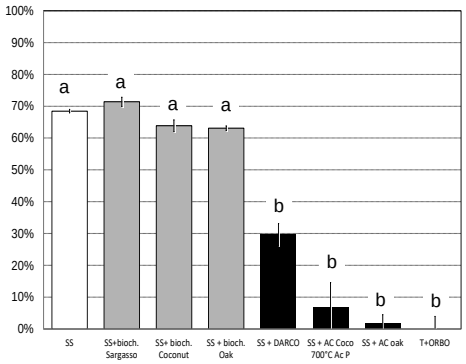

Figure 1: CLD bioaccessibility according amendment (%)
Values correspond to the mean ± SD. Mean values with the same superscript letters (a, b) were no statistically different (P>0.05).

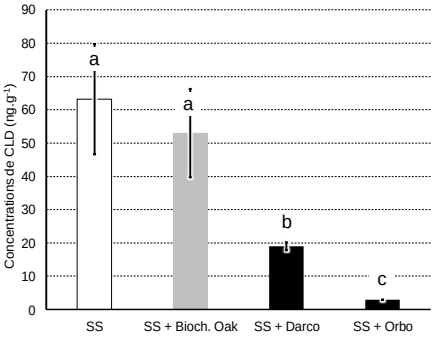

Figure 2: Concentration of CLD in adipose tissue of piglets
Values correspond to the mean ± SD. Mean values with the same superscript letters (a, b) were no statistically different (P>0.05).

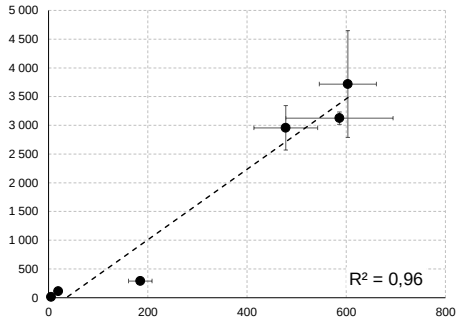

Figure 3: Comparison between availability data and* in vivo *ones
Concentrations of CLD in liver is expressed in $ng.g^{-1}$ of DM (x-axis). Availability data are expressed in ng.mL-1 (n=3).

The surface properties and textural characteristics of ACs drive also the extent of adsorption of CLD. In particular, the surface specific activity is believed to be positively correlated to its binding potentiality [6]. ACs used in the present study are microporous media, even if ORBO© presents a greater part of microporosity (80%) than DARCO© (56%) which can explain the differences of results obtained

Conclusions

This study leads to conclude that (i) AC introduced in CLD contaminated soil should strongly reduces CLD availability; bioaccessibility and bioavailability (ii) Tested biochars showed no reduction of transfert (iii) availability and bioaccessibility tests could be useful screening tests in order to select the appropriate biochar or AC.

4. References

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