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Article

Assessment of an NDL-PCBs Sequestration Strategy in Soil Using Contrasted Carbonaceous Materials through In Vitro and *Cucurbita pepo* Assays

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Featured Application: A sequestration-based remediation strategy using several types of carbonaceous amendments and its potential to reduce transfer to plants.



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Abstract: The present study aims to assess the respective efficiency of Biochars (BCs) and activated carbons (ACs) to limit PCB 101, 138, 153 and 180 transfer to plants. A set of 6 high carbon materials comprising 3 BCs and 3 ACs was tested and used to amend a soil at 2% rate. Then, the two most efficient carbonaceous materials were used as an amendment of an historically contaminated soil sampled in the St Cyprien vicinity (Loire, France). An environmental availability assessment was performed using the ISO/DIS 16751 Part A assay (n = 3). For the in vivo part, *Cucurbita pepo* were grown for 12 weeks. Significant decreases of transfer were found for both assays notably for powdered ACs (up to 98%). By contrast, significantly lower levels of transfer reduction were observed when BCs amendments were performed, ranging from 27 to 80% for environmental availability assessment and 0 to 36% for *C. pepo*. Reduction factors above 90% for the 2 selected materials were found from amended historically contaminated soils. Present results led to consider such a sequestering strategy as valuable to ensure plant production on non-dioxin-like polychlorobiphenyls (NDL-PCBs) contaminated soils.

Keywords: sequestration; transfer; NDL-PCBs; environmental availability

1. Introduction

For a long time, the transfer of highly hydrophobic organic compounds in plants has been neglected, until that many publications claimed that plants are able to take up and accumulate those. Among these plants, species belonging to *Cucurbitaceae* family such as zucchini and pumpkin are able to accumulate polychlorobiphenyls [1,2], dioxins [3] and organochloride pesticides such as chlordecone [4] in roots with high translocation from roots to shoots for varieties of *Cucurbita pepo* [5]. The interception and uptake capacity depend primarily on the physico-chemical characteristics of organic pollutants (including solubility, hydrophobicity and molecular mass), but also on root traits of the species that may influence these criteria [6]. Conditions at the soil-root interface (i.e., rhizosphere) can modify the availability of organic pollutants via the exudation of organic acids as described

in *C. pepo* [7] to favor root interception. The subsequent accumulation of organic pollutants in the root also depends on root characteristics, particularly the composition of membrane and parietal lipids [8] that in relation with hydrophobicity drives the retention in roots. As persistent organic pollutants (POPs) have very marked hydrophobicity characteristics (log Kow greater than 4), in the majority of plant species, these compounds are absorbed and accumulated in the roots without transfer to the vegetative aerial parts and fruits. Nevertheless, some varieties of *Cucurbitaceae* are able to accumulate large amounts of POPs in the aerial parts depending on the number and the position of the non-dioxin-like polychlorobiphenyls (NDL-PCBs) chlorine atoms [1,5].

Thus, acting directly on this transfer to plants would prevent the contamination of food-products. A possible strategy to reduce this halogenated POP transfer to vegetal is to trap them in the soil by making them non-available. In this perspective, several amendment materials have already been evaluated and proven to be effective to limit the POP transfer, as assessed on root vegetables [9,10] or through an in vitro assay [11,12]. To limit transfer to plant, compost amendment of chlordecone (CLD) contaminated soils displayed a reduction of transfer to root vegetables by a factor of 3 to 10 compared to non-amended soils [13]. However, such data is lacking for other POPs, and also for plants known to accumulate halogenated POPs inside their aerial parts like *Cucurbitaceae* family. Activated carbons (ACs) amendment showed also a reduction up to 90% in non-physiological based in vitro environmental availability assays [12,14]. Some studies reported the ability of activated carbons and biochars to trap and reduce the transfer of pesticides, PCBs and other organic pollutants in cucurbits [15–19].

Thus, the subject of the present study is to assess the efficiency of a trapping strategy when using different high carbon matrices as amendment material to ensure plant production on polluted agricultural land. In this frame we aimed to focus on Non-Dioxin-like Polychlorobiphenyls (NDL-PCBs).

2. Materials and Methods

2.1. Experimental Design

The experimental design aimed at assessing an NDL-PCBs sequestration strategy using AC or BC amendment on historically contaminated soils. As several distinct matrices were produced, a preliminary step was to select the most efficient sequestering materials before validation on a soil originating from a known historically contaminated site. Thus, the first step was carried out on an Organization for Economic Co-operation and Development (OECD) soil and the second on a soil originating from St Cyprien (Loire, France), a well-described PCBs contaminated site. In the 22 August 2008 a fire broke on this former facility storing large quantities of contaminated oil. This fire event produced a contamination of the agricultural areas nearby resulting in PCB impregnation of soil and subsequently led to the destruction of various locally produced foodstuffs, including vegetables. Recent contamination data of the site are provided elsewhere [20] and several studies were performed on various soil samples collected from this site during the past decade [20–22]. For each step, the environmental availability and the NDL-PCB transfer to aerial parts (leaves, stems, fruits, flowers) of *Cucurbita pepo* were assessed. Finally, the ability of the quick and cheap in vitro assay to predict the in vivo assay was discussed.

2.2. Production and Acquisition of Condensed Materials

A set of 6 highly carbonaceous materials was obtained from two distinct sources: (i) 3 ACs were obtained from a furnisher (ROTH Sochiel E.U.R.L., Lauterbourg, Grand Est, France) and (ii) 3 biochars were produced by CARBOFRANCE (Montier-sur-Saulx, Lorraine, France). Biochars were produced by pyrolysis using the industrial-scale ovens of CARBOFRANCE (Montiers-sur-Saulx, Lorraine, France). Oak stems originated from various locations of the East part of France, to ensure comparability with the raw material of this industry. Japanese knotweeds (*Reynoutria japonica*) were collected in Laxou (Lorraine, France) using an apparatus designed by Lorexam (Ludres, Lorraine, France).

Ovens were set to perform a pyrolysis at 500 °C or 700 °C. After the pyrolysis process, biochars samples were ground and sieved until <500 µm before their use. Brief description of the condensed materials is provided in Supplemental Information (Table S2) and characterization details of the media are provided elsewhere [11,23].

2.3. Soils Preparation

Two distinct types of soils (OECD soil and St Cyprien soil) were used for this two-step study. Their preparation was carried out at GISFI (Groupement d'Intérêt Scientifique sur les Friches Industrielles, Homécourt-France) and aging was carried out at the Bio-DA (Bioavailability-Bioactivity) platform.

For the first step on OECD soil, two distinct contaminated sets of OECD soils were produced: The first was contaminated by a mix of PCBs 101, 138, 153 and 180 whereas the second remained uncontaminated. An amount of 5% of the total mass of sand was sub-sampled and spiked to achieve the chosen NDL-PCBs concentrations in OECD soil. The spiking methodology used is fully described elsewhere [23]. All constituents of this soil were added and mixed accordingly to the OECD guideline 207 (Table 1) [24]. Concerning the sampled soil from St Cyprien contaminated site (Saint Cyprien, Loire, France), soil was air dried and sieved until <2 mm prior utilization.

Table 1. Composition of the different set of soils. Each percentage represents mass proportion.

Soil Set		Sand	Kaolin	<i>Sphagnum</i> Peat	St Cyprien Soil	Activated Carbon	Biochar
Artificial soils	Standard Soil (SS)	70%	20%	10%			
	PCB 101, PCB 138, PCB 153, PCB 180	SS with oak tree 500 (BC1)	68.6%	19.6%	9.8%		2%
		SS with oak tree 700 (BC2)	68.6%	19.6%	9.8%		2%
		SS with Japanese knotweed (BC3)	68.6%	19.6%	9.8%		2%
		SS with AC1 (AC1)	68.6%	19.6%	9.8%		2%
		SS with AC2 (AC2)	68.6%	19.6%	9.8%		2%
		SS with AC3 (AC3)	68.6%	19.6%	9.8%		2%
	Control- without pollutant	Control	70%	20%	10%		
Sampled soil on an historically contaminated site (St Cyprien, Loire, France)	Standard Soil (SS)				100%		
		SS with AC2			98%	2%	
		SS with AC3			98%	2%	

Then, OECD soil was left to age for 1 month prior to sub-sampling it to 7 aliquots. Finally, 6 out the 7 soil-aliquots were amended by 2% of one of the highly condensed matrices, thoroughly mixed and allowed to age for three additional months. A similar sub-sampling procedure was applied to St Cyprien soil to obtain 3 aliquots. A similar proportion of the two most efficient matrices from the first step was amended. Composition of each soil set and the NDL-PCBs concentrations are presented in Table 1.

2.4. Environmental Availability Assays

Using a balance, a 4 g of test sample of fresh soil was placed in a 50 mL falcon tube. Then a 40 mL volume of a 100 mmol·L⁻¹ Hydroxypropyl-β-cyclodextrin (Kleptose HP oral grade, Roquette, France) extraction solution was added to the tube and shaken horizontally for 20 h (180 rpm, 20 ± 2 °C). Samples were centrifuged at (3000× g for 15 min) to separate the solid phase from the liquid phase (clear supernatant). Supernatant corresponding to the available fraction was extracted 3 times using 20 mL of petroleum ether. The collected petroleum ether was then evaporated until dry and 150 µL of nonane was added to recover analytes. Extracts were quantified as described in [11]. For each soil, 4 replicates were performed.

2.5. Plants Cultivation of *C. pepo*

Seeds of *C. pepo* (Diamant cultivar.) were sown in cups of potting soil and then transplanted at the 1st-leaf stage into 15-cm-diameter pots containing either OECD or historically contaminated soils corresponding to the different tested modalities. Each pot contained between 400 and 500 g of soil and one plant. Plants were grown in a greenhouse under these following controlled environmental conditions cooling above 20 °C. All pots were adjusted each week to 80% of the water holding capacity. After 12 weeks, all aerial parts were harvested, weighted and frozen at −20 °C before analysis.

2.6. Analytical Processes

All chemicals used during the analytical processes were of Pesticide Residues grade and glassware equipment was cleaned with an alkaline detergent (RBS T 105, Carl Roth GmbH, Karlsruhe, Germany).

2.6.1. Extraction Processes of Plant Samples

Biological matrices were extracted by Pressurized-Liquid Extraction (PLE) using a Büchi E-916 Speed-extractor with pure toluene, a temperature of 50 °C, a pressure of 135 Bars and 3 extraction cycles of 5 min.

2.6.2. Purification Step

Potential chromatographic interferences were removed from extracts using Multi-layer silica column (28397-U, Merck KGaA, Darmstadt, Germany). The silver nitrate treated layer removes sulphur-containing compounds; whilst two sulphuric acid treated layers oxidise sample lipids and remove any basic analytes. The potassium hydroxide treated layer removes any acidic sample components. NDL-PCB pass through the silica column unretained. Each extract was evaporated until dryness and dissolved in nonane.

2.6.3. Quantitation Processes

Quantitation of NDL-PCBs was performed on the extracts of the soil environmental availability and plants using a Gas Chromatography (Agilent 6890N, Santa Clara, CA, USA)-High Resolution Mass Spectrometry (Quattro Ultima, Waters, Milford, CT, USA) device in Laboratoire d'Analyse des Sols (Arras, Hauts de France, France). Quantitation was performed by selecting the two most abundant molecular ions for each organic compound, by isotopic dilution and at a resolution close to 10,000. The determination was performed in SIR (Single Ion Recording) mode and ionization by electron impact in low energy positive mode (35.5 eV) and with a trap current between 400 and 600 µA. Program of the column (DB-5MS 60 m, 250 µm internal diameter, 0.25 µm active phase thickness, Agilent J&W, Santa Clara, CA, USA) consisted of successive temperature gradients: 100 °C (1 min); 40 °C·min^{−1} until 200 °C (6 min); 3 °C·min^{−1} until 235 °C (10 min); 8 °C·min^{−1} until 315 °C (9 min). Temperature of the transfer line was set to 250 °C.

2.6.4. Quality Control of the Analytical Processes

Quantitation was performed using isotopic dilution by adding ¹³C internal standards (EC9605-SS, Wellington Laboratories, Guelph, ON, Canada) as described in Environment Canada Method 1/RM/31. Ratio of the molecular ions area of the parent compounds over internal standards of the same chlorine number allowed the quantitation after calibration (Regression coefficient R² > 0.99). In addition, perfluorokerosene (lock mass) was added allowing mass calibration of the mass spectrometer during the whole analysis. Recoveries of the whole analytical steps above 70% were obtained throughout analytical runs using recovery standards (EC9605-RS, Wellington Laboratories, Guelph, ON, Canada). In each run, chromatographic and method blanks were introduced showing no cross-contamination (all < LOQs). A quality control using an independent standard solution (ECPCS3 at 200 µg·L^{−1}, Wellington Laboratories, Guelph, ON, Canada) was introduced showing a <20% deviation. Limits of quantitation for 2 g of soil or plants are 0.2 µg·kg^{−1} for each NDL-

PCB. Each step of the analytical process was performed as documented in the COFRAC quality accreditation of LAS laboratory for the NDL-PCBs quantitation of soil samples.

2.7. Data Analysis

2.7.1. Statistical Analyses

All analyses were carried out using R software (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria). In order to assess the impact of BCs and ACs on pollutant concentrations in both assays from both steps, a Dunnett test (using the package multcomp, Hothorn 2020, version 2) was used in order to compare reductions of NDL-PCBs concentrations against their respective non-amended control (SS) ($n = 3$ for environmental availability and $n = 5$ for plant assay). Then, the ANOVA procedure followed by the Tukey–Kramer post-hoc test (using the package agricolae, Mendiburu 2020, version 1.3.3) were used in order to compare the efficiency of matrices on the groups found significant in the previous test. Differences were considered significant at $p < 0.05$.

2.7.2. Calculations of Relative Environmental Availability and Bioavailability Factors

For each pollutant, relative environmental availability and relative bioavailability factors were calculated as the ratio of each pollutant's concentration from tested amended soil compared to their respective non-amended SS soil as described previously [25].

3. Results

3.1. Test on OECD Soils

A first set of experiments was performed on OECD artificial soil in order to select the most efficient matrices upon their ability to sequester NDL-PCBs.

3.1.1. Environmental Availability

The *in vitro* assay performed on artificial soils demonstrated the efficiency of the amendment strategy to reduce the NDL-PCBs leaching from soil. Indeed, for PCB 101, 138, 153 and 180, environmental availability concentrations from amended soils were significantly lower than those from the non-amended control group (Figure 1), excluding biochar-amended groups for PCB 180. PCB 101 appeared the most susceptible compound to this strategy. Indeed, contrarily to other congeners, even biochar amended soils presented over 50% reduction (62 to 80%, mean) of environmental availability concentrations (Figure 1). Such amendment led to significant but lower reduction for the three most lipophilic compounds: 35 to 42% (mean) for PCB 138, 37 to 42% for PCB 153 and 27 to 33% for PCB 180. As expected, NDL-PCBs were far more sequestered when AC was amended but contrasted results were obtained from granular AC1 compared to powdered AC2, AC3. Indeed, AC1 displayed the lowest reduction potential among ACs as no significant distinction was found between this AC and BC amendment ($p > 0.10$). In contrast, AC2 and AC3 amendment presented the most intense sequestration for all congeners as environmental availability levels were similar to the control levels, i.e., the analytical quantitation limit for each congener. Due to these analytical limits, only minimal reduction factors could be derived from this assay: 79% for PCB 101, 91% for PCB 138, 88% for PCB 153 and 45% for PCB 180. Thus, from this environmental availability assay, the following efficiency hierarchy (reduction of transfer potential) was found between the used sequestration materials: $BC1 = BC2 \leq BC3 = AC1 \ll AC2 = AC3$.

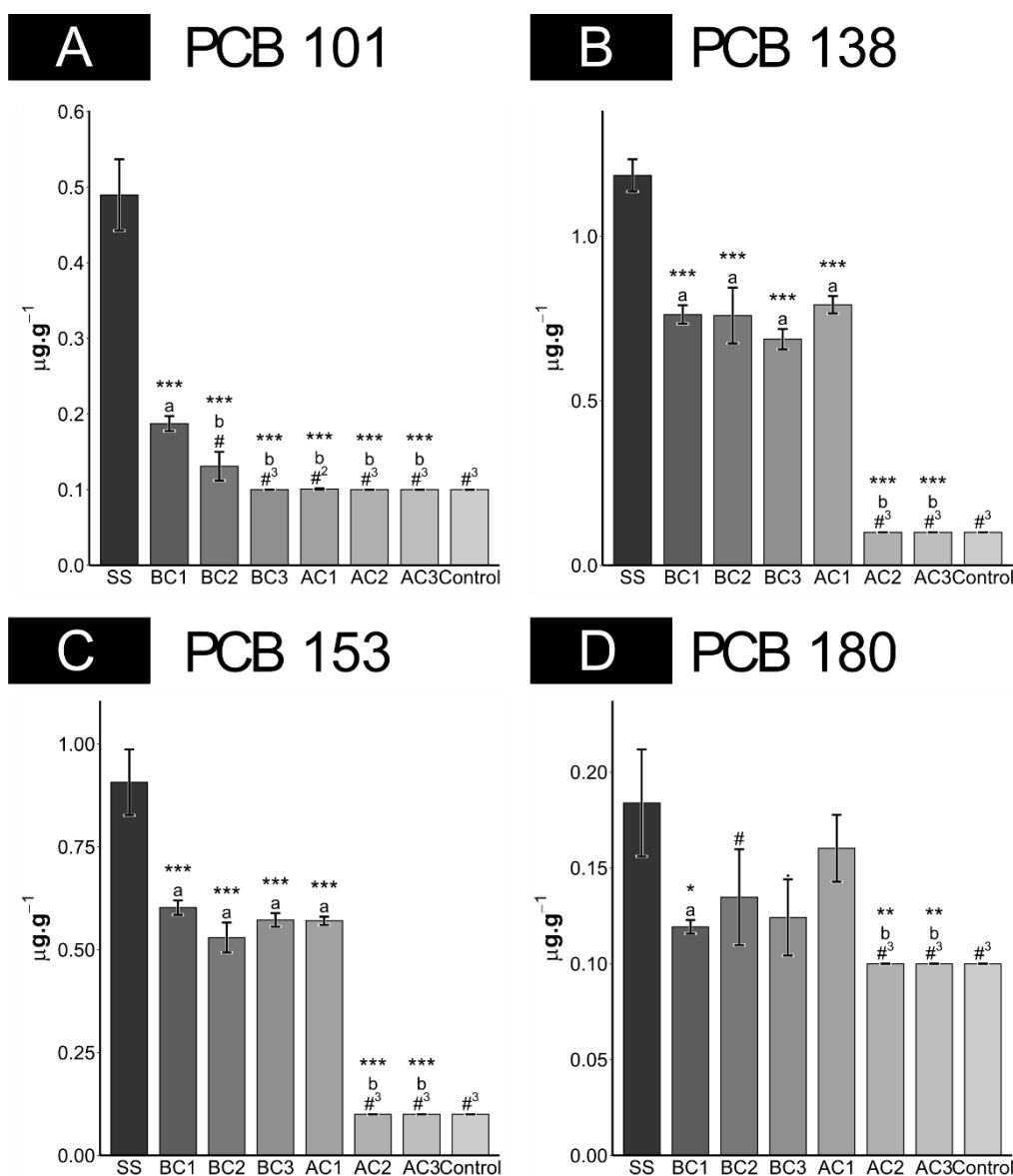


Figure 1. Environmental available concentrations of polychlorobiphenyls (PCB) PCB 101, PCB 138, PCB 153 and PCB 180 present in artificial soil. Values correspond to the mean \pm Standard Error (SE) ($n = 3$) concentrations of (A) PCB 101, (B) PCB 138, (C) PCB 153 and (D) PCB 180 obtained through environmental availability assay. Group mean values with superscript asterisks are statistically different from non-amended but contaminated Standard Soil (SS) (0.05: * > 0.01: ** > 0.001: ***) using a variance analysis and a Dunnett post-hoc test. Controls, representing soil without any spiking of the pollutants, were not included in the tested dataset. Groups mean concentrations with different superscript letters (a, b) are statistically different ($p < 0.05$) from each other using a complementary variance analysis followed by a Tukey post-hoc test on previously found significant groups using Dunnett's. #: Values below Limits of Quantitation (LOQs) were replaced by the corresponding LOQ, superscript numbers indicate the number of replaced values.

3.1.2. *Cucurbita pepo* Assay

The *in vivo* assay on *C. pepo* was performed on the same soils from the *in vitro* assay. Due to technical difficulties inherent to the experimental design (weak soil quantity in each pot), some repetition did not grow properly. Thus, 4 repetitions were obtained from BC2 and BC3 groups, whereas only 1 repetition was obtained from AC3 group instead of the 5 repetitions sown for each modality. As the characteristics of the AC amendment could also strongly influence nutrient availability and water retention, they can interfere

with plant development. Considering transfer, and similarly to previous environmental availability results, BCs and AC1 were the less effective materials. Results were even more contrasted as for all studied NDL-PCBs no significant reductions were found for these materials compared to the SS control group (Figure 2). At the opposite, AC2 and AC3 were also the most effective matrices and interestingly, levels of reduction found were greater than previous measurements as the analytical quantitation limits were far lower than concentrations found in the non-amended control group. Thus, the minimal reduction factors which could be derived from this assay were the following: 93% for PCB 101, 95% for PCB 138, 95% for PCB 153 and 92% for PCB 180. Thus, from this in vivo assay, the following efficiency hierarchy was found between the used sequestration materials: BC1 = BC2 = BC3 = AC1 \ll AC2 = AC3.

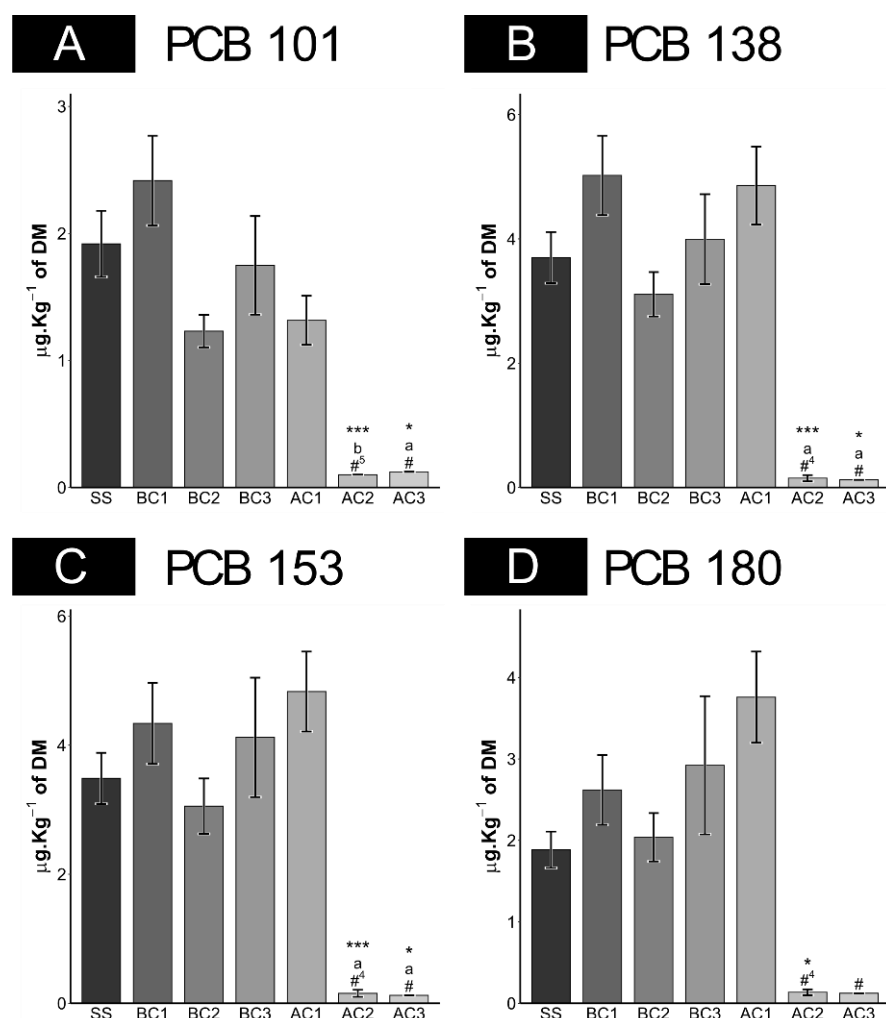


Figure 2. In vivo concentrations of PCB 101, PCB 138, PCB 153 and PCB 180 in aerial parts of *Cucurbita pepo*. Values correspond to the mean \pm SE (n = 5) concentrations of (A) PCB 101, (B) PCB 138, (C) PCB 153 and (D) PCB 180 obtained through the in vivo *Cucurbita pepo* assay. Group mean values with superscript asterisks are statistically different from non-amended soil (SS) (0.05: * > 0.01; ** > 0.001; ***) using a variance analysis and a Dunnett post-hoc test. Controls were not included in the tested dataset. Groups mean concentrations with different superscript letter (a, b) are statistically different ($p < 0.05$) from each other using a complementary variance analysis followed by a Tukey post-hoc test on previously found significant groups using Dunnett's. #: Values below LOQs were replaced by the corresponding LOQ, superscript numbers indicate the number of replaced values. DM stands for Dry Matter.

3.1.3. Selection of the Sequestering Materials

Similar efficiency trends were found between both assays showing that AC2 and AC3 were the two most efficient media. These two media were used as amendment sources for the St Cyprien contaminated soil. However, as AC3 may also present a negative effect on the *C. pepo* growth, AC2 only was assessed in vivo to validate this amendment strategy on an historically contaminated soil.

3.2. Validation on an Historically Contaminated Soil

3.2.1. Environmental Availability

For the first experiment on an historically contaminated soil, the environmental availability in vitro assay confirmed an important sequestration efficiency for NDL-PCBs 138, 153 and 180 (Figure 3). As all results concerning PCB 101 were below LOQs, including non-amended soil (SS), no sequestration potential could be derived for this specific compound. For the three most lipophilic compounds, intense reductions were obtained as all environmental values except one (PCB 138, AC2 amendment) were below LOQs. Thus, high levels of reduction were obtained and a minimum reductions factor of (i) 83 to 90% (mean) for PCB 138, (ii) 91% for PCB 153 and (iii) 27 to 44% for PCB 180 were obtained (Table 2). These results provided evidence that even in a known historic contamination context (over a decade between contamination context and sampling of the soil) such a strategy could efficiently limit the environmental availability of these pollutants.

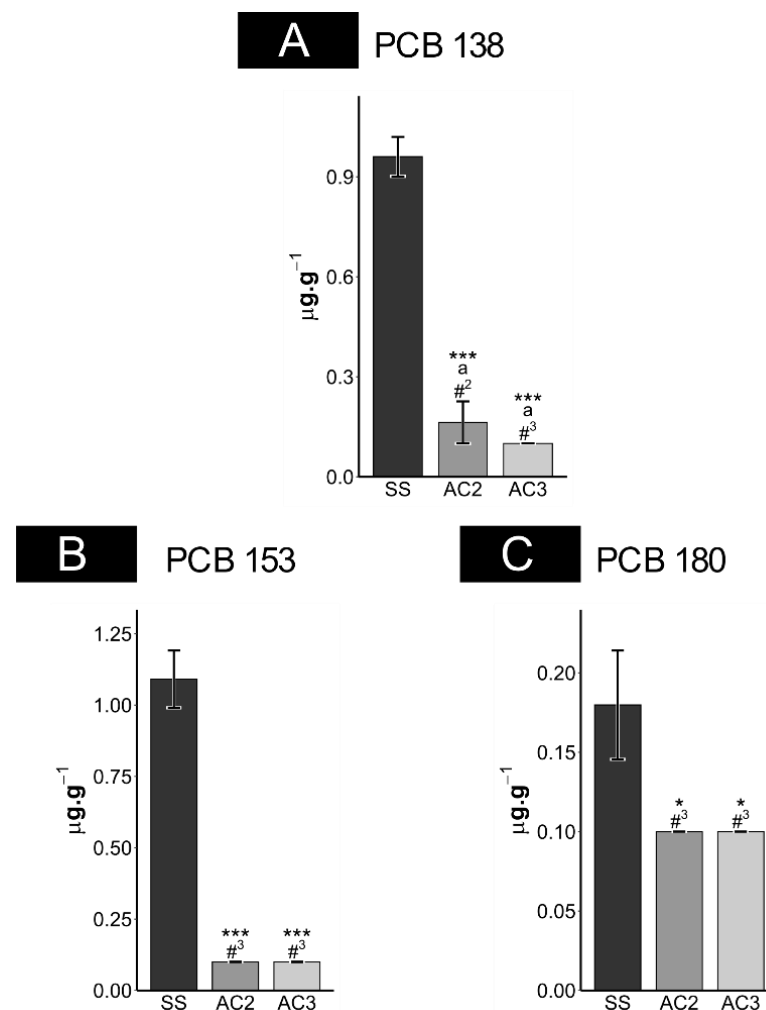


Figure 3. Environmental available concentrations of PCB 138, PCB 153 and PCB 180 present in St Cyprien soil. Values correspond to the mean \pm SE ($n = 3$) concentrations of (A) PCB 138, (B) PCB 153 and (C) PCB 180 obtained through the environmental availability assay. As all concentrations

obtained for PCB 101 were below LOQ, data were not analyzed. Group mean values with superscript asterisks are statistically different from non-amended St Cyprien soil (SS) (0.05: * > 0.01: ** > 0.001: ***) using a variance analysis and a Dunnett post-hoc test. Groups mean concentrations with different superscript letters (a) are statistically different ($p < 0.05$) from each other using a complementary variance analysis followed by a Tukey post-hoc test on previously found significant groups using Dunnett's. #: Values below LOQs were replaced by the corresponding LOQ, superscript numbers indicate the number of replaced values.

Table 2. Transfer reduction found on an historically contaminated soil.

			Mean Concentrations in $\mu\text{g}\cdot\text{g}^{-1}$	Min-Max Concentrations in $\mu\text{g}\cdot\text{g}^{-1}$ (Number of Repetition)	Mean or Minimal Reduction Factor (a)
Environmental availability	PCB101	SS	0.10	all values < LOQ (n = 3)	
		AC2	0.10	all values < LOQ (n = 3)	0% ^a
		AC3	0.10	all values < LOQ (n = 3)	0% ^a
	PCB138	SS	0.96	[0.84–1.03] (n = 3)	
		AC2	0.16	[0.10–0.29] (n = 3)	83%
		AC3	0.10	all values < LOQ (n = 3)	90% ^a
	PCB153	SS	1.09	[0.96–1.29] (n = 3)	
		AC2	0.10	all values < LOQ (n = 3)	91% ^a
		AC3	0.10	all values < LOQ (n = 3)	91% ^a
	PCB180	SS	0.18	[0.12–0.23] (n = 3)	
		AC2	0.10	all values < LOQ (n = 3)	44% ^a
		AC3	0.10	all values < LOQ (n = 3)	44% ^a
In vivo bioavailability	PCB138	AC2	0.10	all values < LOQ (n = 4)	94% ^a
		SS	1.73	[0.86–2.73] (n = 5)	
	PCB153	AC2	0.10	all values < LOQ (n = 4)	96% ^a
		SS	2.23	[1.05–3.59] (n = 5)	
	PCB180	AC2	0.10	all values < LOQ (n = 4)	90% ^a
		SS	1.04	[0.56–1.63] (n = 5)	

^a: Reduction factor.

3.2.2. *Cucurbita pepo* Assay

The second set of experiments on AC2 amended soil was performed on *Cucurbita pepo* plantations (n = 4) (Figure 4). Similarly, to environmental availability results, high reduction of NDL-PCBs transfer was observed for amended soil, except for PCB 101 for which all results, even for non-amended soil, were below LOQs. Minimal reduction levels derived from this assay were the following: (i) 94% (mean) for PCB 138, (ii) 96% for PCB 153 and (iii) 27 to 90% for PCB 180, confirming the reduction levels found on OECD soils.

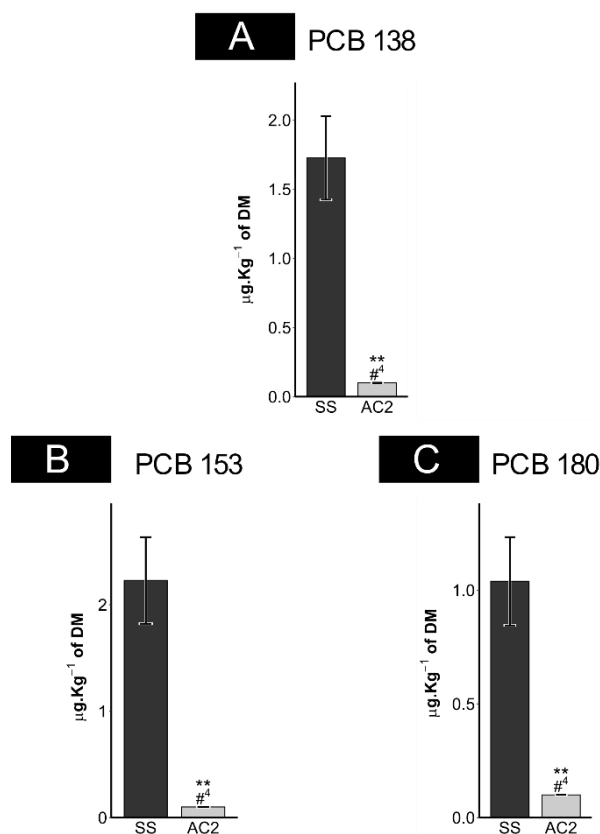


Figure 4. In vivo concentrations of, PCB 138, PCB 153 and PCB 180 present in *C. pepo* aerial parts when grown on St Cyprien soil. Values correspond to the mean \pm SE ($n = 4$) concentrations of (A) PCB 138, (B) PCB 153 and (C) PCB 180 obtained through the in vivo *Cucurbita pepo* assay. As all concentrations obtained for PCB 101 were below LOQ, data were not analyzed. Group mean values with superscript asterisks are statistically different from non-amended soil (SS) (0.05: * $>$ 0.01: ** $>$ 0.001: ***) using a variance analysis and a Dunnett post-hoc test. #: Values below LOQs were replaced by the corresponding LOQ, superscript numbers indicate the number of replaced values.

4. Discussion

4.1. In Vitro Assessment of Interactions between Pollutants and Carbonaceous Materials

The environmental availability test was used to express the portion of the pollutant that is desorbed from the soil and available in the soil solution for interception and uptake by plant roots. Considering this test, overall, BCs were less effective than ACs in trapping PCBs due to their limited microporous and mesoporous surfaces. The efficiency was of the same order in both soils, suggesting that the physicochemical properties of the soils did not influence this efficiency and that the OECD artificial soil realistically mimics the behavior of natural soil. There was no impact of biochar type regardless of the PCB considered, suggesting that the nature of the biomass and pyrolysis temperature did not influence sequestration potential even though BC1 had a 10-fold lower specific surface area than the other two.

For AC materials, AC2 and AC3 were the most effective in reducing PCB concentrations given their larger specific surface area and mesoporous surface, while AC1 was less effective due to its granular shape. This result confirms that surface properties and textural characteristics impact the sorption properties of carbonaceous materials in contaminated soils [11,12,14,26,27]. Regarding the type of pollutants, no difference could be shown in terms of sequestration efficiency by the different carbonaceous materials probably because the physicochemical properties related to adsorption in soil such as lipophilicity are of the same order (about $\log K_{ow}$ of 7).

4.2. Effect of Plant on the Interactions between Pollutants and Carbonaceous Materials

The former environmental availability assay reflects the ability of BC and AC to increase pollutant sorption on these materials, but it did not consider plant physiological processes that may influence this interaction. Among plants, it is well known that the Cucurbita family accumulate considerable amounts of POPs in their shoots [28]. For this reason, *C. pepo* was chosen in this study because it highly extracts and accumulates POPs including PCB from contaminated soils [2,29–33]. Plant is a major driver of pollutants availability through a variety of root functions such as uptake, exudation that can alter biogeochemical parameters of the soil (e.g., pH and redox potential, concentrations of pollutants, complexing or chelating compounds) in the vicinity of the roots [34]. Roots release protons, responsible for a substantial decrease of rhizosphere pH, that could in turn change the sorption of PCB congeners on soil substrate that is maximum between pH 6.5 and 7.5 [35]. Root exudates like organic acids also play a great role on the sorption/desorption of pollutants from soil components and organo-mineral complexes and subsequently on their availability for plant uptake (Figure 5). The amendment of soil with citric acid that is strongly exuded in *Cucurbita pepo* ssp. *pepo* allowed a significant increase of shoot PCB concentration [36] probably due to PCB desorption [32] and/or to root absorption of PCB via complexation [7]. POPs are transported from roots to shoots via the xylem sap [5,37,38] and other transfer pathways to shoots are negligible such as direct soil contamination, atmospheric deposition and volatilization from soil and subsequent redeposition on shoots [39]. Our results showed that only the addition of activated carbons to the soil decreases significantly POPs transfer to shoot and thus their extraction from soil by *C. pepo* roots. Previous studies also showed a reduction of PCB concentration in *C. pepo* shoot tissue: 63% of PCB (Aroclor 1254) with 12.5% AC amendment [18] and 54% of PCB with 11.1% BC amendment [16]. Furthermore, the addition of AC (0.1 and 0.23%; *w/w*) reduced from 73% to 98% of heptachlor epoxide in the shoot of winter squash (*Cucurbit maxima*) [40]. AC amendment (0.02 to 0.08%) also reduced up to 66% of the dieldrin concentration in cucumbers (*Cucumis sativus* L.) [17].

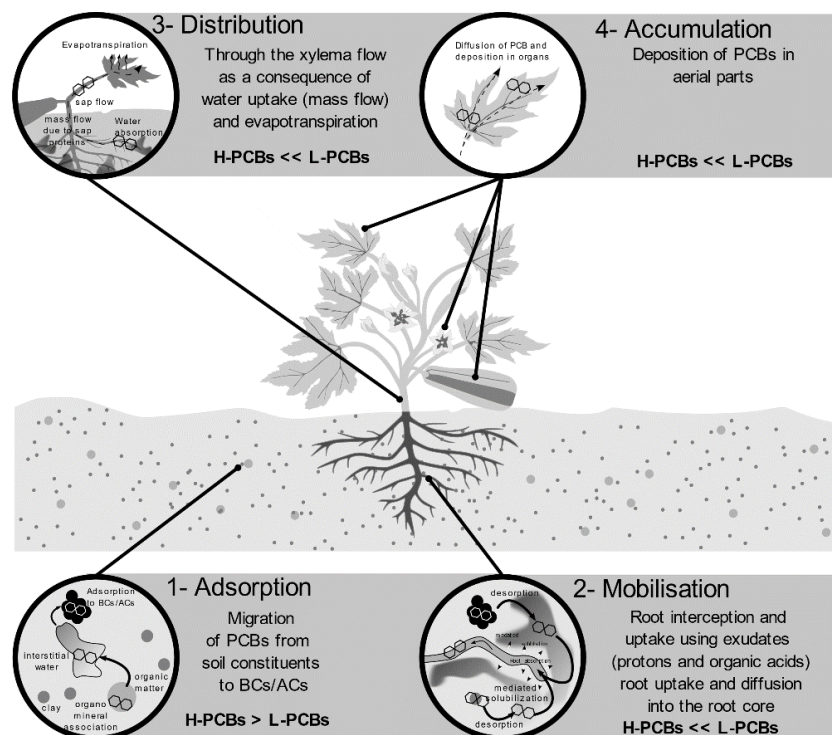


Figure 5. In vivo processes leading to PCB contamination of *C. pepo* aerial parts. Respective tendencies of each category of PCBs (high weight NDL-PCBs–H-PCBs as PCB 180, 153 and 138 or Low weight NDL-PCBs–L-PCBs as PCB 101) to follow each step were mentioned.

It may be hypothesized that reduction of PCB concentration in shoot tissue is due to the sorption of PCB into the surface of activated carbon, which prevent these molecules from being available in the soil pore water and absorbed into the root, and thus translocated from the root to shoot tissue. This assay involves the use of a sorbent phase acting as an infinite sink for pollutants. Concerning the in vivo assays, the present study used *C. pepo* as a model plant which extracts pollutants from the water solution and accumulates them in aerial parts.

5. Conclusions

Sequestration strategy involving high carbonized material appeared to significantly reduce the transfer of NDL-PCBs to *Cucurbita pepo* aerial parts, appearing promising to ensure vegetable cultivation. However, no significant reduction of transfer was observed when BC was used, few when granular AC was amended, showing that only powdered AC may be suitable to that purpose. In that particular case, reduction levels above 90% have been observed from these particularly micro- and mesoporous media. Regardless of the type of soil, in vivo and in vitro experiments showed a noticeable similarity of responses leading to consider that (i) the congener effect (ii) the nature of the sequestering material were the main parameters playing on the efficiency of such an amendment strategy. The convenient in vitro assay showed an interesting correlation needing to be further assessed to predict in vivo transfer to *Cucurbita pepo* plants. Such an assay appears as a promising tool in the frame of testing before the in situ application of such materials.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app12083921/s1>, Table S1: Physico-chemical characterization of ACs and BCs; Table S2: Transfer reduction found on an OECD soil.

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References

1. Inui, H.; Wakai, T.; Gion, K.; Kim, Y.-S.; Eun, H. Differential Uptake for Dioxin-like Compounds by Zucchini Subspecies. *Chemosphere* **2008**, *73*, 1602–1607. [[CrossRef](#)]
2. Zeeb, B.A.; Amphlett, J.S.; Rutter, A.; Reimer, K.J. Potential for Phytoremediation of Polychlorinated Biphenyl-(PCB)-Contaminated Soil. *Int. J. Phytoremediation* **2006**, *8*, 199–221. [[CrossRef](#)]
3. Hülster, A.; Marschner, H. Transfer of PCDD/PCDF from Contaminated Soils to Food and Fodder Crop Plants. *Chemosphere* **1993**, *27*, 439–446. [[CrossRef](#)]

4. Clostre, F.; Letourmy, P.; Turpin, B.; Carles, C.; Lesueur-Jannoyer, M. Soil Type and Growing Conditions Influence Uptake and Translocation of Organochlorine (Chlordecone) by Cucurbitaceae Species. *Water Air Soil Pollut.* **2014**, *225*, 2153. [CrossRef]
5. Greenwood, S.J.; Rutter, A.; Zeeb, B.A. The Absorption and Translocation of Polychlorinated Biphenyl Congeners by *Cucurbita pepo* Ssp *Pepo*. *Environ. Sci. Technol.* **2011**, *45*, 6511–6516. [CrossRef]
6. Zhang, C.; Feng, Y.; Liu, Y.; Chang, H.; Li, Z.; Xue, J. Uptake and Translocation of Organic Pollutants in Plants: A Review. *J. Integr. Agric.* **2017**, *16*, 1659–1668. [CrossRef]
7. White, J.C.; Mattina, M.I.; Lee, W.-Y.; Eitzer, B.D.; Iannucci-Berger, W. Role of Organic Acids in Enhancing the Desorption and Uptake of Weathered *p,p'*-DDE by *Cucurbita pepo*. *Environ. Pollut.* **2003**, *124*, 71–80. [CrossRef]
8. Paterson, S.; Mackay, D.; Tam, D.; Shiu, W.Y. Uptake of Organic Chemicals by Plants: A Review of Processes, Correlations and Models. *Chemosphere* **1990**, *21*, 297–331. [CrossRef]
9. Fernandes, P.; Jannoyer-Lesueur, M.; Soler, A.; Achard, R.; Woignier, T. Effects of Clay Microstructure and Compost Quality on Chlordecone Retention in Volcanic Tropical Soils: Consequences on Pesticide Lability and Plant Contamination. In Proceedings of the 19th World Congress of Soil Science, Soil Solutions for a Changing World, Brisbane, Australia, 1–6 August 2010.
10. Woignier, T.; Duffours, L.; Colombel, P.; Dieudonné, P. Nanoporous Clay with Carbon Sink and Pesticide Trapping Properties. *Eur. Phys. J. Spec. Top.* **2015**, *224*, 1945–1962. [CrossRef]
11. Delannoy, M.; Yehya, S.; Techer, D.; Razafitianamaharavo, A.; Richard, A.; Caria, G.; Baroudi, M.; Montargès-Pelletier, E.; Rychen, G.; Feidt, C. Amendment of Soil by Biochars and Activated Carbons to Reduce Chlordecone Bioavailability in Piglets. *Chemosphere* **2018**, *210*, 486–494. [CrossRef]
12. Ranguin, R.; Jean-Marius, C.; Yacou, C.; Gaspard, S.; Feidt, C.; Rychen, G.; Delannoy, M. Reduction of Chlordecone Environmental Availability by Soil Amendment of Biochars and Activated Carbons from Lignocellulosic Biomass. *Environ. Sci. Pollut. Res.* **2020**, *27*, 41093–41104. [CrossRef]
13. Woignier, T.; Clostre, F.; Fernandes, P.; Rangon, L.; Soler, A.; Lesueur-Jannoyer, M. Compost Addition Reduces Porosity and Chlordecone Transfer in Soil Microstructure. *Environ. Sci. Pollut. Res.* **2015**, *28*, 1–11. [CrossRef]
14. Ranguin, R.; Delannoy, M.; Yacou, C.; Jean-Marius, C.; Feidt, C.; Rychen, G.; Gaspard, S. Biochar and Activated Carbons Preparation from Invasive Algae *Sargassum* Spp. for Chlordecone Availability Reduction in Contaminated Soils. *J. Environ. Chem. Eng.* **2021**, *9*, 105280. [CrossRef]
15. Denyes, M.J.; Rutter, A.; Zeeb, B.A. In Situ Application of Activated Carbon and Biochar to PCB-Contaminated Soil and the Effects of Mixing Regime. *Environ. Pollut.* **2013**, *182*, 201–208. [CrossRef]
16. Denyes, M.J.; Langlois, V.S.; Rutter, A.; Zeeb, B.A. The Use of Biochar to Reduce Soil PCB Bioavailability to *Cucurbita pepo* and *Eisenia fetida*. *Sci. Total Environ.* **2012**, *437*, 76–82. [CrossRef]
17. Hilber, I.; Wyss, G.S.; Mäder, P.; Bucheli, T.D.; Meier, I.; Vogt, L.; Schulin, R. Influence of Activated Charcoal Amendment to Contaminated Soil on Dieldrin and Nutrient Uptake by Cucumbers. *Environ. Pollut.* **2009**, *157*, 2224–2230. [CrossRef]
18. Langlois, V.S.; Rutter, A.; Zeeb, B.A. Activated Carbon Immobilizes Residual Polychlorinated Biphenyls in Weathered Contaminated Soil. *J. Environ. Qual.* **2011**, *40*, 1130–1134. [CrossRef]
19. Mandl, A.; Lindner, W. Organochlorine Pesticide Residues in Styrian Soils and Seeds of the Styrian Oil Pumpkin: An Inventory and a Concept for Their Reduction. *Int. J. Environ. Anal. Chem.* **1999**, *73*, 1–13. [CrossRef]
20. ADEME. TROPHE-Transferts et Risques des Organiques Persistants pour l'Homme et les. ADEME. 2017. Available online: <https://www.ademe.fr/trophe-transferts-risques-organiques-persistants-lhomme-ecosystemes> (accessed on 3 April 2022).
21. Fournier, A.; Feidt, C.; Travel, A.; Bizec, B.L.; Venisseau, A.; Marchand, P.; Jondreville, C. Relative Bioavailability to Laying Hens of Indicator Polychlorobiphenyls Present in Soil. *Chemosphere* **2012**, *88*, 300–306. [CrossRef]
22. Delannoy, M.; Fournier, A.; Tankari Dan-Badjo, A.; Schwarz, J.; Lerch, S.; Rychen, G.; Feidt, C. Impact of Soil Characteristics on Relative Bioavailability of NDL-PCBs in Piglets. *Chemosphere* **2015**, *139*, 393–401. [CrossRef]
23. El Wanny, N.; Le Roux, Y.; Fournier, A.; Baroudi, M.; Woignier, T.; Feidt, C.; Delannoy, M. Organochlorine POPs Sequestration Strategy by Carbonaceous Amendments: Toward a Better Understanding of the Transfer Reduction to Laying Hens. *J. Hazard. Mater.* **2022**; in press. [CrossRef]
24. OECD. Test No. 207: Earthworm Acute Toxicity Test. In *Section 4: Effects on Biotic Systems*; Guideline for Testing of Chemicals; OECD Publishing: Paris, France, 1984; Volume 4.
25. Feidt, C.; El Wanny, N.; Ranguin, R.; Gaspard, S.; Baroudi, M.; Yacou, C.; Rychen, G. In Vitro and in Vivo Assessment of a CLD Sequestration Strategy in Nitisol Using Contrasted Carbonaceous Materials. *Environ. Geochem. Health*, **2021**; in press. [CrossRef] [PubMed]
26. Rittenhouse, J.L.; Rice, P.J.; Spokas, K.A.; Koskinen, W.C. Assessing Biochar's Ability to Reduce Bioavailability of Aminocyclopyrachlor in Soils. *Environ. Pollut.* **2014**, *189*, 92–97. [CrossRef] [PubMed]
27. Saito, T.; Otani, T.; Seike, N.; Murano, H.; Okazaki, M. Suppressive Effect of Soil Application of Carbonaceous Adsorbents on Dieldrin Uptake by Cucumber Fruits. *Soil Sci. Plant Nutr.* **2011**, *57*, 157–166. [CrossRef]
28. Otani, T.; Seike, N.; Sakata, Y. Differential Uptake of Dieldrin and Endrin from Soil by Several Plant Families and *Cucurbita* Genera. *Soil Sci. Plant Nutr.* **2007**, *53*, 86–94. [CrossRef]
29. Low, J.E.; Whitfield Åslund, M.L.; Rutter, A.; Zeeb, B.A. Effect of Plant Age on PCB Accumulation by *Cucurbita pepo* Ssp. *Pepo*. *J. Environ. Qual.* **2010**, *39*, 245–250. [CrossRef]

30. Lunney, A.I.; Zeeb, B.A.; Reimer, K.J. Uptake of Weathered DDT in Vascular Plants: Potential for Phytoremediation. *Environ. Sci. Technol.* **2004**, *38*, 6147–6154. [[CrossRef](#)]
31. White, J.C. Inheritance of *p,p'*-DDE Phytoextraction Ability in Hybridized *Cucurbita pepo* Cultivars. *Environ. Sci. Technol.* **2010**, *44*, 5165–5169. [[CrossRef](#)]
32. White, J.C.; Parrish, Z.D.; Isleyen, M.; Gent, M.P.N.; Iannucci-Berger, W.; Eitzer, B.D.; Kelsey, J.W.; Mattina, M.I. Influence of Citric Acid Amendments on the Availability of Weathered PCBs to Plant and Earthworm Species. *Int. J. Phytoremediation* **2006**, *8*, 63–79. [[CrossRef](#)]
33. White, J.C.; Wang, X.; Gent, M.P.N.; Iannucci-Berger, W.; Eitzer, B.D.; Schultes, N.P.; Arienzo, M. Mattina Subspecies-Level Variation in the Phytoextraction of Weathered *p, p'*-DDE by *Cucurbita pepo*. *Environ. Sci. Technol.* **2003**, *37*, 4368–4373. [[CrossRef](#)]
34. Hinsinger, P.; Plassard, C.; Jaillard, B. Rhizosphere: A New Frontier for Soil Biogeochemistry. *J. Geochem. Explor.* **2006**, *88*, 210–213. [[CrossRef](#)]
35. Adeyinka, G.C.; Moodley, B. Kinetic and Thermodynamic Studies on Partitioning of Polychlorinated Biphenyls (PCBs) between Aqueous Solution and Modeled Individual Soil Particle Grain Sizes. *J. Environ. Sci.* **2019**, *76*, 100–110. [[CrossRef](#)] [[PubMed](#)]
36. Gent, M.P.N.; Parrish, Z.D.; White, J.C. Nutrient Uptake among Subspecies of *Cucurbita pepo* L. Is Related to Exudation of Citric Acid. *J. Am. Soc. Hortic. Sci.* **2005**, *130*, 782–788. [[CrossRef](#)]
37. Goto, J.; Iwabuchi, A.; Yoshihara, R.; Kodama, N.; Matsui, T.; Hirota, M.; Eun, H.; Inui, H. Uptake Mechanisms of Polychlorinated Biphenyls in *Cucurbita pepo* via Xylem Sap Containing Major Latex-like Proteins. *Environ. Exp. Bot.* **2019**, *162*, 399–405. [[CrossRef](#)]
38. Mattina, M.I.; Eitzer, B.D.; Iannucci-Berger, W.; Lee, W.-Y.; White, J.C. Plant Uptake and Translocation of Highly Weathered, Soil-Bound Technical Chlordane Residues: Data from Field and Rhizotron Studies. *Environ. Toxicol. Chem.* **2004**, *23*, 2756–2762. [[CrossRef](#)] [[PubMed](#)]
39. Whitfield Åslund, M.L.; Rutter, A.; Reimer, K.J.; Zeeb, B.A. The Effects of Repeated Planting, Planting Density, and Specific Transfer Pathways on PCB Uptake by *Cucurbita pepo* Grown in Field Conditions. *Sci. Total Environ.* **2008**, *405*, 14–25. [[CrossRef](#)] [[PubMed](#)]
40. Murano, H.; Otani, T.; Makino, T.; Seike, N.; Sakai, M. Effects of the Application of Carbonaceous Adsorbents on Pumpkin (*Cucurbita Maxima*) Uptake of Heptachlor Epoxide in Soil. *Soil Sci. Plant Nutr.* **2009**, *55*, 325–332. [[CrossRef](#)]