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Letter

First Evidence of Environmental Formation of Sulfonated PCBs

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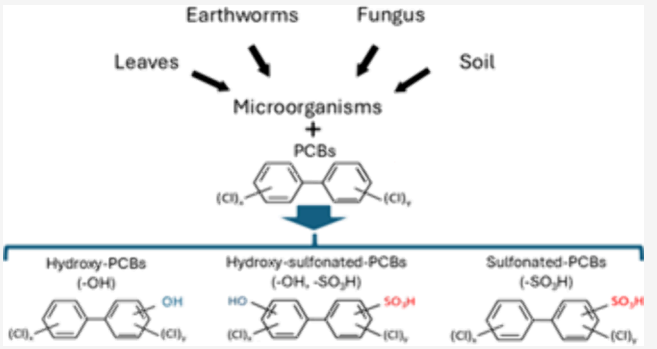
ABSTRACT: Several types of metabolites can be produced from the environmental degradation of PCBs such as OH-PCBs, sulfated PCBs, methoxylated PCBs, and others. However, while sulfonated and OH-sulfonated PCBs were recently found in soil, little information is available on their formation and their environmental path. In this study, the environmental formation of OH-sulfonated and sulfonated PCBs was shown for the first time in treatments using eight PCB congeners as a carbon source in a flask experiment. Here, 10 environmental inocula from different sources were tested to evaluate, in a semiquantitative way, the formation of OH-sulfonated and sulfonated PCBs. OH-PCBs were also monitored, as they represent one of the first degradation steps of PCBs. All inocula could generate these metabolites, although the number of congeners varied greatly. In general, OH-PCBs and OH-sulfonated PCBs were produced at the highest concentrations. For sulfonated PCBs, the highest number of congeners was produced from an inoculum derived from plant leaves and from a PCB-contaminated site. The results show that OH-sulfonated and sulfonated PCBs can be environmentally formed by microbial inocula, even by the generalist ones, not specifically selected in PCB rich environments.



KEYWORDS: *hydroxy-sulfonated PCBs, sulfonated PCBs, sulfated PCBs, LC-HRMS identification, congeners*

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a widespread class of old industrial chemicals, well-known for being persistent organic pollutants (POPs)[1] and for their toxic effects on both human health and the environment.[2,3] Over the years, attention to their metabolites, such as methoxylated,[4,5] hydroxylated,[6−9] and sulfated PCBs,[10−13] has also increased, since they are persistent in the environment and induce toxicity, as their parent compounds.[12] Recently, two new classes of PCB metabolites were found: sulfonated polychlorinated biphenyls (S-PCBs) and hydroxy-sulfonated polychlorinated biphenyls (OH-S-PCBs). S-PCBs were identified for the first time in polar bear serum,[14] and shortly thereafter, they were detected, together with the OH-S-PCBs, in agricultural soil samples collected from a heavily PCB-contaminated site located in northern Italy, i.e., the National Priority Site (SIN) for Remediation SIN Brescia-Caffaro.[15] Here, soils were collected in agricultural areas that were contaminated for more than 50 years by irrigation water receiving wastewater of one of the largest PCB-producing factories in Europe.[16−20] In 2021, Li and co-workers[21] investigated the importance of microorganisms living in the gastrointestinal tract of mice fed with a PCB mixture in the formation of PCB metabolites, including S-PCBs. By analyzing samples of feces, collected 24 h after PCB administration, the authors observed a higher production of S-PCBs in mice with a microbiome than in germ-free ones, suggesting that gut microorganisms could play a role in the formation of these new contaminants. Regarding their environmental fate, it was shown that they can bioaccumulate in earthworms[22] and plant roots.[23] In addition, Palladini and colleagues[23] investigated their persistence in soil by analyzing soil samples subjected to rhizoremediation with different plants for 18 months. The results revealed that after 18 months of rhizoremediation their concentrations in soil did not appreciably decrease in any sample. Although their potential toxicological and ecotoxicological effects are not known yet, human health toxicity and ecotoxicity can be expected, as for other PCB metabolites.[12] Although the PCB degradation capability of various microbial strains has been tested in several studies[24−33] to date, no experiments have been performed to investigate the ability of microorganisms in the formation of these two new classes of metabolites in the environment. Therefore, the aim of this paper is to test for the first time the potential environmental production of S-PCBs and OH-S-

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Table 1. Types of Inocula Used in This Experiment[a]

treatment	description	environmental representativeness
LIL	nonsterilized flask (low inoculum), light conditions	evaluation of a generic opportunistic inoculum, under light conditions
LID	nonsterilized flask (low inoculum), dark conditions	evaluation of a generic opportunistic inoculum, under dark conditions
G1	inoculum from the gut of earthworms living in a PCB-contaminated site	evaluation of a PCB-adapted and possibly selected inoculum
B1	culturable bacterial inoculum from G1 after growth on specific culture media	evaluation of the enriched bacterial fraction of G1
F1	inoculum from *Pleurotus ostreatus* fungus	evaluation of a specific fungal inoculum from mycelia
F2	culturable fungal inoculum from G1 after growth on specific culture media	evaluation of an enriched fungal fraction of G1
S1	inoculum from PCB-contaminated site soil	evaluation of a microbial community selected in a soil highly contaminated with PCBs
M2	inoculum from a mix of *Quercus ilex* and *Photinia* sp. leaves	evaluation of a mixed phyllosphere activity
M1	culturable bacterial and fungal inocula mix from S1 after growth on specific culture media	evaluation of a selected and enriched fungal and bacterial community from a PCB-contaminated site
L1	inoculum from only *Q. ilex* leaves	evaluation of a single-species phyllosphere inoculum activity

[a]Two additional (sterilized) controls not shown. More details in Table SI-2.

PCBs by microbial communities. While no efforts were made to identify microbial species or consortia, precise metabolite concentrations, parent compound disappearance, or specific pathways, our aim was to test for the first time the ability of different inocula, from several environmental matrices, to produce metabolites and provide evidence for formation in the environment starting from PCB parent compounds.

METHODS AND MATERIALS

Experimental Design and Setup. Ten different inocula were tested with a PCB mix of eight congeners (PCB 3, PCB 15, PCB 31, PCB 40, PCB 155, PCB 116, PCB 175, and PCB 209) as a carbon source. These congeners were one for almost each chlorination class (from mono- to hepta-PCBs, plus the deca-PCB) (more details in Table SI-1). Continuously stirred 250 mL flasks (Table 1) were used to host inocula from different microbial communities or species hosting the communities. Flasks were kept at 20 ± 1 °C and 250 rpm. Most of the treatments (G1, B1, F1, F2, S1, M1, M2, and L1) (Table 1) were selected to evaluate the metabolite formation ability of specific inocula, such as those from a PCB-contaminated site, from earthworms living in the same contaminated site, fungi, or the leaf phyllosphere. Two treatments (LIL and LID) were instead selected to test the metabolite formation ability of a generalist environmental microbial contamination (nonsterilized flasks). More details about the environmental representativeness of the inocula are available in Table 1. Two additional flasks were used as sterile controls to assess abiotic degradation under dark/light conditions. Each flask was filled with 100 mL of sterilized mineral medium (MM), spiked with a native PCB mix and added with the respective inoculum (apart from controls).

Samples were collected and analyzed at different times (3, 17, 31, 80, and 213 days) to observe the formation of PCB degradation products during shorter (weeks) and longer (months) time periods.

Extraction and Analysis of PCB Metabolites. Samples were extracted according to the method of Bagnati et al.[23] Briefly, aqueous sample extraction was performed with ENVI-C18 SPE cartridges by acidifying the samples with 0.1% formic acid and eluting them with methanol and cyclohexane. Solid samples were centrifuged and extracted as reported in Text SI-1. Analyses were performed using a Thermo Fisher Scientific HPLC-HRMS instrument, a Vanquish LC system coupled to an Orbitrap Exploris 120 high-resolution mass spectrometer, run at 60 000 resolution. The identification of S-PCBs and OH-S-PCBs was extensively described in a previous paper that reported their first discovery in soil samples.[15] Briefly, it was based on accurate mass measurements in MS and MS^2 mass spectra (sulfonated PCBs and OH-sulfonated PCBs) and additionally on comparisons with data obtained with a mixture of sulfonation products of an industrial PCB mixture (sulfonated PCBs). The confidence levels of these identifications were based on the paper by Schymanski et al.:[34] "level 1b" (confirmed by the synthesis of a mixture of isomers) for sulfonated PCBs and "level 3" (tentative candidates) for OH-sulfonated PCBs. The LOQs were determined for the different congeners to be about 2 ng/L, based on the smallest measurable peak areas in the corresponding ion chromatograms extracted with a 5 ppm window. More details about the analytical and experimental procedures are given in Texts SI-1 and SI-2.

Quality Assurance/Quality Control (QA/QC). OH-PCB-187 and S-PCB 155 (the only sulfonated standard available to the best of our knowledge, previously synthesized by our group[35]) were used to construct calibration curves, which were applied also to the quantification of other detected metabolites (more information about the quantitative analysis is given in Text SI-1). To evaluate possible cross-contamination, laboratory blanks (solvents) were included at a rate of one per sample batch and extracted following the same procedures as for samples. The extraction recovery of the internal standard from the aqueous phase was complete (139 ± 15%),[23] whereas it was 75 ± 10% for soil,[15] leaves, and pellet samples. The spike solution was also analyzed for metabolites, and if found, they were subtracted from those in the experimental samples. A thorough validation of the quantitative method for all of the metabolites was not possible, because of the lack of most reference standards. When using the available standards, S-PCB 155 and OH-PCB-187, the percent coefficients of variation (CV %) for three replicates were 1.77% and 3.78%, respectively.

RESULTS AND DISCUSSION

Formation of OH-PCBs, OH-S-PCBs, and S-PCBs in Each Treatment and Control. Given the lack of S-PCB and OH-S-PCB standards and considering the number of parent PCBs in the mix, the following description will give an

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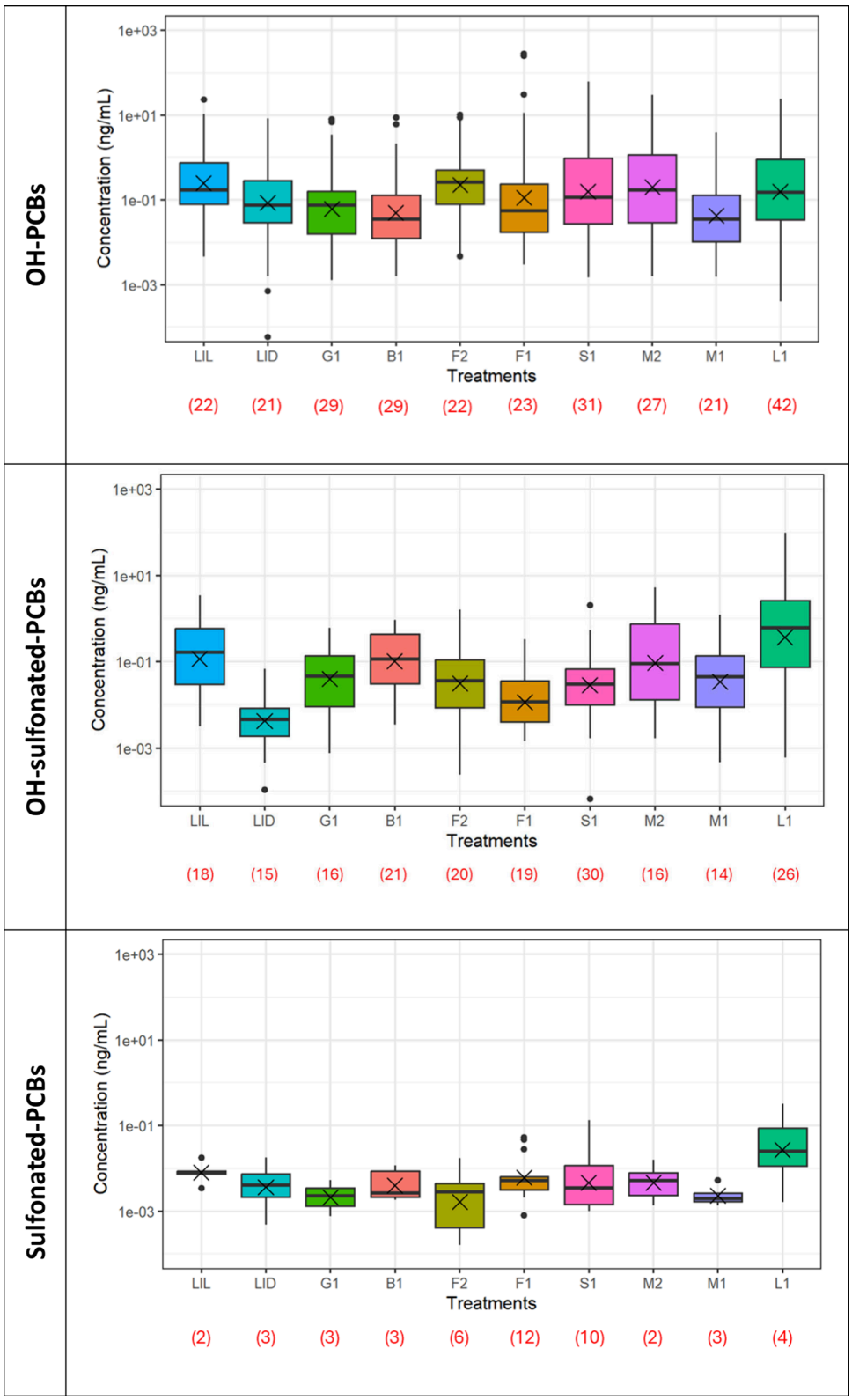

Figure 1. Box plots of concentrations of OH-PCBs, OH-S-PCBs, and S-PCBs per treatment on a log scale. Dots over and below the box plots represent the outliers. Red numbers in brackets below each box plot indicate the number of unique congeners detected for each treatment.

overview of the time of formation, general concentrations, and number of congeners formed. No metabolites were detected in sterilized (light and dark conditions) control samples. While for OH-PCBs the average and median concentrations produced were substantially oscillating around 0.1 ng/mL, a different picture appeared for OH-S-PCBs and S-PCBs (Figure 1). In fact, for these two classes, a larger difference among the median concentrations could be observed among treatments in terms of concentrations. The L1 treatment seemed to be the most efficient in forming OH-S-PCBs (almost 1 ng/mL) with

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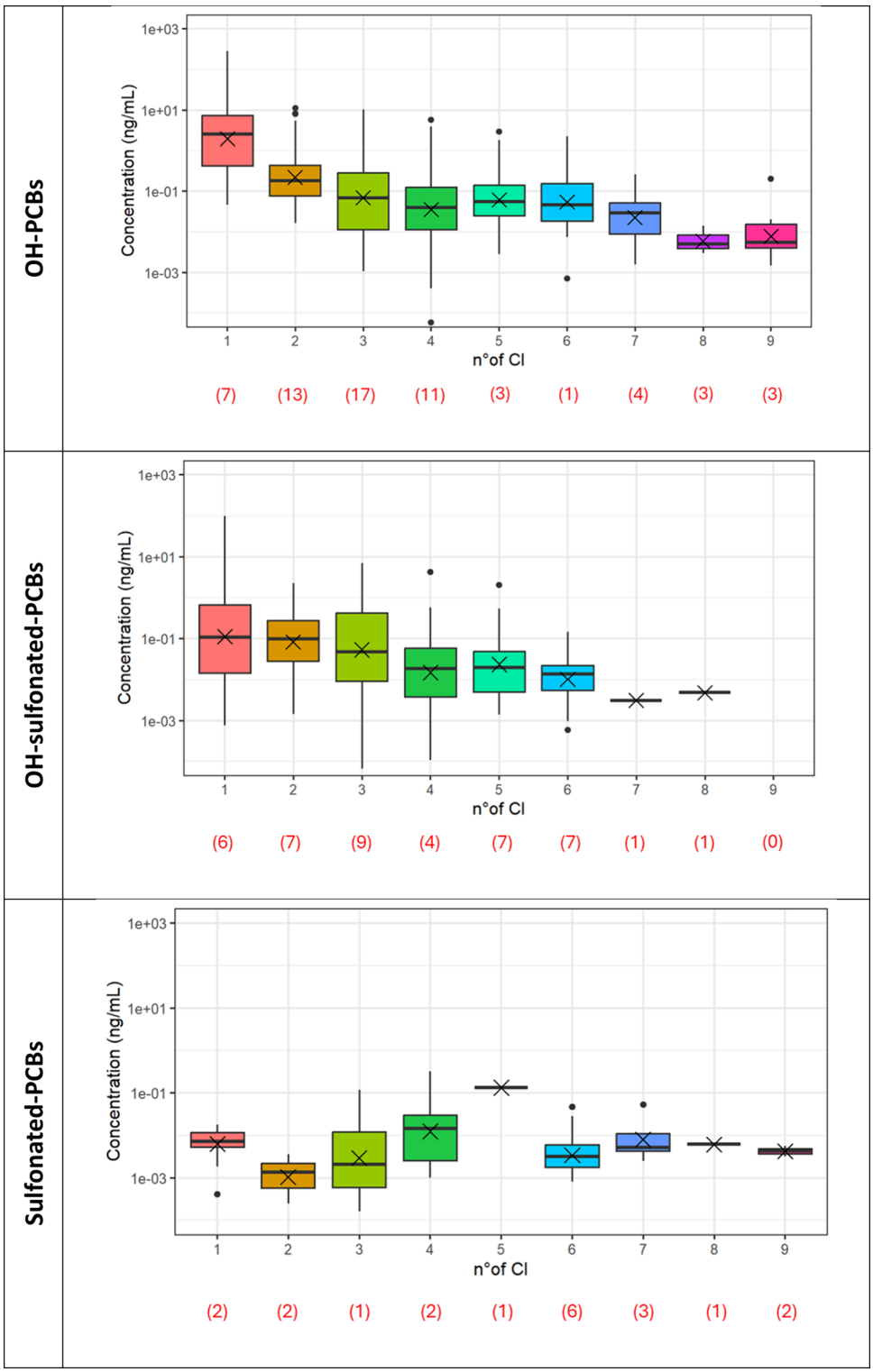

Figure 2. Box plots of concentrations of all chlorination families of OH-PCBs, OH-S-PCBs, and S-PCBs on a log scale, regardless of the treatment. Red numbers in brackets below each box plot indicate the number of unique congeners detected for each chlorination family.

the other treatments generally differing within 1−2 orders of magnitude: for OH-S-PCBs around 10^{-1} ng/mL for B1, LIL, and M2, between 10^{-1} and 10^{-2} ng/mL for M1, G1, F2, and S1, and less than 10^{-2} ng/mL for F1 and LID. When evaluating S-PCB formation, L1 was still the best performing in terms of median concentration (around 6×10^{-2} ng/mL), followed by LIL, F1, M2, S1, B1, G1, and M1 (between 10^{-2} and 10^{-3} ng/mL). However, the ranking slightly changed when looking at the total of different congeners per class, where S1 (the inoculum derived from a PCB-contaminated site) was first with 30 congeners, L1 was second with 26 congeners for OH-sulfonated PCBs, while M1 was last with 14 congeners. Looking at S-PCBs, the maximum number of congeners was reached by the fungal inoculum (F1) with 12 congeners, followed by S1 with 10 congeners. The low-inoculum treatments, especially LIL, seemed to confirm the ability of a

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generalist inoculum to degrade PCBs, although not at the fastest pace, and with a reduced number of congeners (~2−3).

Leaves as One of the Best Inocula. The unexpected degradation ability of L1 can be explained by the presence of a high diversity of microorganisms on their surface, which constitute the phyllosphere.[36] This community includes bacteria, mycelial fungi, and yeasts. Many studies dealing with the ability of leaf-associated bacteria in PAH and BTEX degradation are available.[37−44] To the best of our knowledge, no studies on PCB degradation on leaf surfaces are available. In fact, the efficiency of metabolite formation by L1 was surprising. It can be hypothesized that leaf-associated microorganisms, in this case of *Q. ilex* leaves, in addition to being able to degrade PAHs,[42] can also metabolize PCBs. On the other hand, it is known that PCBs can be degraded by adapted microbial communities living in PCB-contaminated soils, such as the SIN Brescia-Caffaro,[17,45] and by *P. ostreatus*, even in liquid medium.[46−49]

OH-PCBs, OH-S-PCBs, and S-PCBs within Each Chlorination Family. Looking within the chlorination families of the individual classes, regardless of the treatment (Figure 2), the less chlorinated families (from mono to tri or tetra) generally predominate over the others, especially for OH and OH-S-PCBs, with concentrations higher by up to a few orders of magnitude. More specifically, OH-PCB concentrations span more than 4 orders of magnitude, with levels generally decreasing with an increase in the number of chlorines. With OH-S-PCBs, concentrations also decreased with an increase in the number of chlorines, but generally within less than 2 orders of magnitude. For S-PCBs, no clear trend can be observed, with the concentration generally oscillating within 2 orders of magnitude, with a maximum for pentachlorinated and a minimum for di- and trichlorinated. For a fair comparison, it must be remembered that concentrations in the spike mix of parent PCBs for penta-, hepta-, and deca-PCBs were 1/5, 1/5, and 1/20 of the other families, respectively, and octa- and nona-PCBs were not present in the spike mix (Table SI-1), so this could explain some of the difference in concentrations.

Comparison with the Environmentally Measured Concentration Fingerprints. While a comparison between laboratory and environmental concentrations was not possible, due to the nature of this experiment and the few environmental measurements available, a fingerprint (comparison of congener class abundance) analysis was instead feasible. The trends observed here for the metabolite families differed from those reported for contaminated soils,[15] where, in general, penta- and hexachlorinated metabolite families were present at the highest concentrations. This might be because PCBs with a low number of chlorine substitutions (1−3) are more easily degraded[50] and, according to their physicochemical properties, more easily leached into soil than the highly chlorinated and more hydrophobic PCBs, which may accumulate over time, especially in the top soil layer.[16] Additionally, often the concentrations of PCBs in the environment reflect the original aroclor or other PCB mixture composition, where penta- and hexachlorinated congeners often prevail.[17]

OH-S-PCB versus Sulfated PCBs. Some considerations can be made on the formation sequence and chemical identities since no nona- or octa-PCB congeners were used in the spike mix, so their formation would be derived from the decachloro-PCB, PCB 209, present in the mix. There is a current debate[14,15] on the identity of OH-S-PCBS since they are indistinguishable in HRMS from sulfated PCBs, given the same exact mass. The chemicals described as OH-S-PCBs can also be sulfated PCBs or a mixture of OH-S-PCBs and sulfated PCBs. The degradation of PCB 209 can perhaps furnish some hints on the potential identity, since nonachloro- and octachloro-S-PCB were formed, but no $C_{12}H_2O_4Cl_9S$ (which in this case can only be nonachloro-sulfated PCB) was formed, in any treatment (Figure 2). Indeed, no formation would have been possible for a nonachloro-OH-S-PCB, while the dechlorination product, identified as octachloro-OH-S-PCB (Figure 2) appears, possibly indicating its identity as OH-S-PCB. Further studies are ongoing to confirm this hypothesis.

Temporal Trend of Degradation Products in the Different Treatments. Details about the formation of congener classes by the different treatments in time are available in Tables SI-3 and SI-4 and Figures SI-1−SI-3. While a precise evaluation of concentration changes would require single-congener experiments, the present setup allows for a first and comparative evaluation of the degrading capability of different inocula. For OH-PCBs (Figure SI-3), nearly all inocula seem to be capable of producing an array of metabolites, even at the very beginning (t = 3 to t = 31), with an increase of 1−2 orders of magnitude: most up to heptachlorinated PCBs and some (such as F1) up to nonachloro-OH-PCBs. For OH-S-PCBs (Figure SI-2), the situation is slightly different with most inocula requiring up to 80 or 213 days to produce highly chlorinated formation products and mostly limited to penta- or hexachlorinated OH-S-PCBs. For S-PCBs, a general large variability appears, with metabolites that emerged at the end of the experimental period (213 days).

Degradation Product Fingerprint of Different Inocula. Principal component analyses (PCAs) were performed (Figure SI-4) to determine which treatment would produce specific congener classes. PCAs were produced for the end of the experimental period (213 days), since at this time most metabolites were formed, especially the highly chlorinated ones. Figure SI-4 shows that L1 and S1 treatments have a specific ability to form tri-, tetra- and hexa-OH-PCBs, while F1 appears to be more capable of forming highly chlorinated congeners, such as octa and nona, while the other treatments do not show specific abilities. Looking at OH-S-PCBs, F1 again confirms the higher efficiency of formation of hexa- to octachlorinated congeners, S1 for penta and G1 for di and tetra, while the rest of the treatments show a similar behavior mostly characterized by tri and mono. For S-PCBs, F1 is again best in producing highly chlorinated congeners (from hexa- to octachlorinated), while L1 and S1 are characterized by the formation of di-, tri-, and tetra-S-PCBs. LIL and LID treatments unexpectedly reveal the capability of producing also highly chlorinated metabolites (up to penta- and hexachlorinated), showing that a generalist and opportunistic inoculum could form these metabolites.

Outlook for Future Studies. This work has shown the ability of different microbial inocula to produce S- and OH-S-PCBs. However, many more studies are needed to elucidate, for example, if they are formed under aerobic or anaerobic conditions as well as their precise identity, such as OH-S-PCBs or sulfated PCBs. To verify these conditions, more single-congener standards should be available or techniques capable or recognizing congeners formed during the mass spectrometric analysis. In addition, more studies are needed to evaluate the microbial species responsible for the degradation, possibly with the need for metagenomic techniques.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.5c00139.

Additional information about the experimental procedures and methods, including the congeners and amount used in the experiment, details about the inocula used, and concentrations detected in all treatments in tabular and chart form (PDF)

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Notes

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