

**Appendix for
City of Midland Pilot Corrective Action Work Plan**

Bioaccumulation of Polychlorinated Dibenzo-*p*-dioxins/dibenzofurans in *E. foetida* from Floodplain Soils and the Effect of Activated Carbon Amendment

Sonja K. Fagervold,[#] Yunzhou Chai,^{\$} John W. Davis,^{\$} Michael Wilken,[†] and Upal Ghosh^{#*}

[#] Department of Civil and Environmental Engineering, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250 USA

^{\$} Toxicology and Environmental Research and Consulting, The Dow Chemical Company, 1803 Building, Midland, MI 48674 USA

[†] Environmental Analytical Support, The Dow Chemical Company, 1602 Building, Midland, MI, 48674 USA

* Corresponding author phone: 410-455-8665; Fax: 410-455-6500; e-mail: ughosh@umbc.edu

ABSTRACT

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (dioxins and furans, or PCDD/F) have been found in the floodplain soils along the Tittabawassee River downstream of Midland, MI. One potential way of containing PCDD/Fs and reducing their bioaccumulation potential is the addition of strong sorbents like activated carbon (AC) to the soil. Laboratory studies were conducted to evaluate the bioaccumulation of aged dioxins and furans in soil at the base of the terrestrial food chain using earthworms (*E. foetida*) as model organisms. This research also assessed the effect of AC addition to soil on bioaccumulation in the earthworms and passive uptake in polyoxymethylene samplers. Two soil samples from Tittabawassee River floodplain were used in this study that had different soil textures and PCDD/F congener profiles, one being a natural levee soil with typical pattern associated with chloralkali production, and the other a wetland soil with possible sources from both chloralkali and chlorophenol production. In the untreated floodplain soils, biota sediment accumulation factors (BSAFs) ranged from 0.17 for 2,3,7,8-TCDD to 0.02 for some of the higher chlorinated congeners, which were substantially lower than would be predicted using conventional equilibrium partitioning model. The addition of AC to the two floodplain soils reduced the BSAF values to generally lower than 0.02. The amendment of the wetland soil (SW-265) with 2% and 5% AC resulted in 77.7 and 91.2% reduction of TEQ in earthworms, respectively. More strikingly, the amendment of the natural levee soil (SW-20) with 2% and 5% AC showed 99.4 and 99.9% reduction of TEQ in earthworms, respectively. The effectiveness of the AC amendment is more profound for soil SW-20 which has low fraction of organic carbon phase that competes with AC for the sorption of dioxins and furans. Aqueous concentrations of dioxins and furans in soil slurries decreased with AC treatment.

INTRODUCTION

Low levels of anthropogenic chemicals in terrestrial soils over large areas from past industrial activities pose serious challenges in risk assessment and the development of appropriate and protective remedies. Some of these legacy contaminants such as polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs, or dioxins and furans) are chemically very stable and are highly hydrophobic compounds with logarithm of octanol-water partitioning coefficients above five (Saçan *et al.*, 2005). PCDD/Fs have a high affinity to organic matter and have limited mobility unless transported in association with particulate organic matter. However, these compounds are bioaccumulative, can be found in the terrestrial food chain, and have been reported to cause observable impacts to animals at the higher trophic levels.

Containment is one possible remediation strategy for PCDD/Fs. This might be a reasonable strategy due to the high costs and impacts to existing ecosystem from excavation and incineration of large areas of low concentration PCDD/F contaminated soils. One possible way of containing PCDD/F and reducing bioaccumulation potential would be the addition of strong sorbents like activated carbon (AC) to the soil. The addition of AC has been shown to decrease the accumulation of hydrophobic contaminants in aquatic sediments (Millward *et al.*, 2005; Sun and Ghosh, 2007). This decrease is generally through decreased aqueous phase concentration (Zimmerman *et al.*, 2004) and decreased fraction of the contaminant that is bioavailable resulting in decreased accessibility of the contaminant to the organism (Millward *et al.*, 2005; Sun and Ghosh, 2007). However, little work has been done with activated carbon in terrestrial environments.

Cornelissen *et al.* (Armitage *et al.*, 2008) studied the available fraction of PCDD/Fs in soils at a former wood impregnation site in Sweden and found that although the concentrations were relatively high, the available fraction was considerably less than previously thought due to strong sorption to black carbon. Brändli *et al.* (Brändli *et al.*, 2008) found that the addition of AC to soil slurries decreased the freely dissolved aqueous concentration of native PAHs up to 99%. Both these studies (Armitage *et al.*, 2008; Brändli *et al.*, 2008) used passive samplers [55 µm thin polyoxymethylene (POM)] to measure the freely dissolved concentration of contaminants. The use of passive samplers as way of measuring porewater concentrations has been widely used

(Jonker and Koelmans, 2001a; Cornelissen *et al.*, 2008) and this technology may be useful in interpreting bioavailability differences across soils. However, it is still uncertain if POM or other passive sampling techniques can predict the bioaccumulation potential of hydrophobic contaminants in soil the same way as earthworm bioassays.

Earthworms are in close contact with soil particles and are at the base of the food chain. They are therefore an important link in the transport of environmental contaminants in the soil and organisms higher up in the terrestrial food web. Earthworms are commonly used for bioaccumulation tests in soil. Earthworms can either be exposed to dioxins and furans through skin contact, where pore water concentration is important, or via ingested solids. Although pore water is thought to be an important route of exposure, ingestion might play a dominant role for the more hydrophobic chemicals like PCBs, dioxins, and furans (Loonen *et al.*, 1997).

Biota to soil accumulation factor (BSAF) is a common way of describing the relationship between the soil concentration and the concentration in earthworms. Typical BSAF values for PCDD/Fs in terrestrial earthworms are 0.09 to 1.1 (Matscheko *et al.*, 2002). Similar ranges (0.09 to 0.28) were found in 28-day bioaccumulation tests with *N. virens* in dredged sediments (Schrock *et al.*, 1997) while higher BSAF values for dioxins have been reported for aquatic species (<http://el.erdc.usace.army.mil>). BSAF values for other hydrophobic compounds in terrestrial earthworms are generally higher. For example Blankenship *et al.* (Blankenship *et al.*, 2005) found BSAF values for PCBs in earthworms of 0.48 and 2.4, depending on the level of contamination and soil type in aged soil.

The soil in the floodplain of Tittabawassee River and sediment in the river have been found to contain elevated levels of PCDD/Fs due to historic discharge from The Dow Chemical Company facilities located in Midland, MI that have been in operation since 1897. The dioxins and furans contamination is likely to be associated with chloralkali process and chlorophenol production process in the early 20th century. Prior to the installation of the wastewater treatment plant at the site in mid 1930s, wastes from the manufacturing operations were incinerated, discharged to waste management ponds that were occasionally breached during flooding events or discharged directly to the Tittabawassee River. In order to develop a sound management strategy for the

soil in the floodplain, studies are being performed to understand the fate, transport and biouptake processes that determine the exposure risk of the dioxins and furans to ecological system and the risk to human health (Zwiernik *et al.*, 2008; Garabrant *et al.*, 2009). This paper presents one element of the effort by investigating the bioaccumulation of dioxins and furans to terrestrial organism, *E. foetida*, and the effect of activated carbon amendment.

The objectives of this study were to i) evaluate the bioaccumulation of the aged PCDD/Fs at the base of the food chain, ii) investigate the effect of AC addition to the soil on bioaccumulation, and iii) assess whether passive samplers can be used to predict bioaccumulation potential of PCDD/Fs in soil. A 28-day bioaccumulation test was conducted using two distinct Tittabawassee River soil samples with different soil textures and PCDD/F congener profiles. This study used earthworms as an indicator organism to assess bioaccumulation at the base of the terrestrial ecosystem. Passive samplers [55 μm thin polyoxymethylene (POM)] were used to measure the freely dissolved PCDD/F concentrations. Both passive sampler and bioaccumulation test results were used to determine the available fraction of PCDD/Fs and thus bioaccumulation potential of these compounds.

MATERIALS AND METHODS

Site Description and Soil Samples

Soil samples were collected from the Tittabawassee River floodplain at different depths from two locations with different geomorphic settings. Soil samples were taken using a hand auger after the overlying grass and leaves had been removed. Soil SW-20 was collected at a location on natural levee/intermediate terrace at 5-6 ft below ground surface. Soil SW-265 was collected at a location in wetland area at 0-0.8 ft below ground surface. Soil samples were sieved through a 2 mm sieve after collection to remove large objects such as gravel, leaves and grass roots, and were homogenized and stored at 4°C in sealed containers until further use. An artificial soil (AS) was used as a control for weight loss of the worms during the 28 day incubation, as well as a background control for dioxin/furan analysis. The AS comprised of (by dry weight) 10% sphagnum peat, 20 % kaolin clay and 70 % silica sand and pH was adjusted with calcium carbonate to pH 7 (ASTM Standard E1676, 2004).

Water holding capacity of the soils was determined according to a method described by Bernard et al. (Bernard, 1965), with minor modifications. Briefly, triplicate soil samples were soaked in water for 48 h inside a column with cheese cloth in the bottom, removed and drained for 48 h on damp sand. The columns were covered with damp paper tissue to minimize evaporation. The soil was then weighed before and after drying in an oven at 105 °C for 48 h. The water holding capacity of AS, SW-20, and SW-265 was determined to be 30.2 (\pm 0.6)%, 21.8 (\pm 0.3)% and 36.8 (\pm 0.1)%.

Total Organic Carbon Content of Soil

Three aliquots (~ 2 g dry wt for each) were taken from each soil, dried in an oven at 105 °C, then pulverized and pooled. The weight percentage of carbon, hydrogen and nitrogen (CHN) in each soil were determined using a Perkin Elmer 2400 CHN Analyzer. Soil samples were acidified with 6N HCl to remove the inorganic carbon in the samples before CHN analysis. As a result the elemental carbon content from this CHN analysis represents the total organic carbon content (f_{oc}) of the soil sample. The organic carbon contents were 5.6% for SW-265 and 0.38% for SW-20.

Earthworms

Mixed age adult (>90 days) worms, *Eisenia fetida*, were obtained from Aquatic Research Organisms (Hampton, NH) and were cultured in an aquarium (51 cm by 26 cm by 31cm) filled with peat moss. The pH was adjusted to 7 with calcium carbonate and hydrated with water according to ASTM method E1676 (ASTM Standard E1676, 2004). Worms were fed every 4 days with alfalfa pellets that had been aged in test water for 2 weeks. At the start of the experiment, adult worms with well-developed clitella were transferred from the culturing media, washed and allowed to depurate on wet tissue paper for 24 h. They were then washed, gently dried and weighed before being transferred to test beakers.

Activated Carbon Amendment

Granular activated carbon (type TOG LF 80 x 325, Calgon Corp. Pittsburg, PA) was added to AS, SW-20, and SW-265 at two doses of 2% and 5% of dry weight, and rolled on a Cell production roller (Bellco, Vineland, NJ) at 3.25 rpm for 14 days before the start of the bioaccumulation study. The soil was moistened to 75% of the water holding capacity 24 h before the start of the test. The soil without AC amendment was denoted as NT, soil with 2% AC amendment was denoted as 2% AC, and soil with 5% AC amendment was denoted as 5% AC.

Bioaccumulation Test

The bioaccumulation test was performed in 400 ml glass beakers with the equivalent of 200 g dry soil which was moistened with the appropriate amount of test water. Three treatments (NT, 2% AC, and 5%AC) were designed for each of the three soils (AS, SW-20, and SW-265). Four replicates beakers were incubated with earthworms for each soil per treatment. In total, 36 beakers were set up for worm exposure.

At the start of the bioaccumulation test, six worms (five for PCDD/F analysis and one for lipid analysis) were added to each beaker and covered with nylon mesh to prevent worms from escaping. No food was added during the bioaccumulation study. The beakers were weighed

every 3-4 days to ensure proper moisture content. At the end of the 28-day incubation, the earthworms were washed and allowed to depurate in clean AS for 24 h to ensure the clearing of the gut of particles containing PCDD/Fs. After another 24 h depuration on wet tissue paper, the worms were cleaned, gently dried, weighed and immediately frozen. Worms were then freeze dried overnight in a Freeze Mobile 6 (Virtis, Gardiner, NY) before chemical analysis. Quadruplicate samples of earthworms were subjected to PCDD/Fs analysis separately, while the four replicates soil samples were pooled after extraction for PCDD/Fs analysis.

Lipid Content of Worms

Total lipids were extracted by crushing one freeze-dried worm from each beaker in a mortar and pestle with 5 ml 1:1 chloroform/methanol and 0.5 g anhydrous sodium sulfate. The lipid concentration in the supernatant was determined as described in van Handel et al. (van Handel, 1985) with calibration using soybean oil. Spectrophotometric analysis was performed on a Genesys 10 spectrophotometer (Thermo Electron Corp. Waltham, MA) at 525 nm.

Biota-soil Accumulation Factor (BSAF) Calculations

Biota-soil accumulation factors of dioxins and furans were calculated by dividing the lipid-normalized concentrations in earthworms by the concentration in soil normalized to organic carbon.

$$BSAF = \frac{C_{worm} / f_{lipid}}{C_{soil} / f_{oc}}$$

Where C_{worm} is the concentration of individual PCDD/F congener in dry worms, C_{soil} is the concentration of individual PCDD/F congener in soils, f_{oc} is the fraction of organic carbon in soil and f_{lipid} is the fraction of lipid in dry worm. The concentration of PCDD/F and lipid content were based upon quadruplicate samples for each soil per treatment, while the concentration of PCDD/F in soil was based upon one pooled sample for each soil per treatment.

Polyoxymethylene (POM)

Polyoxymethylene strips with a thickness of 55 μm were kindly supplied by Dr. Gerard Cornelissen (Norwegian Geotechnical Institute, Oslo, Norway) and cleaned by Soxhlet extraction for 12 h with hexane before use. Ten grams (dry weight) of soils SW-20 and SW-265, as well as 2 % AC and 5 % AC amended soils were added to 300 ml amber glass bottles. Water containing 25 mg/L sodium azide and 0.01 M calcium chloride (Jonker and Koelmans, 2001b), was added to these bottles in addition to 0.2 g POM per bottle. The bottles were horizontally shaken on an orbital shaker (Bellco, Vineland, NJ) at 3.2 rpm for 120 d to ensure equilibration with sediment. After equilibration, the POM strips were removed, cleaned with a moist tissue and extracted according to the method described in Jonker et al. (Jonker and Koelmans, 2001b).

Freely dissolved PCDD/Fs were determined based upon the following equation:

$$C_w = \frac{C_{POM}}{K_{POM}}$$

Where C_{POM} is the concentration of individual PCDD/F congener in POM, C_w is the porewater concentration of the corresponding congener and K_{POM} is the POM-water distribution ratio. Values of K_{POM} from Cornelissen et al (Armitage *et al.*, 2008) were used in the present paper.

PCDD/Fs analysis

Sample extraction and purification were based on U.S. Environmental Protection Agency (U.S. EPA) method 1613B (U.S. Environmental Protection Agency, 1994). Briefly, freeze-dried worm samples (100-200 mg) were digested using 75 ml concentrated HCl at ambient temperature for approximately 18 h after addition of all seventeen ^{13}C -labeled 2,3,7,8-substituted PCDD/Fs standards. The digested worm samples and soil samples (10-30 g) after addition of all seventeen ^{13}C -labeled 2,3,7,8-substituted PCDD/Fs standards were extracted in a Soxhlet apparatus with toluene (~250 ml) as a solvent for 20 h. An aliquot of the extract was purified using a multi-step cleanup with a mixed silica gel/acid/base column, silica gel/silver nitrate column and activated aluminum oxide. The solvent in the purified extract was exchanged to nonane after blow-down using high purity nitrogen. Polyoxymethylene (POM) samples were dissolved in half-concentrated sulfuric acid. After addition of all seventeen ^{13}C labeled 2,3,7,8-substituted

PCDD/Fs standards the solution was extracted with a benzene/hexane mixture (5% benzene by volume) followed by the clean-up and solvent exchange described above. Seventeen 2,3,7,8-substituted PCDD/Fs congeners and total homologues were quantified using high resolution gas chromatography/high resolution mass spectroscopy (HRGC/HRMS) following U.S. EPA method 1613B.

The PCDD/Fs commonly refer to 49 polychlorinated dibenzo-*p*-dioxins (PCDDs or dioxins) and 87 polychlorinated dibenzofurans (PCDFs or furans) containing four to eight chlorines. There are 17 congeners with chlorines in the 2,3,7,8-positions that are considered to be of toxicological significance and their relative potencies are estimated using the toxic equivalent factor (TEF) (Van den Berg *et al.*, 2006). The toxic equivalent (TEQ) is the concentration of individual congener multiplied by the TEF for the congener. The following abbreviations are used in this paper to address PCDD/F congeners or homologs. Tetra-, penta-, hexa-, hepta- and octa-chlorinated dibenzo-*p*-dioxins are denoted as TCDD, PeCDD, HxCDD, HpCDD, and OCDD. Tetra-, penta-, hexa-, hepta- and octa- chlorinated dibenzofurans are denoted as TCDF, PeCDF, HxCDF, HpCDF, and OCDF. The total PCDD/Fs concentration in this paper refers to the total of tetra- through octa- dioxins and furans.

RESULTS AND DISCUSSION

PCDD/Fs in Soils

The two different floodplain soils evaluated in this study exhibited both different soil textures and different PCDD/F profiles. Soil SW-20, which is more typical of the soil type along the Tittabawassee River floodplain, was sandy (sand 81%, silt 11%, and clay 5%) with a very low organic carbon content (f_{oc} of 0.38%). Conversely, Soil SW-265, which was taken from a wetland area along the river, had a much lower sand content (sand 32%, silt 44%, and clay 20%) and an approximate 15-fold higher organic carbon content (f_{oc} of 5.6%). The total concentrations of PCDD/Fs in the soils were 363 and 56.7 $\mu\text{g}/\text{kg}$, the total concentrations of seventeen 2,3,7,8-substituted congeners were 196 and 42.6 $\mu\text{g}/\text{kg}$, and the WHO-TEQs were

23.9 and 1.16 $\mu\text{g}/\text{kg}$ for soils SW-20 and SW-265, respectively. The concentration of each homolog group of dioxins and furans in these two soils are presented in Figure 1.

The soil SW-20 was primarily impacted by tetra-, penta- and hexa- chlorinated furans, with the 2,3,4,7,8-PeCDF, 2,3,7,8-TCDF, and 1,2,3,4,7,8-HxCDF congeners comprising ~ 90% of the TEQ profile (data not shown). In contrast, the dioxin congeners were either near or below the detection limit and did not contribute any significant TEQ for this soil. These data are consistent with the observation of Wilken *et al.* (Wilken *et al.*, 2006) where they reported that these three congeners were the dominant contributor to the TEQ profile along the banks of the Tittabawassee River and were likely produced from chloro-alkali production that began in the late 19th century. In contrast, soil SW-265 was somewhat impacted by hepta- and octa-chlorinated dioxins and furans in addition to tetra, penta, and hexa- chlorinated furans (Figure 1), which indicates sources from chlorophenol production processes in addition to the chloralkali production (Wilken *et al.*, 2006). Three furan congeners 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF and 2,3,7,8-TCDF contributed approximately 75% of the TEQ for soil SW-265 while the dioxin congeners 2,3,7,8 TCDD and 1,2,3,7,8 PeCDD contributed approximately 13% of the TEQ for this soil (data not shown). Laboratory prepared AS used in bioaccumulation experiments had a low total concentration of dioxins and furans (0.2 ng/g) as shown in Figure 1. The dominant homologs present in the AS were OCDD and OCDF comprising 93% of the total.

Earthworm Bioaccumulation

The total homolog concentrations of dioxins and furans in soil and worms after 28 d of incubation with AS, SW-20 and SW-265 are shown in Figure 2. The concentrations of total dioxins and furans in soils were 363, 56, and 0.2 $\mu\text{g}/\text{kg}$ and total concentrations in worms were 346, 2.6, and 0.67 $\mu\text{g}/\text{kg}$ for SW-20, SW-265, and AS respectively. Generally, the total homolog concentration profiles in worms exposed to soils with out AC amendment reflected the homolog concentration profiles in the corresponding soils. The exception was that the total TCDDs and total PeCDDs were relatively enriched in worms exposed to both untreated SW-20 and SW265, whereas total HpCDFs and OCDF concentrations were unproportionally lower in worms exposed to untreated SW-265. The amendment of AC in soils reduced the bioaccumulation of dioxins and furans in various degrees, therefore, the dioxins and furans homolog concentration

profiles in worms exposed to AC treated soil were apparently not consistent with the profiles in the corresponding untreated soils. Overall bioaccumulation was two orders of magnitude higher in soil SW-20 compared to soil SW-265, likely attributable to an order of magnitude higher total concentration of PCDD/Fs in soil and an order of magnitude lower fraction organic carbon content in SW-20 compared to SW-265.

The dioxins and furans concentrations in the AS were four orders of magnitude lower compared to soil SW-20 and three orders of magnitude lower than soil SW-265. Correspondingly, the dioxins and furans in worms exposed to the untreated AS were much lower compared with the concentrations in worms exposed to soils SW-20 and SW-265. Note that the elevated dioxins and furans concentrations in worms exposed to AS amended with 5% AC was primarily driven by one replicate which was 3.6 µg/kg and the average \pm standard deviation for the rest three replicates was 0.44 ± 0.06 µg/kg. It is possible that the worms accumulated some background dioxins and furans from food and culture media before they were exposed to the AS. Fortunately, this low background values do not impact the interpretation of the bioaccumulation results for soil SW-20 and SW-265.

BSAF values in untreated soils

The BSAF values for the two untreated soils were similar and ranged from 0.17 for 2,3,7,8-TCDD to 0.02 for some of the higher chlorinated congeners (Figure 3). The BSAF values were generally lower for the higher chlorinated congeners for both dioxins and furans. These BSAF values were substantially lower than would be predicted using conventional equilibrium partitioning model. Similar range of BSAFs (0.09 to 0.28) has been reported in 28-day bioaccumulation tests with *N. virens* in dredged sediments (Schrock *et al.*, 1997). However, the BSAF values measured in the present study were lower than the BSAF values reported in indigenous worms from agricultural soils in Sweden, which ranged from 0.09 to 1.1 (Matscheko *et al.*, 2002). The BSAF values for the native PCDD/Fs in soils that have presumably aged in the field for several decades are generally lower compared to reported values for other hydrophobic organics such as PCBs. Sun and Ghosh (Sun and Ghosh, 2007) found that BSAFs for PCBs increased initially with increasing log Kow and decreased for log Kow values higher than 6.7. Thus, for PCDD/Fs that have log Kow values in the range of 6.5-8.5, the BSAF values are

expected to be in the lower range compared to PCBs and decrease with increasing hydrophobicity. As shown in Supplemental Figure S2 for both soil samples, BSAF values showed a decreasing trend with increasing congener K_{ow} value.

Effect of activated carbon amendment on bioaccumulation

Addition of activated carbon to soils greatly reduced the bioaccumulation of PCDD/Fs in the earthworms (Table 1 and figure 2). For example, the earthworms exposed to untreated SW-20 contained an average of 210 $\mu\text{g}/\text{kg}$ of total TCDFs, but only 1.98 $\mu\text{g}/\text{kg}$ for worms exposed to SW-20 with 2 % AC, a 99% decrease. AC addition to soils decreased the concentration of dioxins or furans in all worm samples, with more pronounced decrease for soil SW-20. This could be due to its higher initial PCDD/Fs concentration or its lower organic carbon content. For both soils, after amendment with 5% AC, the uptake of total PCDD/Fs in earthworms was similar to background (i.e., the uptake measured in laboratory AS exposures).

The percentage decrease of PCDD/F concentrations in the worms in soil with AC compared to untreated soils is shown in Supplementary Figure S4. For the SW-20 soil, TCDFs and PeCDFs were the dominant homologs and the decrease in worms from soils with AC amendment was around 99%. The OCDD was present at greater abundance in SW-265 soil and the decrease in worm concentrations in AC amended soils for OCDD was around 60%. Generally, percent reductions in bioaccumulation with AC amendment were higher for the lower chlorinated congeners (tetra- to hexa- furans) compared to the highly chlorinated OCDD/F for each soil. The tetra- to hexa- furans are the major contributors to TEQ, thus reduction of these congeners is important to have a net impact on toxicity reduction. Previous work with PCBs in sediments also demonstrated a similar phenomenon of greater effectiveness for the lower chlorinated congeners after short periods of sediment/AC contact (Millward *et al.*, 2005; Sun and Ghosh, 2007). This phenomenon might be attributed to the lower aqueous solubility and slower mass transfer rate of the higher chlorinated congeners between soil/sediment and AC particles.

The addition of AC to the two floodplain soils substantially reduced the BSAF values to generally lower than 0.02 (Figure 3). The percent reduction in BSAF values for 2,3,7,8-TCDD

and 2,3,7,8-TCDF were over 99% in soil SW-20 that had a lower native organic carbon content. The percent reduction of BSAF for 2,3,7,8-TCDD and 2,3,7,8-TCDF was 99% and 75% in soil SW-265 after amendment with 2% AC. Higher reductions in BSAF values were observed at a 5% dose of AC to the soil. Based on the large reductions of measured BSAF values, especially for the SW-20 soil, it appears that a much smaller dose of AC to soil in the range of 1% by weight may be effective in binding up and reducing the uptake of the available fraction of the PCDD/Fs in sandy soils with low native organic carbon content.

TEQ Reduction

The addition of activated carbon resulted in a large decrease of TEQ in the earthworms (Table 1). The amendment of soil SW-265 with 2% and 5% AC exhibited 77.7 and 91.2% reduction of TEQ in earthworms, respectively. More strikingly, the amendment of soil SW-20 with 2% and 5% AC showed 99.4 and 99.9% reduction of TEQ in earthworms, respectively. The soil SW-20 had low organic carbon content compared to soil SW-265. Thus, the effectiveness of the AC amendment is more profound for SW-20 which has low organic carbon phase that competes with AC for the sorption of dioxins and furans.

Possible effect of AC on earthworms

All treatments, except for soil SW-265 with no AC amendment, exhibited weight loss of worms during the 28-day incubation (Supplemental Figure S6). The weight loss averages were approximately 2% for worms incubated with untreated AS to about 30 % for worms incubated with SW-20 amended with 5% AC. Worms incubated with SW-265 exhibited a small increase in weight after the 28-day incubation. This might be attributed to the relatively high organic carbon content of this soil (5.6 %) compared to SW-20 that had an organic carbon content of only 0.38 %. Poor nutritional quality of the soils, especially after amendment with AC and the absence of feeding during the test may be responsible for the weight loss of the worms. Some of the worms may also have released egg cocoons during the exposure period that may have resulted in an apparent weight loss of the organisms. However, we did not find a correlation between the weight loss and the lipid % in the different treatments (Supplemental Figure S4), indicating that the loss of storage lipids might not be the main weight loss mechanism. Millward

et al. (Millward *et al.*, 2005) also observed a decrease in wet weight of the polychaete *Neanthes arenaceodentata* after 28 days of incubation with sediment amended with 3.4 % AC with no change in the lipid content.

Generally, worms in soils with AC amendment exhibited more weight loss than worms in soils without amendments. The reason for this elevated weight loss is unclear. Earthworms feed on organic debris and one can speculate to whether the AC absorbs organic compounds that the earthworms would otherwise, either directly or indirectly, use for nutrition. Another mechanism is avoidance of the soil in the beakers with AC and thus the earthworms would not ingest enough soil for weight maintenance. However, if reduction in feeding rate could explain the reduction in PCDD/F biouptake in the worms after AC amendment, we would expect a much more drastic reduction in the biouptake of the hexa- and hepta- congeners that are less soluble and are primarily uptaken by ingestion. Rather, the effects of 2% AC amendment in soil SW-265 on the reduction of the biouptake in worms were 40% for HpCDFs and 80% for TCDFs. Thus, it is unlikely that reduced feeding rate can explain the reduction in biouptake of PCDD/F congeners in soil.

POM

Porewater concentrations were calculated based upon the concentration in POM and published POM-water partition coefficients (Armitage *et al.*, 2008). The porewater concentration was as expected 6-7 orders of magnitude lower than the soil concentrations (Figures 1 and 4). The percent mass of PCDD/F congeners transferred to POM during the 3-month batch equilibrium test was less than 2% for soil SW-265 and less than 7% for soil SW-20. Thus depletion of PCDD/Fs from soil during the POM-equilibrium experiment was small. For the furans, the lower chlorinated (tetra- and penta-) congeners generally dominated in the aqueous phase. For dioxins, the octachloro congener was dominant in the soil phase and was also found to be dominant in the aqueous phase.

When soil was amended with AC, the porewater concentrations were greatly reduced. Similar to the reduction in worm concentration, the porewater concentration decreased between

approximately 70 % for congener OCDD in SW-20 samples and almost 100% for some of the lower chlorinated congeners as illustrated in Figure 4. The congeners with highest concentration in soils and porewater, such as the TCDFs and PeCDFs, exhibited 93-99 % reduction in pore water concentration and around 99% reduction in worm concentration when amended with 2% AC compared to the non treated soils.

Correlation between PCDD/F concentration in worm and concentration in soil or POM.

The relationship between PCDD/F uptake in worms and concentration in soil or passive uptake in POM samplers are shown in Figure 5. Generally, there is a greater degree of scatter in the plots showing worm concentration vs. soil concentration, with the impact of AC treatment not being explained by just the concentration in whole soil. When plotted against uptake in the passive sampler, the correlation is stronger compared to that with soil. Passive uptake by POM is driven by the water phase concentration and partition constants for the PCDD/F congeners and thus reflects the bioavailability and relative potential for each congener to bioaccumulate in organism lipids. However uptake in living organisms is more complicated than a simple concentration gradient driven process, and involves a balance between uptake and elimination rate processes that may result in a dynamic equilibrium state that is different from what may be achieved in a passive sampler.

These results from laboratory exposure studies could be confirmed through pilot-scale studies in the field that would present ideal platforms for addressing several remaining questions. A field study may involve control and treated plots similar to those conducted at a tidal Mudflat in San Francisco Bay (Cho *et al.*, 2009) where control plots without AC amendment was tracked along treated plots where AC was amended. Application of AC in terrestrial soil should be relatively easier compared to application in submerged areas and may involve common agricultural spreaders and tilling equipment. Based on the results of this study, a low dose of AC in the order of 1% by weight of sandy floodplain soil should result in an order of magnitude reduction of PCDD/F bioaccumulation in soil invertebrates. Remaining questions that may be addressed through a pilot-scale study include: 1) applicability of the observations to the field scale, 2) evaluation of the physical persistence of AC in the terrestrial soil environment, 3) Long-term

effectiveness of the AC in the terrestrial environment, and 4) Testing of alternate application methods of AC in the field.

REFERENCES

- (1) Saçan, M. T., Özkul, M. and Erdem, S. S. (2005). Physico-chemical properties of PCDD/PCDFs and phthalate esters. *SAR & QSAR in Environmental Research* **16**(5): 443-459.
- (2) Millward, R. N., Bridges, T. S., Ghosh, U., Zimmerman, J. R. and Luthy, R. G. (2005). Addition of Activated Carbon to Sediments to Reduce PCB Bioaccumulation by a Polychaete *Neanthes arenaceodentata* and an Amphipod *Leptocheirus plumulosus*. *Environ. Sci. Technol.* **39**(8): 2880-2887.
- (3) Sun, X. and Ghosh, U. (2007). PCB Bioavailability Control in *Lumbriculus Variegatus* through Different Modes of Activated Carbon Addition to Sediments. *Environ. Sci. Technol.* **41**(13): 4774-4780.
- (4) Zimmerman, J. R., Ghosh, U., Millward, R. N., Bridges, T. S. and Luthy, R. G. (2004). Addition of Carbon Sorbents to Reduce PCB and PAH Bioavailability in Marine Sediments: Physicochemical Tests. *Environ. Sci. Technol.* **38**(20): 5458-5464.
- (5) Armitage, J. M., Cousins, I. T., Persson, N. J., Gustafsson, O., Cornelissen, G., Saloranta, T., Broman, D. and Naes, K. (2008). Black Carbon-Inclusive Modeling Approaches for Estimating the Aquatic Fate of Dibenzo-p-dioxins and Dibenzofurans. *Environmental Science & Technology* **42**(10): 3697-3703.
- (6) Brändli, R. C., Hartnik, T., Henriksen, T. and Cornelissen, G. (2008). Sorption of native polyaromatic hydrocarbons (PAH) to black carbon and amended activated carbon in soil. *Chemosphere* **73**(11): 1805-1810.
- (7) Jonker, M. T. O. and Koelmans, A. A. (2001a). Polyoxymethylene solid phase extraction as a partitioning method for hydrophobic organic chemicals in sediment and soot. *Environmental Science and Technology* **35**(18): 3742-3748.
- (8) Cornelissen, G., Pettersen, A., Broman, D., Mayer, P. and Breedveld, G. D. (2008). Field testing of equilibrium passive samplers to determine freely dissolved native polycyclic aromatic hydrocarbon concentrations. *Environmental Toxicology and Chemistry* **27**(3): 499-508.

- (9) Loonen, H., Muir, D. C. G., Parsons, J. R. and Goverst, H. A. J. (1997). Bioaccumulation of polychlorinated dibenzo-p-dioxins in sediment by oligochaetes: influence of exposure pathway and contact time *Environ. Toxicol. Chem.* **16**(7): 1518-1525.
- (10) Matscheko, N., Tysklind, M., deWit, C., Bergek, S., Andersson, R. and Sellstrom, U. (2002). Application of sewage sludge to arable land-soil concentrations of polybrominated diphenyl ethers and polychlorinated dibenzo-p-dioxins, debenzofurans, and biphenyls, and their accumulation in earthworms. *Environ. Toxicol. Chem.* **21**(12): 2512-2525.
- (11) Schrock, M. E., Barrows, E. S. and Rosman, L. B. (1997). Biota-to-sediment accumulation factors for TCDD and TCDF in worms from 28-day bioaccumulation tests. *Chemosphere* **34**(5-7): 1333-1339.
- (12) Blankenship, A. L., Zwiernik, M. J., Coady, K. K., Kay, D. P., Newsted, J. L., Strause, K., Park, C., Bradley, P. W., Neigh, A. M., Millsap, S. D., Jones, P. D. and Giesy, J. P. (2005). Differential Accumulation of Polychlorinated Biphenyl Congeners in the Terrestrial Food Web of the Kalamazoo River Superfund Site, Michigan. *Environ. Sci. Technol.* **39**(16): 5954-5963.
- (13) Zwiernik, M. J., Kay, D. P., Moore, J., Beckett, K. J., Khim, J. S., Newsted, J. L., Roark, S. A. and Giesy, J. P. (2008). Exposure and effects assessment of resident mink (*Mustela vison*) exposed to polychlorinated dibenzofurans and other dioxin-like compounds in the Tittabawassee River basin, Midland, Michigan, USA. *Environmental Toxicology and Chemistry* **27**(10): 2076-2087.
- (14) Garabrant, D. H., Franzblau, A., Lepkowski, J., Gillespie, B. W., Adriaens, P., Demond, A., Hedgeman, E., Knutson, K., Zwica, L., Olson, K., Towey, T., Chen, Q., Hong, B., Chang, C.-W., Lee, S.-Y., Ward, B., LaDronka, K., Luksemburg, W. and Maier, M. (2009). The University of Michigan dioxin exposure study: predictors of human serum dioxin concentrations in Midland and Saginaw, Michigan. *Environmental Health Perspectives* **117**(5): 818-824.
- (15) ASTM Standard E1676 (2004). Standard guide for conducting laboratory soil toxicity or bioaccumulation tests with the lumbricid earthworm *Eisenia Fetida* and the enchytraeid potworm *Enchytraeus albidus*, ASTM International, West Conshohocken, PA, 2004, DOI: 10.1520/E1676-04 www.astm.org.
- (16) Bernard, J. M. (1965). Forest floor moisture capacity of the new jersey pine barrens. *Ecology* **44**(3): 574-576.
- (17) van Handel, E. (1985). Rapid determination of total lipids in Mosquitoes. *J. Am. Mosq. Control Assoc.* **1**(3): 302-304.
- (18) Jonker, M. T. O. and Koelmans, A. A. (2001b). Polyoxymethylene Solid Phase Extraction as a Partitioning Method for Hydrophobic Organic Chemicals in Sediment and Soot. *Environ. Sci. Technol.* **35**(18): 3742-3748.

- (19) U.S. Environmental Protection Agency (1994). Method 1613B: Tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS (revision B), U.S. Environmental Protection Agency, Washington DC, USA.
- (20) Van den Berg, M., Birnbaum, L. S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N. and Peterson, R. E. (2006). The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Sciences* **93**(2): 223-241.
- (21) Wilken, M., Martin, G., Lamparski, L., Denney, P. and Baker, B. (2006). Pattern recognition in floodplain samples. *Organohalogen Compounds* **68**: 2371-2374.
- (22) Cho, Y.-M., Ghosh, U., Kennedy, A. J., Grossman, A., Ray, G., Tomaszewski, J. E., Smithenry, D. W., Bridges, T. S. and Luthy, R. G. (2009). Field application of activated carbon amendment for In-Situ stabilization of polychlorinated biphenyls in marine sediment. *Environmental Science & Technology* **43**(10): 3815-3823.

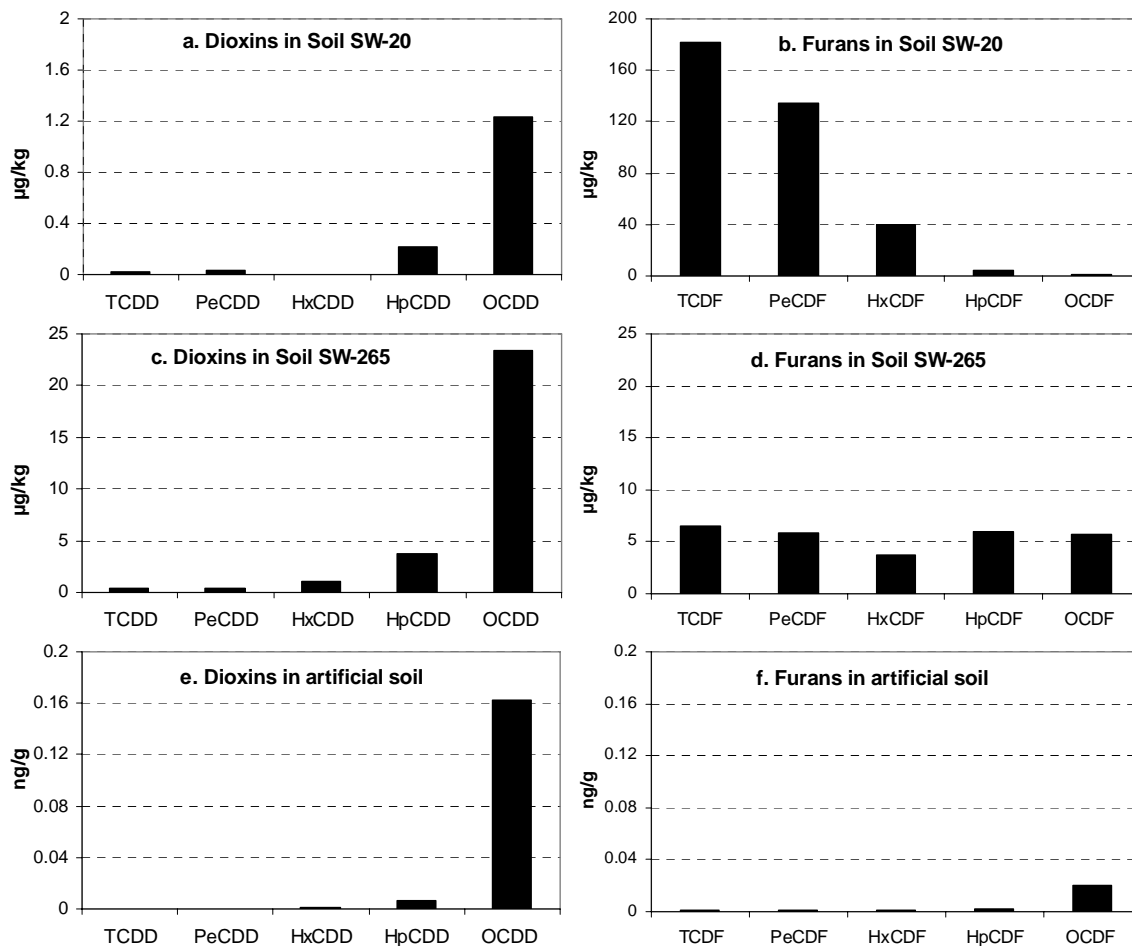


Figure 1. Total homolog concentration of dioxins and furans in the two field soils from Tittabawassee River floodplain and one clean artificial soil prepared in the laboratory. Note the difference of scales for dioxins and furans in soil SW-20. Soil concentrations of total dioxins and furans were 363, 56.7 and 0.2 µg/kg for soil SW-20, SW-265, and artificial soil, respectively.

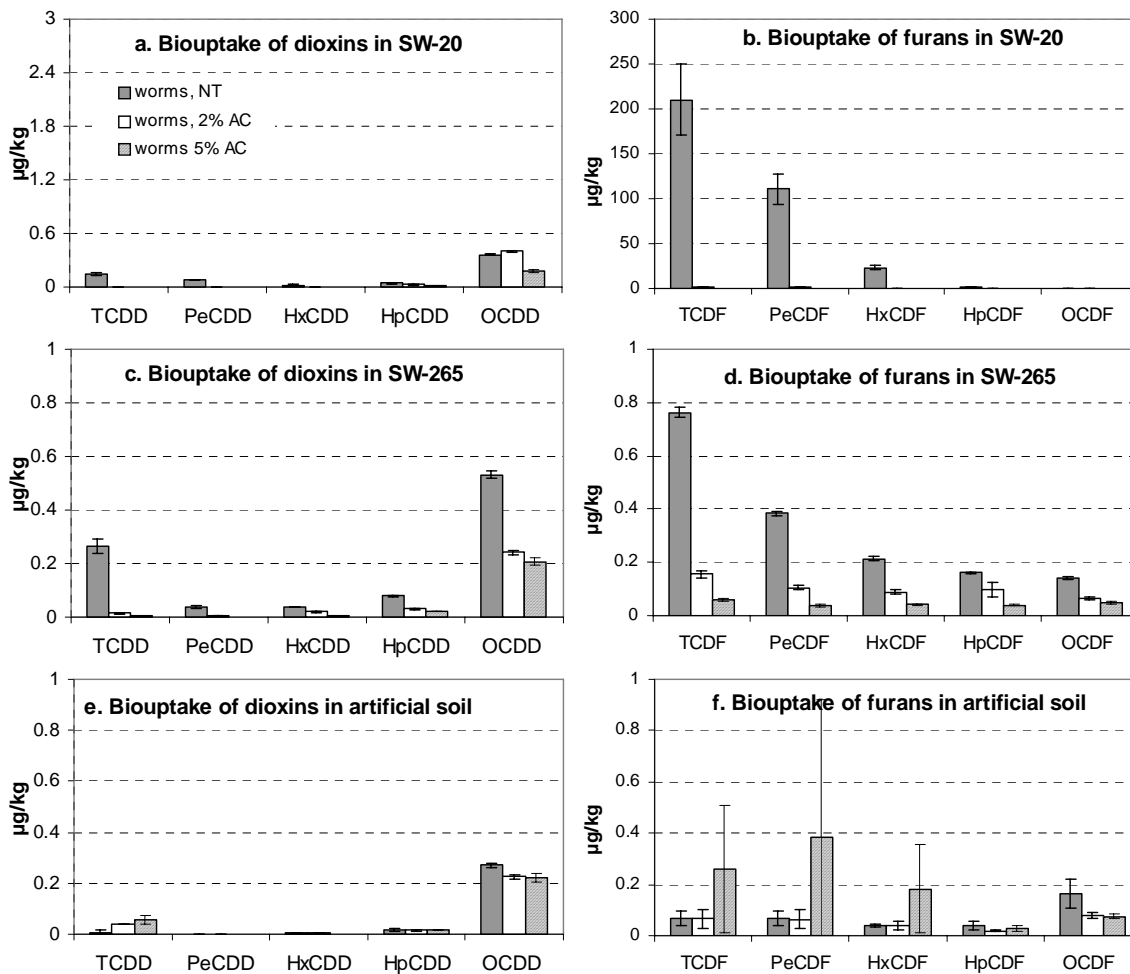


Figure 2. Total homolog concentration of dioxins and furans in worms after 28 days of incubation. Solid, open and hatched bars represent the worms exposed to untreated soil, soil with 2% activated carbon, and soil with 5% activated carbon, respectively. Note the difference in scales. Error bars show ± 1 standard error.

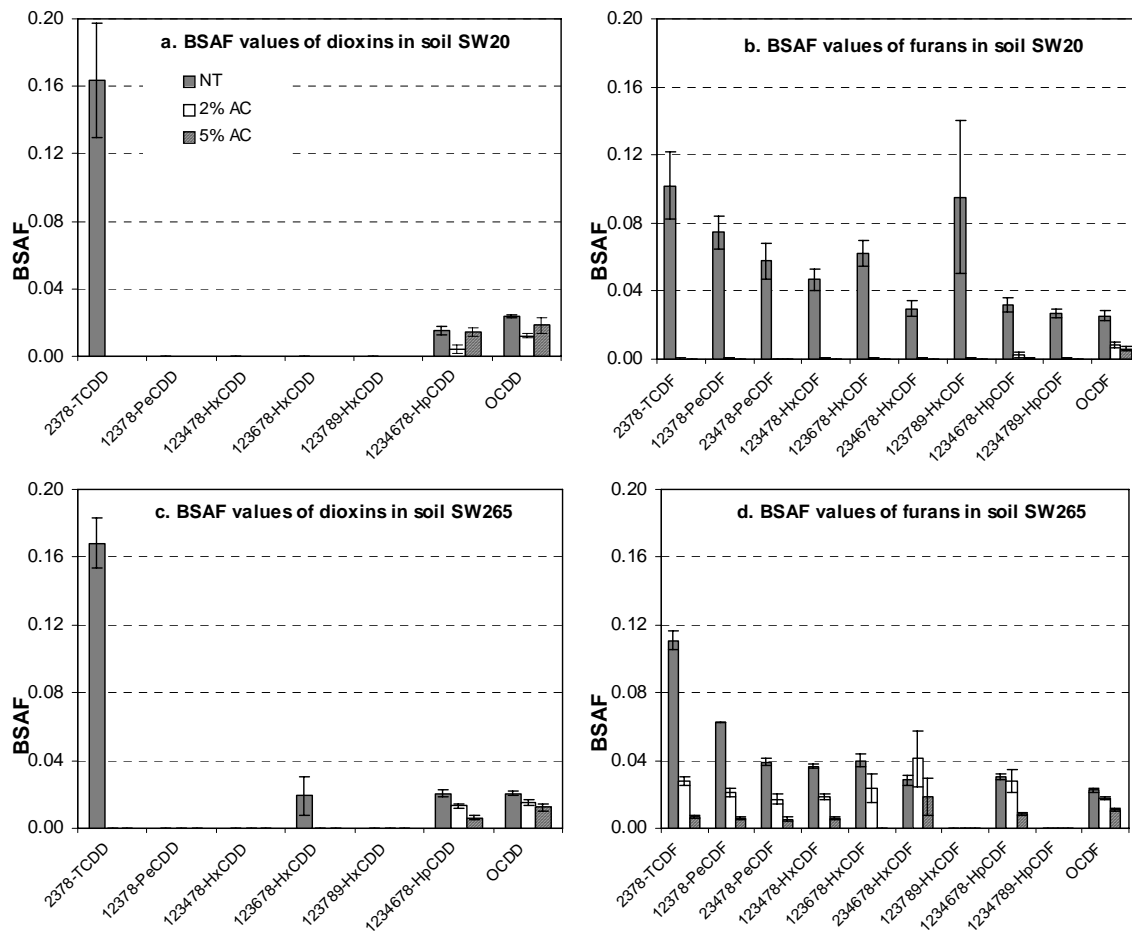


Figure 3. Biota-soil accumulation factors (BSAF) for individual dioxin and furan congener in soils SW20 and SW-265 with different activated carbon amendments. Solid, open, and hatched bars represent BSAF values for worms exposed to untreated soil, soil amended with 2% activated carbon, and soil with 5 % activated carbon, respectively. Error bars show ± 1 standard error.

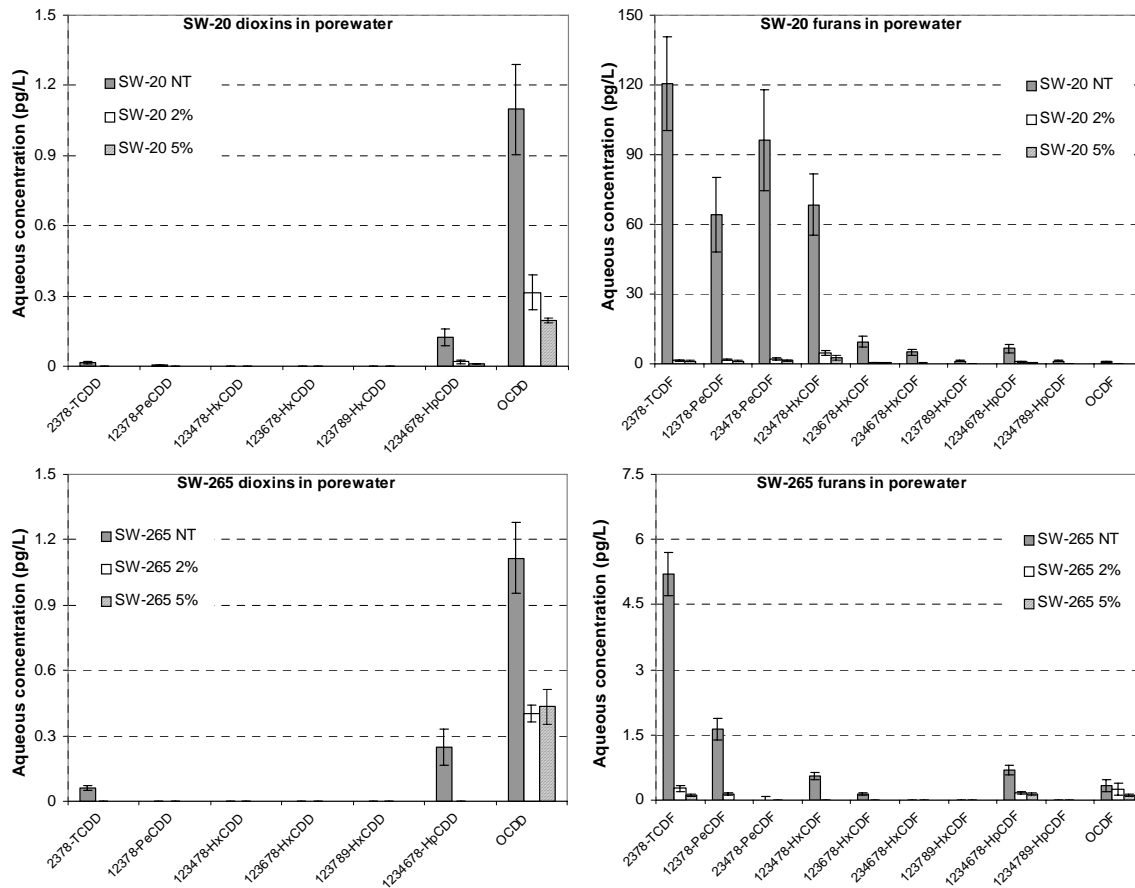


Figure 4. Dioxin and furan aqueous concentrations estimated from POM passive samplers in soil slurries of untreated and activated carbon treated soils. Solid, open, and hatched bars represent aqueous concentrations corresponding to untreated soil, soil amended with 2% activated carbon, and soil with 5% activated carbon, respectively. Error bars show ± 1 standard error.

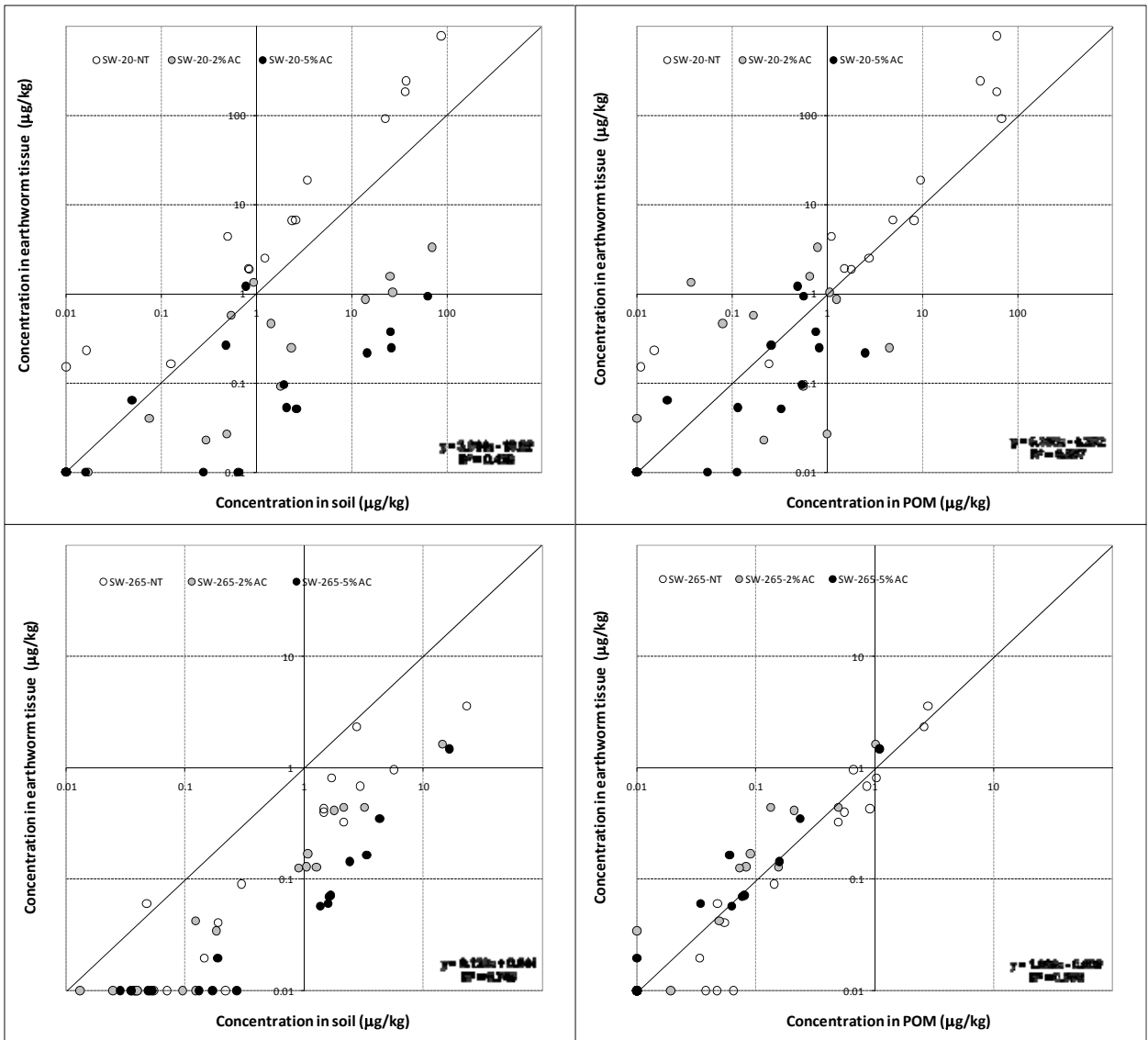


Figure 5. Correlation between dioxin and furan concentrations in earthworm tissue and soil or passive sampler.

Table 1. Toxic equivalent (TEQ) values for soils and worms

		TEQ ^a (ng/kg)		
		Artificial Soil	SW265	SW20
Soil	NT^b	0.3	1160	23900
	2%AC	1.0	807	17800
	5%AC	0.5	1220	17000
Worm	NT	11.3 ± 8.8 ^c	75.9 ± 2.5	22000 ± 8120
	2%AC	10.8 ± 12.6	16.9 ± 2.4	123 ± 41.1
	5%AC	64.3 ± 125 (1.7 ± 2.4) ^d	6.7 ± 1.4	32.4 ± 8.5

^a: TEQ (ng/kg) was determined by WHO 2005 TEF values, non-detect = 0

^b: NT denotes no treatment with AC

^c: Worm data was average ± standard deviation (n=4), soil data was pooled from 4 replicates

^d: This elevated level was primarily driven by one replicate which was 252 ng/kg, the average ± standard deviation for the rest 3 replicates was 1.7 ± 2.4 ng/kg

SUPPLEMENTARY INFORMATION

Table S1. Absolute concentrations of dioxins and furans in the different soil types and amendments. Standard deviations are given in parenthesis

	Worm concentrations (pg/g)					
	SW 20	SW 20 2% AC	SW 20 5% AC	SW 265	SW 265 2% AC	SW 265 5% AC
TCDD	141 (12.8)	ND	ND	264 (27.4)	14 (2.61)	4 (0.45)
PeCDD	81 (1.73)	ND	ND	39 (5.40)	4 (0)	ND
HxCDD	23 (2.42)	7 (0.28)	5 (1.59)	36 (0.49)	1 (4.31)	7 (0.76)
HpCDD	45 (6.02)	25 (2.47)	1 (1.21)	81 (2.67)	31 (2.79)	22 (2.10)
OCDD	362 (10.1)	400 (7.53)	185 (16.5)	532 (15.1)	242 (7.55)	208 (15.3)
TCDF	210361 (39181)	1980 (179)	293 (26.4)	763 (17.1)	155 (12.0)	60 (3.36)
PeCDF	110751 (16632)	1580 (131)	19 (21.9)	385 (8.87)	105 (7.81)	38 (5.20)
HxCDF	22793 (2504)	616 (42.0)	88 (7.85)	213 (8.54)	88 (8.63)	4 (3.17)
HpCDF	1402 (173)	202 (52.3)	26 (3.59)	161 (4.04)	98 (25.9)	40 (2.91)
OCDF	268 (37.3)	170 (21.8)	40 (6.04)	143 (4.46)	65 (7.25)	49 (4.39)

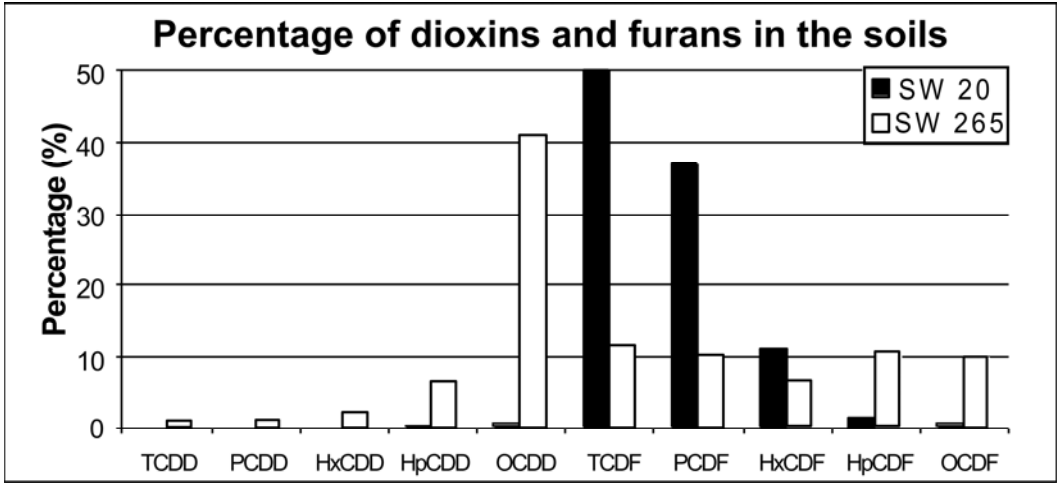


Figure S1. Percentage of dioxin and furan in the two different soil types. Solid bars represent soil SW-20 and open bars represent soil SW-265. Soil concentrations of total dioxins and furans were 363 $\mu\text{g}/\text{kg}$ and 56.7 $\mu\text{g}/\text{kg}$ for soils SW-20 and SW-265, respectively

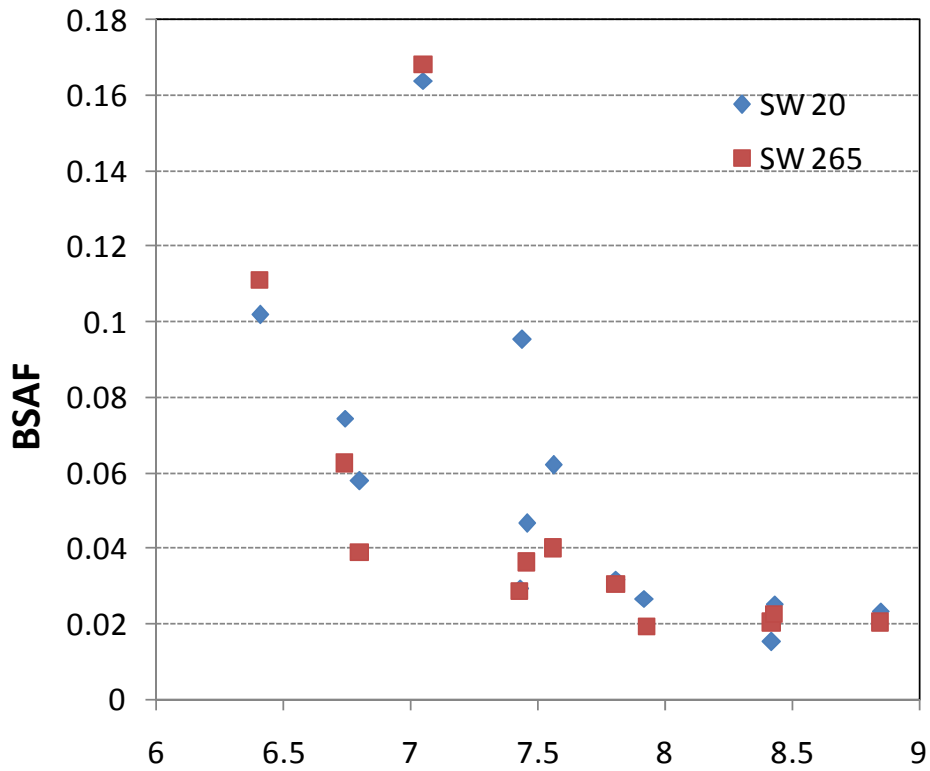


Figure S2. Biota Sediment Accumulation Factors vs. log Kow

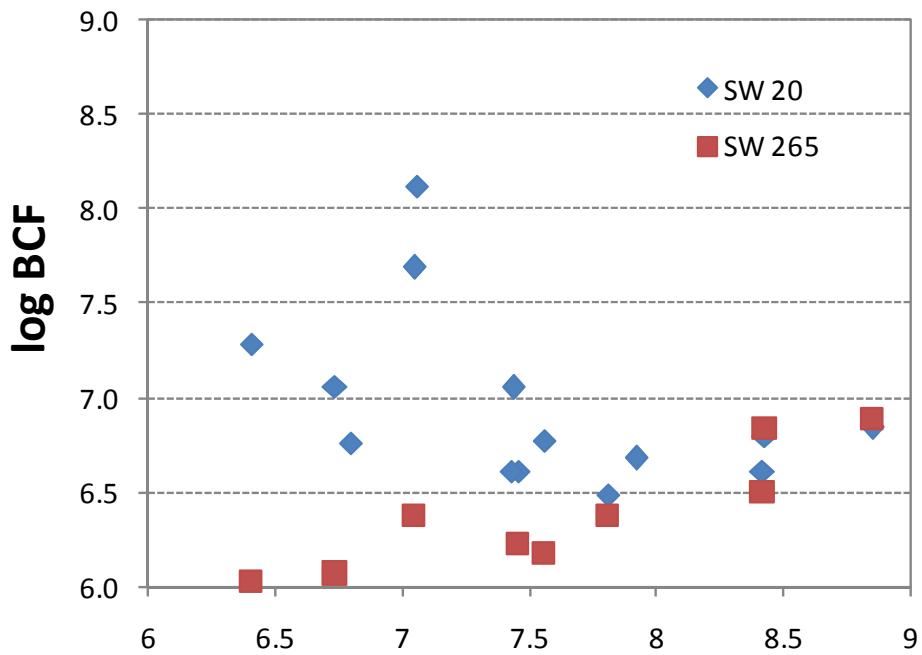


Figure S3. Bioaccumulation factor versus log Kow

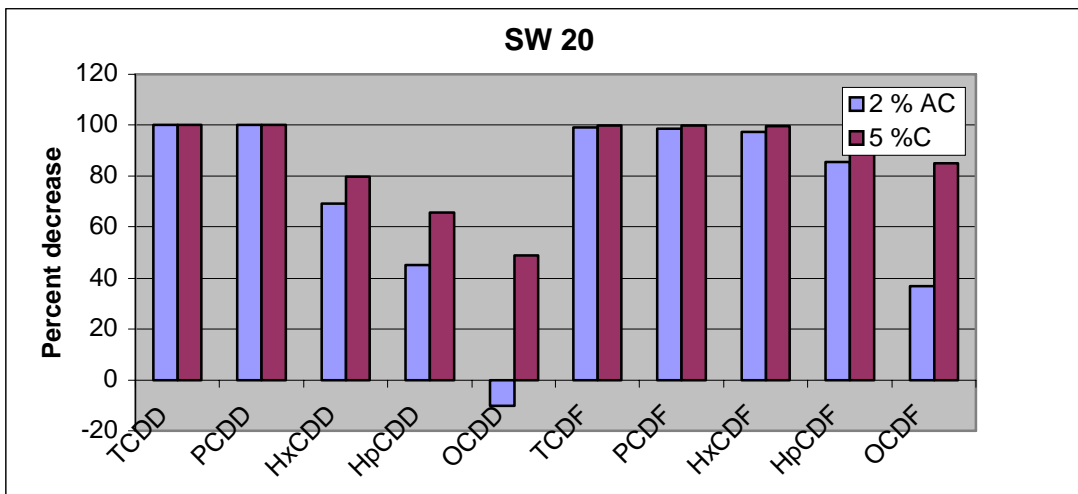
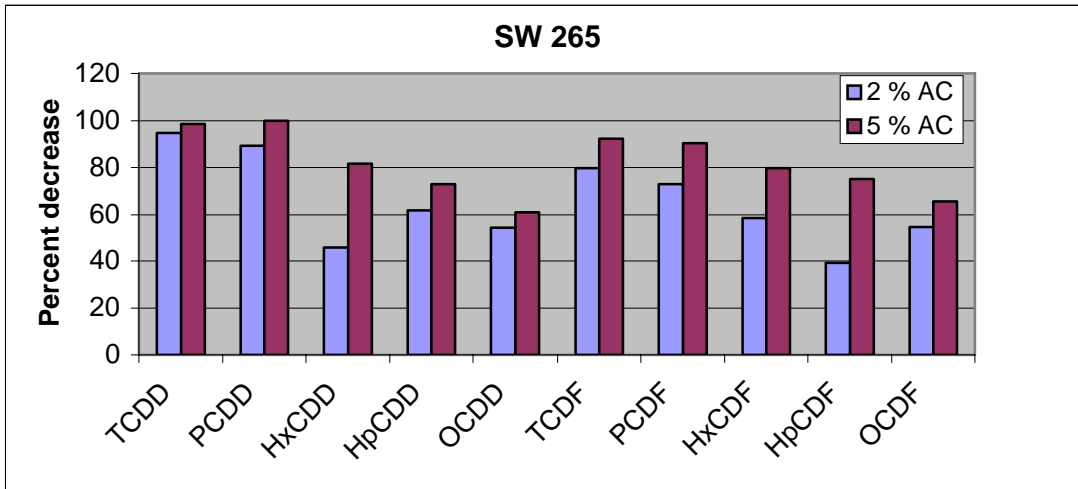


Figure S4. Percent decrease of the concentration in worms compared to the worms from the non-treated soils. Some of lower chlorinated congeners were not detected or were very close to the detection limit in samples with 2 or 5% AC.

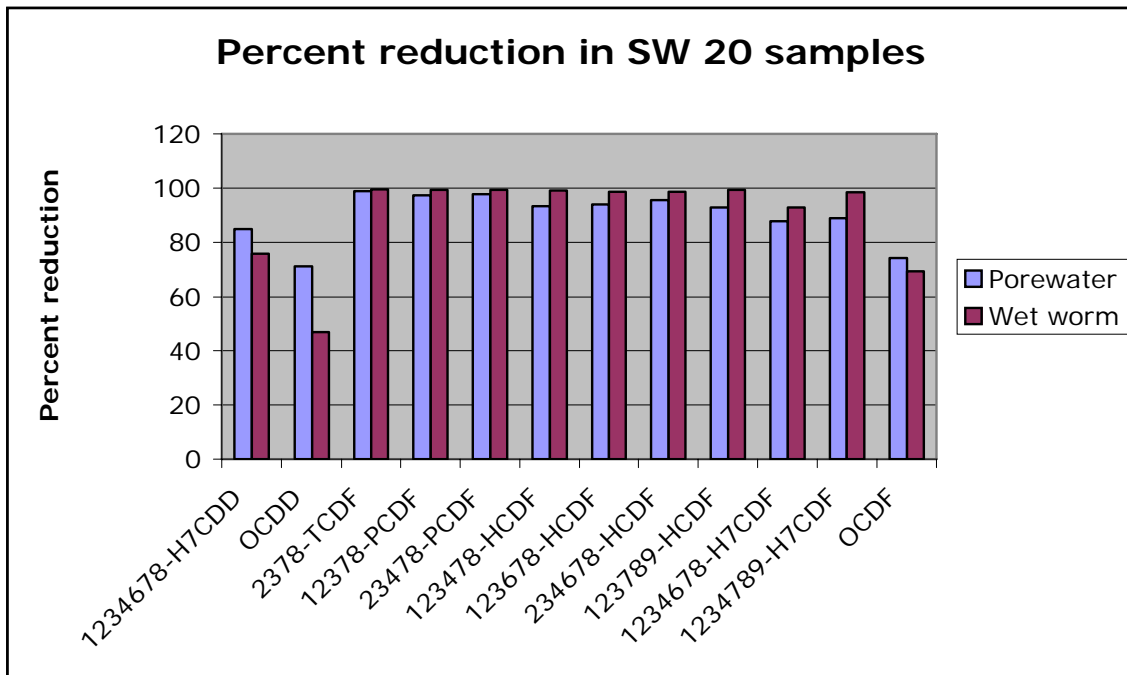


Figure S5. Percent reduction in worm and porewater concentration in SW-20 with 2% AC compared to the no treatment control.

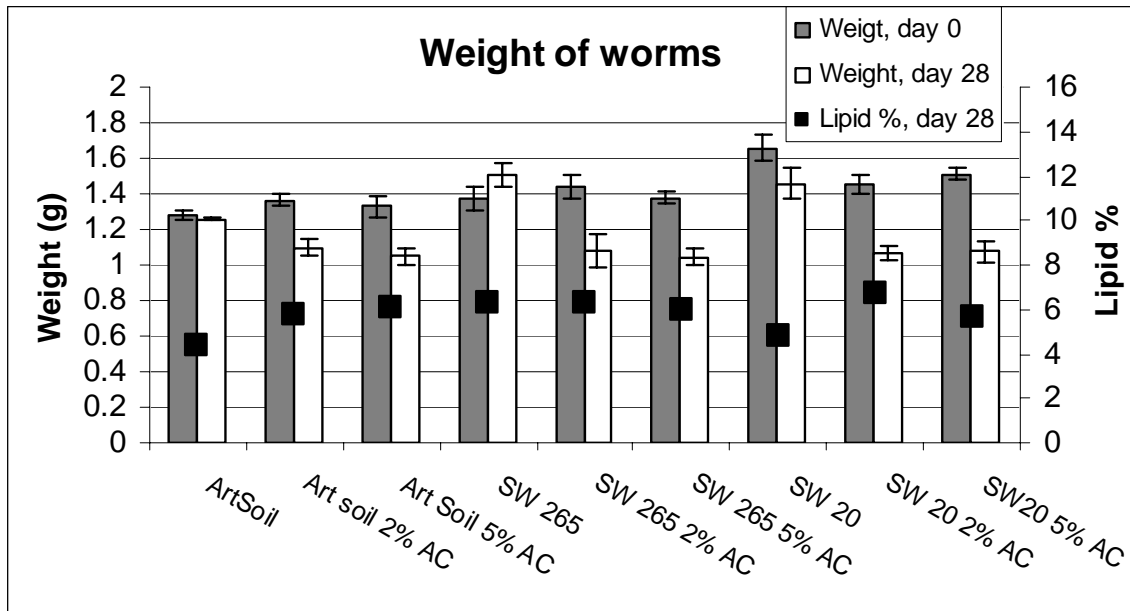


Figure S6. Weight before and after incubated and lipid % of worms after 28 days of incubation. Grey bars represents weight at day 0 and open bars represents weight at day 28. Solid squares represents lipid % of the worms at the end of the bioaccumulation test. (error bars will come on lipid %)

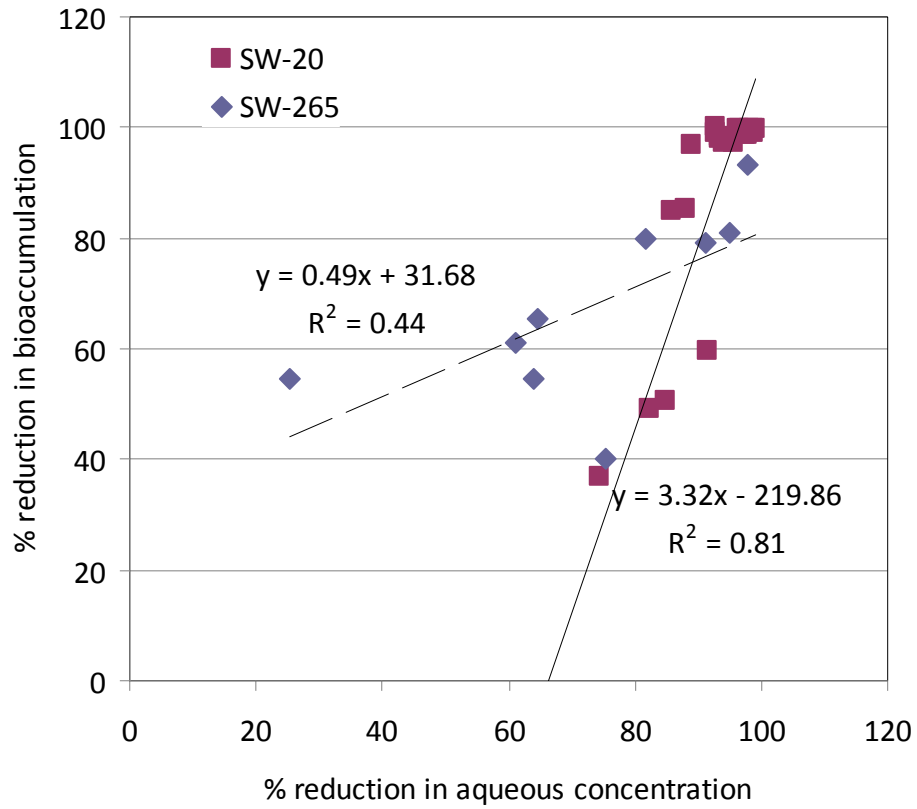


Figure S7. Correlation between percent reduction of bioaccumulation and aqueous equilibrium of dioxins and furans with AC amendment to soil.