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# Can Physicochemical and Microbial Soil Properties Explain Enantiomeric Shifts of Chiral Organochlorines?

MARTINA KOBLIČKOVÁ,<sup>†</sup>  
LADISLAV DUČEK,<sup>†,‡</sup> JIČI JARKOVSKÝ,<sup>†,‡</sup>  
JAKUB HOFMAN,<sup>†</sup> THOMAS D. BUCHELI,<sup>§</sup>  
AND JANA KLÁNOVÁ<sup>\*,†</sup>

RECETOX - Research Centre for Environmental Chemistry and Ecotoxicology, Masaryk University, Kamenice 126/3, 625 00 Brno, Czech Republic, Institute of Biostatistics and Analyses, Masaryk University, Kamenice 126/3, 625 00 Brno, Czech Republic, and Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstrasse 191, CH-8046 Zurich, Switzerland

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Enantiomeric fractions (EF) of PCB 95, 132, 149, and 174,  $\alpha$ -HCH,  $o,p'$ -DDD, and  $o,p'$ -DDT were analyzed in 112 soil samples using two-dimensional gas chromatography and triple-quadrupole mass spectrometry. To assess the soil conditions that facilitate enantioselective fractionation of chiral compounds, EF values of selected PCBs were further correlated with a wide range of physicochemical and microbial soil parameters in an attempt to identify the influential factors and their mutual relations. It was evident that soils where nonracemic ratios of investigated compounds were found were more carbon rich but they also contained significantly more humic and fulvic acids and total nitrogen. These specific physicochemical properties were accompanied by significantly increased values of all key biotic variables, the amount of microbial biomass, and its respiration activity (both basal and substrate-induced). Therefore, the shifts from racemic ratios appeared to be associated with more sustainable and active soil microflora. Among other abiotic characteristics, most significant differences were detected in the soil texture. Soil samples with significant shifts contained increased amount of clay component and correspondingly decreased proportion of sand fraction. These differences can also be associated with more intensive microbial activity, because clay content and texture with an increased amount of microaggregates are known to be favorable for soil microflora and its viability.

## Introduction

Persistent organic pollutants (POPs) are a group of compounds receiving continuous attention due to their long half-lives in the environment and their potential harmful effects. To assess their potential environmental and human risks, not only their current levels in various matrices but also many factors influencing their future fate have to be considered, such as their low photochemical and biological degradability,

potential for long-range transport, and accumulation in biotic matrices. What makes the problem even more complex is the fact that many of these POPs are chiral and occur in the environment as two (or more) isomers with different optical activities. Nineteen out of 209 PCB congeners, or organochlorine pesticides such as  $\alpha$ -HCH, *trans*-chlordane, *cis*-chlordane, and  $o,p'$ -DDT, are chiral compounds composed of two stereoisomers which are identical in atoms and bindings but with different three-dimensional structures that are not superimposable. Enantiomers have identical physicochemical properties and abiotic degradation rates; but because of different molecular configurations, they may differ in binding to structure-sensitive biological receptors, and as a result undergo biotic degradation at different rates (1–3). The chirality of biologically active compounds is of special relevance due to the fact that most of these compounds are introduced into the environment as racemic mixtures (1:1), but their uptake and metabolism by organisms may be selective for individual enantiomers (4, 5).

In the last 20 years, particular attention has been paid to the enantiomeric ratios (ER) of POPs in biotic samples. A variety of chiral compounds was analyzed in zooplankton and benthos (3), rainbow trout (6), and Baltic herring and guillemot egg (7), in their internal organs, e.g. shark livers (4), or blubber (8), eggs, or human milk (7, 9, 10). Since the ERs often change between the trophic levels, some researchers have assessed their values throughout the food chain (3).

Another subject of numerous investigations has been soil as a matrix serving as a sink of the atmospheric pollution and preserving the long-term record of contamination. ERs of various chiral pollutants were measured in several soil types from a variety of sampling sites, since the microbial degradation in soil also leads to the shifts in their values (7, 11–17). Deviations from racemic mixtures were observed in both directions, but racemic mixtures (i.e., EF = 0.5) were also found in many cases. Some authors have attempted to compare the chiral signatures found in air samples with the ERs in soil for source apportionment of the atmospheric pollution and determination of pollutant flux directions (11, 12, 14–16).

The activity of the microbial community and its degradation preferences were thought to be responsible for fluctuation of ERs in soils, but the factors influencing this fluctuation have never been addressed in detail. Wiberg et al. (17) measured EFs of organochlorine pesticides in soils and assessed their correlation with total organic carbon (TOC) content, assuming that microbial activity depends on TOC. Kurt-Karakus et al. (17) studied correlations between EF deviations, soil organic matter (SOM) content, and mean annual temperatures. Wiberg et al. found no differences between EF in silt loam and sandy loam soils (13) and concluded that the variability in the enantiomeric composition could not be explained by differences in soil grain size composition. Bucheli and Brändli (7) found soil pH to be correlated with enantiomeric shift of atropisomeric PCBs in soils, and Buerge et al. (18) observed the same for chiral pesticide metalaxyl.

Studies of enantioselective degradation of chiral compounds conducted under laboratory conditions have also failed to produce a clear explanation. Garcia-Ruiz et al. (19) observed no significant variations of atropisomeric ratios during the biodegradation process indicating nonstereoselective degradation of PCBs by microorganisms. On the contrary, Singer et al. (20) showed that enantioselectivity varied with respect to strain, congener, and substrate. The observed shifts of enantioselectivity for the same strain and

\* Corresponding author e-mail: klanova@recetox.muni.cz.

<sup>†</sup> RECETOX - Research Centre for Environmental Chemistry and Ecotoxicology, Masaryk University.

<sup>‡</sup> Institute of Biostatistics and Analyses, Masaryk University.

<sup>§</sup> Agroscope Reckenholz-Tänikon Research Station ART.

substrate, but for different PCB congeners, were explained by the specific chlorination pattern of each PCB congener that predetermines a degree of interaction with the enzyme (20).

Identification of factors controlling enantioselective processes in the real environment is, however, crucial for a better understanding of the sources of enantioselectivity observed in environmental samples. In attempt to identify such influential factors and their mutual relations, we correlated the EF values with a wide range of physicochemical and microbial soil parameters from a variety of soil samples. To do this, and in contrast with most of the earlier studies, we relied on a highly selective quantification method (7).

## Materials and Methods

**Soil Sampling and Characterization.** Top soils ( $n = 112$ ) were sampled, manipulated, and stored in accordance with ISO standard (21). They were collected from various sampling sites (mostly grasslands) in the Czech Republic ( $n = 60$ ) (22), former Yugoslavia ( $n = 33$ ) (22), and the Sultanate of Oman ( $n = 19$ ) in 2005–6 covering a range of soil types and contamination levels. For microbiological analysis, fresh soils were sieved ( $<2$  mm) and stored at  $4^{\circ}\text{C}$ . For physicochemical and chemical analyses, soils were air-dried at laboratory temperature. The following physicochemical characteristics of samples were measured by standard methods described, e.g., in Forster (23): soil pH values ( $\text{pH}(\text{H}_2\text{O})$  and  $\text{pH}(\text{KCl})$ ), total organic carbon (TOC), total soil nitrogen ( $N_{\text{tot}}$ ),  $\text{CaCO}_3$  content, humic compounds (HCs) in 0.1 M sodium pyrophosphate soil extracts (humic acids, HA; fulvic acids, FA), cation exchange capacity (CEC), base saturation (BS), Q4/6 parameter, particle size analysis: clay ( $<0.002$  mm), fine soil particles ( $<0.01$  mm), coarse soil dust (0.01–0.05 mm), fine sand (0.05–0.1 mm), and sand (0.1–2.0). The following soil microbial parameters were measured: microbial biomass content ( $C_{\text{bio}}$ ) (24), extractable carbon ( $C_{\text{ext}}$ ) (24), basal respiration (BR) (25), substrate-induced (SIR) respiration (26), and biomass-specific respiration rate ( $\text{qCO}_2$ ).

**Sample Preparation.** Each dry soil sample (5 g) was extracted with dichloromethane (DCM, pesticide residue analysis grade, Pestiscan, Lach-Ner, Neratovice, Czech Republic) in a Büchi System B-811 automatic extractor (Büchi Labortechnik AG, Flawil, Switzerland). A glass column (30 cm length, 1 cm I.D.) filled with 5 g of silica gel (Merck, Darmstadt, Germany, activated overnight at  $150^{\circ}\text{C}$ , and modified with sulfuric acid) was used for cleanup. PCBs and OCPs were eluted using DCM: *n*-hexane (pesticide residue analysis grade, Pestiscan, Lach-Ner, Neratovice, Czech Republic) (1:1) mixture (30 mL), and samples were concentrated under a gentle stream of nitrogen (grade 4.7, SIAD, Czech Republic) to the final volume of 1 mL.

**Chemicals.** Chiral PCBs 95, 149, 132, and 174 were purchased from LGC Promochem (Wesel, Germany) as individual solutions;  $\alpha$ -HCH, *o,p'*-DDT, and *o,p'*-DDD were from Dr. Ehrenstorfer GmbH (Germany). Solvents were purchased from Lach-Ner (Neratovice, Czech Republic) in the pesticide grade quality. Deionized water was purified using a Milli-Q Gradient A10 water purification system (Millipore, Billerica, MA).

**GC–GC–MS–MS.** The method developed by Bucheli and Brändli (7) for quantification of atropisomeric PCBs was adapted to include several chiral organochlorine pesticides (see Supporting Information (SI) for more information). PCBs were detected with a triple quadrupole mass spectrometer Varian 1200 (Varian, Walnut Creek, CA) in the electron impact mode with 70 eV ionization energy. Multiple reaction monitoring was performed with  $[\text{M}]^+$  as precursor ion and  $[\text{M} - 2\text{Cl}]^+$  as product ion for all PCBs. For  $\alpha$ -HCH, *o,p'*-DDT, and *o,p'*-DDD, collision energy was set to  $-30$  V using

argon as collision gas. Peak assignment, retention times, heart-cut windows, and monitored ions are listed in SI (Table S1).

**Determination of Enantiomeric Fractions.** The enantiomeric fraction (EF) rather than enantiomeric ratio (ER) was employed in the analyses. As demonstrated by Harner et al. (27) the enantiomeric fraction has substantial advantages when compared to enantiomeric ratio. It is based on a bounded additive scale that is linear, finite (ranging from 0 to 1), and symmetric in sample distribution around the racemic value of 0.5 (27–29).

The enantiomeric fraction (EF) is used as a descriptor of enantiomeric signatures:

$$\text{EF} = E_+/(E_+ + E_-) \text{ or } E_1/(E_1 + E_2)$$

where  $E_1$  and  $E_2$  was the first and the last eluting enantiomer when the identity of (+) and (–) forms was unknown. All EFs were determined using the peak areas of the respective enantiomers ( $E_1$ ,  $E_2$ , and  $E_+$ ,  $E_-$ , respectively).

Enantiomeric ratios ( $\text{ER} = E_+/E_-$  or  $E_1/E_2$ ). They are often used in the literature and can be transferred to EFs:

$$\text{EF} = \text{ER}/(1 + \text{ER})$$

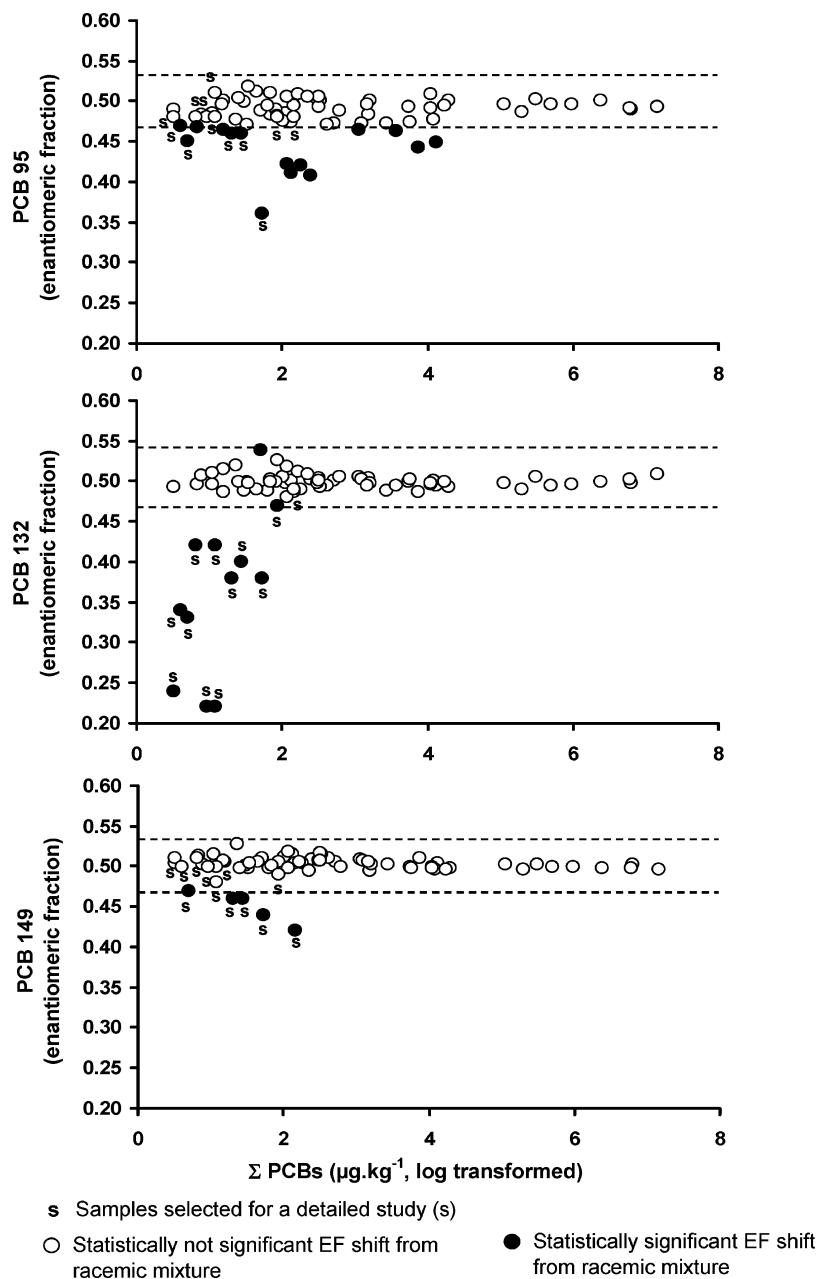
For PCBs 132, 149, and 174, and  $\alpha$ -HCH, the elution order of individual enantiomers was specified using the enantiopure standards. In the case of PCB 95, *o,p'*-DDD, and *o,p'*-DDT,  $E_1$  and  $E_2$  values were used for the calculation of EF.

**Statistical Analyses.** The standard set of nonparametric descriptive statistics (median, percentiles) was used to describe primary values of both abiotic and biotic measures. All concentration parameters were found to be log-normally distributed with occasional very outlying values in the upper tail. The influence of extreme maxima was eliminated using 10th–90th percentile range in any comparative analysis. Primary values were further log transformed  $[\ln(X + 1)]$  to reach normal distribution and homogeneity of variance prior to any parametric testing. Standard *t* test and one-way ANOVA were used to compare two or more experimental variants. Pearson's product-limit correlation was used to inspect mutual association among examined parameters. The analyses were performed using Statistica data analysis software system, version 8 (2007).

## Results and Discussion

**A Pilot Study.** Soil samples from the Czech Republic, former Yugoslavia, and the Sultanate of Oman were used for the initial analysis of the EFs. Ranges of EF values for the individual chiral POPs measured in this study are listed in SI, Table S2. There was no significant difference in the variability of the EF determined in the three regions, and measured values were also similar to the ones published previously (7, 11–17) (Table S2).

To study factors controlling enantioselective processes, we had to distinguish significant EF shifts from the racemic value ( $\text{EF} = 0.5$ ) from nonsignificant deviations of the analytical technique. A stochastic definition of racemic samples can be derived from the relative standard deviation of EF quantification. Bucheli and Brändli (7), for instance, found that apparatus precision of their methodology for determination of enantiomeric fractions of various PCB congeners ranged from 0.2 to 2.2%. Accepting the 2.2% value as a relative standard deviation of EF quantification, and  $\mu \pm 3\sigma$  as limits of distribution range, we can stochastically define samples with EFs between 0.467 and 0.533 as racemic. Therefore, only samples with EF outside this range of normal distribution can be considered nonracemic. Organic carbon content was determined in 77 soil samples as a next step, together with levels of overall POP contamination (totals of 7 indicator PCBs,  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ -HCHs, *o,p'* and *p,p'*-DDE, DDD,



**FIGURE 1. A relationship between enantiomeric fractions of PCB 95, PCB 132, and PCB 149, and a total concentration of PCBs in soil ( $n = 77$  soils). Dashed lines (EFs of 0.467 and 0.533) mark the interval inside of which the samples are considered to be racemic.**

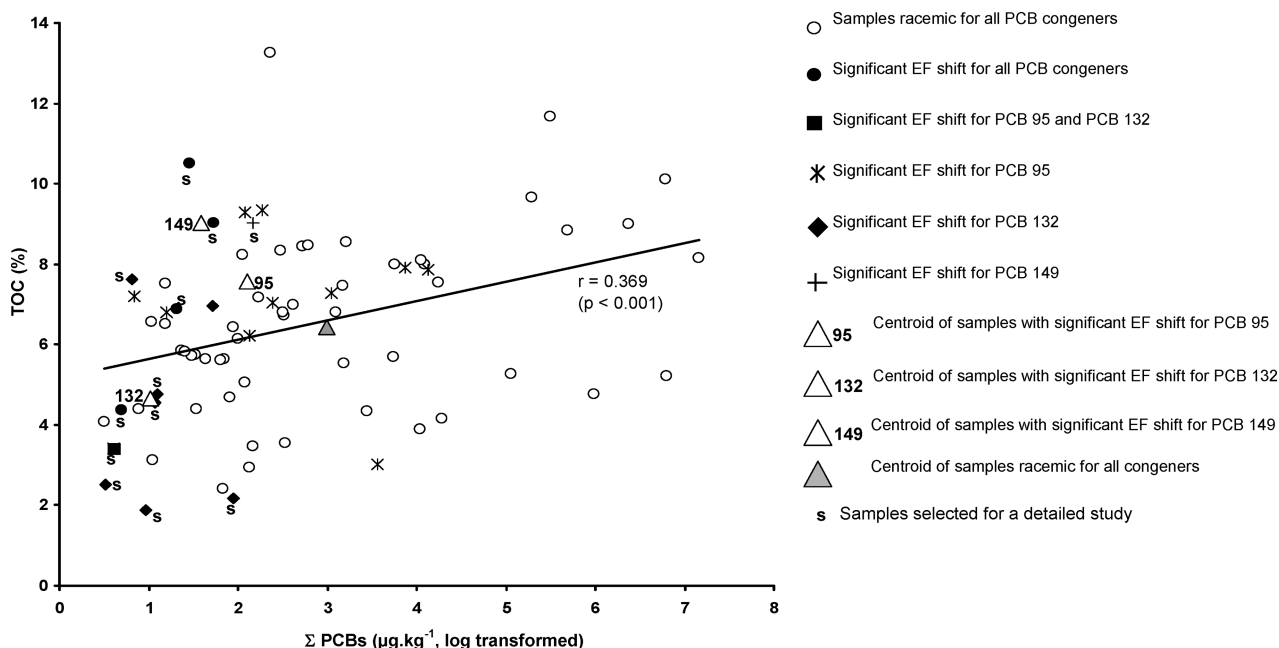
and DDT). A final data set provided a wide range of values of these key parameters. Most soils were grasslands ( $n = 71$ ; 92.2%) covering the whole range of TOC values as summarized in SI, Table S3 (median TOC value 6.4%). The survey further included four carbon-rich forest soils (median 8.9%) and two arable soils with typically decreased TOC content (median 2.7%). Forest and arable samples did not cause any systematic bias in the TOC and PCB content because of the wide range in the majority of grasslands (10th to 90th percentile range was 3.4–9.0% for TOC content and 1.6–197.9  $\mu\text{g kg}^{-1}$  for  $\Sigma\text{PCBs}$ ).

Three PCB congeners (PCBs 95, 132, and 149) were selected to be model compounds for a detailed study. Correlation scatter plots of enantiomeric fractions related to total concentrations of PCBs revealed no significant quantitatively expressed correlation (Figure 1.) Instead, all samples with  $\Sigma\text{PCBs} > 50 \mu\text{g kg}^{-1}$  manifested EFs very close to the racemic mixture (ranging from 0.47 to 0.52), while nonracemic EFs appeared to be increasingly probable in soils with

relatively low PCB concentrations where some concentration-related trends occurred.

These results lead us to the conclusion that we should work with enantiomeric fractions from a stochastic point of view rather than to search for quantitative correlation patterns. Nonracemic samples were selected from the database based on the criteria defined above (EFs outside the interval 0.467–0.533) for detailed assessment of the conditions facilitating enantioselective processes. Table S4 (SI) separates the soil samples without significant EF shifts (group A) from the soil samples with significant EF shifts for one (group B), two, or three (group C) PCB congeners. It has been documented that significant EF shifts for one or more congeners are always found in soils with low PCB concentrations.

Figure 2 shows a distribution analysis of the soil samples indicating statistically supported ( $r = 0.34$ ,  $p < 0.01$ ) relationship between the PCB levels and TOC values. As can be seen from this two-dimensional map, samples with



**FIGURE 2.** Scatter plot localization of the soil samples in a space given by TOC content and PCB concentration. Centroid is defined as a median value of all samples in the given group.

significant EF shifts for PCB 132 had lower TOC content while those of PCB 95 and PCB 149 were found in carbon rich soils. It can also be concluded from Figure 2 (and from Table S4) that significant EF shifts of all three congeners were more frequent in carbon rich soils. This trend was, however, not supported by consistent statistical significance. In fact, the overall correlation between EF and TOC was hard to interpret when applied to the whole data set ( $EF_{PCB95}$ :  $r = -0.194$ ,  $p = 0.100$ ;  $EF_{PCB132}$ :  $r = 0.310$ ,  $p = 0.010$ ;  $EF_{PCB149}$ :  $r = -0.174$ ,  $p = 0.138$ ). None of the correlation coefficients numerically exceeded value 0.5 and only  $EF_{PCB132}$  demonstrated significant positive increase with increasing TOC values. This resulted from the fact that PCB 132 provided a widest range of EF values (0.22–0.54) among all PCB congeners (Figure 1).

The lack of significant correlations between EFs and TOC (also observed in previously published studies) (13, 17) can be assigned to the large number of soil samples ( $n = 59$ , 76.6%) with racemic (0.47–0.53) EF values. These racemic samples did not contribute to the correlation pattern simply for numerical reasons; they masked any reasonable product-limit correlation inevitably resulting in the low interpretation value. Another reason is that even though TOC content is a good predictor of the soil microbial activity and biomass (30, 32), it does not fully explain the variance of EF shifts. Further soil parameters should be investigated both individually and in correlation for their potential to explain the variability of EFs.

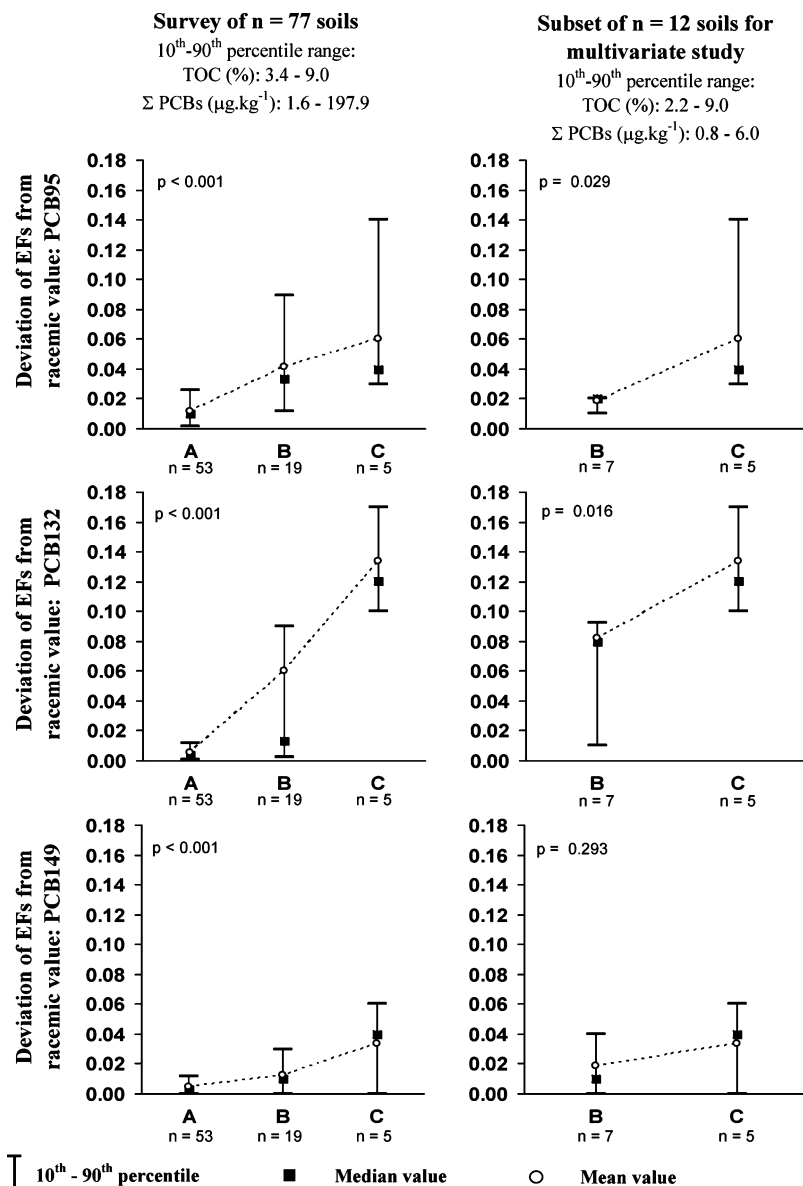
**A Detailed Assessment of Soil Parameters.** To achieve this, a reduced but well documented set of 12 soils with low levels of PCB contamination and a range of EFs (Figure 1) was identified among the original collection. A selection appeared to be representative in TOC values since a TOC range of a subset was similar to the one of a primary survey (Figure 3.) For every soil sample, at least one of three investigated PCB congeners manifested a significant EF shift, while five samples had nonracemic EF values simultaneously for two or three PCB congeners. The abiotic and biotic characteristics (SI, Table S5) derived from the long-term biomonitoring were available for all sites since these soils have been sampled and analyzed for 16 soil physicochemical properties and 8 microbial parameters four times a year since 2000. While data from a single sampling campaign would suffer from time related variability especially in biotic

attributes, this 6-year time series provided reasonable background for relevant site description. Monitoring data were aggregated on the basis of rank statistics with the overall median which allowed us to assess a situation without a masking effect of seasonal or temporal fluctuations.

There are basically two families of microbial parameters indicating sustainability and ecophysiological activity of the microbial communities which are also used to benchmark soil fertility (33) and mineralization potential (34). As expected, microbial biomass content ( $C_{bio}$ ) and all measures of respiration activity (BR, SIR) as representatives of the first group are strongly intercorrelated ( $r$  ranged from 0.66 to 0.90,  $p < 0.01$ ) and also significantly correlated with growing TOC content (all correlation coefficients  $r > 0.75$ ,  $p < 0.01$ ). Estimated content of extracellular organic carbon ( $C_{ext}$ ) forms the second dimension, independent of the biomass content, respiration activity, or TOC. A biomass-specific respiration rate ( $qCO_2$ ) relates a basal respiration (BR) to microbial biomass content ( $C_{bio}$ ). Elevated values of this quotient indicate the stress conditions, especially when combined with decreasing  $C_{bio}$ . Indeed, in our samples  $qCO_2$  negatively correlated with  $C_{bio}/C_{org}$  ratio ( $r = -0.714$ ;  $p < 0.01$ ) and the profile suggested a typical hyperbolic shape, well-known from the literature (35).

To assess soil conditions that facilitate enantioselective behavior, soils were aggregated in two groups similar to Table S4 (B: soils, where EF of one PCB congener was significantly shifted from racemic value; C: soils with significant EF shifts for two to three PCB congeners). A multivariate typology of both soil groups is presented in SI, Tables S6 and S7. It is evident, that group C samples were more carbon rich (median value of 5.7% TOC) than group B samples (3.1%), but they also contained significantly more humic (HA) (0.6 vs 0.4%) and fulvic (FA) (0.8 vs 0.4%) acids and total nitrogen ( $N_{tot}$ ) (0.3 vs 0.2). These differences were accompanied by significantly increased values of all key biotic variables: microbial biomass (824.7 vs 586.4  $\mu g C g_{dm}^{-1}$ ) and its respiration activity, both basal and substrate-induced (2.4 vs 1.4  $\mu g CO_2 - C g_{dm}^{-1} h^{-1}$  for basal, and 18.7 vs 13.4  $\mu g CO_2 - C g_{dm}^{-1} h^{-1}$  for substrate-induced respiration). More intensive degradation processes therefore appeared to be associated with more sustainable and active soil microflora. This finding was also supported by decreased value of  $C_{ext}/C_{bio}$  ratio in C group





**FIGURE 3.** Deviations of enantiomeric fractions of selected PCB congeners from the racemic value (0.5) for three groups of soil samples. Soil group A: soils, where all PCB congeners were racemic; B: soils, where EF of one PCB congener was significantly shifted from racemic value (0.5); C: soils with significant EF shifts for two to three PCB congeners.

samples (171 μg C mg C<sub>bio</sub><sup>-1</sup>) when compared to B group (208 μg C mg C<sub>bio</sub><sup>-1</sup>). Although this difference was not statistically significant, it indicated more intensive mineralization processes in the soil matrix.

Among other abiotic characteristics, most significant differences between group B and group C soils were detected in the soil texture. Soil samples with significant EF shifts for two or three PCB congeners (group C) contained very significantly increased amount of clay component (8.7% vs 6.3%) and correspondingly decreased proportion of sand fraction (38.7 vs 54.4%) when compared with the group B. These differences can also be associated with more intensive microbial activity, because clay content and texture with an increased amount of microaggregates are known to be favorable for soil microflora and its viability (30).

However, it can also be hypothesized whether these abiotic soil parameters (high TOC, HA, and FA, and clay content) also create specific conditions for other processes affecting a fate of chiral compounds in soils. Since soils clustered in group C have also significantly lower pH values (median of 5.7) when compared to B (median of 7.0), we can speculate that a high content of humic substances or clay can facilitate

specific sorption processes which can be further altered by the soil pH value. That would mean that measured EFs reflect not only a true enantiomeric ratio of two isomers in soil caused by enantioselective degradation but also enantioselective sorption of two isomers to the soil fractions (36).

A stochastic methodical approach searching for conditions that facilitate the enantioselective processes was strongly preferred to standard correlation methods which would necessarily suffer from the small size of the sample set. Categorization of soils according to the number of congeners, demonstrating significant EF shifts, proved to be a meaningful approach which allowed us to search for specific parameters affecting the enantioselective processes in soils. As can be seen from Figure 3, group C soils (significant shifts for two to three of the PCB congeners) are also the ones with the highest EF deviations from the racemic value. The same pattern was found both in a large soil survey with 77 soils and in a small subset of sites (Figure 3).

This study is, of course, limited in power due to the low number of samples in the detailed experiment and should be regarded as a pilot indication rather than a final result. However, it introduced a new approach to the problem. A

coupling of the chiral analysis to biomonitoring data brought a significant new piece of information. It has been suggested that intensive degradation processes can be predicted from specific soil conditions of C group soils (high TOC, HA, FA,  $N_{\text{tot}}$ , clay) as well as biotic parameters (BR, SIR) indicating viability of the soil microflora.

A relationship between parameters predicting the soil degradation potential and the actual EF of the individual compound in soil is, however, not so trivial since these parameters only describe the readiness of soil microorganisms to degrade chemicals. They cannot predict variability of microbial cultures with their dietary preferences. Sampling sites with multiple degrading strains can provide an excellent degradation potential yet very low shift from the racemic ratios. Moreover, there are other processes not necessarily connected to the microbial degradation of chemicals but to their sorption behavior (36). Additional experiments are needed to determine to which extent the shifts in EFs are related to abiotic or biotic processes. They have to be focused on enantioselectivity of microbial degradation of chiral compounds as well as on the enantioselective sorption of chiral compounds to humic substances.

Another problem is that we cannot fully rely on laboratory simulations with a single strain and specific substrate. Most of our knowledge about the enantioselective degradation of POPs comes from the laboratory experimental systems involving pure cultures of bacteria in laboratory media containing a single compound (37). It is, however, increasingly evident that the metabolism of substrates in natural conditions occurs via complex metabolic networks involving multiple community members exchanging metabolites and regulating carbon flow according to the availability of other substrates and nutrients, as well as to the physicochemical conditions (37). Laboratory studies are very important for revealing mechanisms but they may not reflect what is happening in the environment.

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## Supporting Information Available

GC–GC–MS–MS method (Table S1), overview of EF values measured in the soil samples from this and previously published studies (Table S2), basic characteristics of 77 soil samples from the pilot study (Table S3), TOC content and PCB concentration in the groups of soil samples without/with significant EF shifts (Table S4), detail characterization of 12 soils from the multivariate study (Table S5), abiotic (Table S6) and biotic (Table S7) parameters of investigated 12 soils stratified in two groups according to measured EFs of PCB 95, 132, and 149, and scatter plot of enantiomeric fractions of PCB 95, 132, and 149 against the PCB levels in soils (Figure S1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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