WHICH FACTORS AFFECT THE FATE OF CHIRAL PERSISTENT ORGANIC POLLUTANTS IN THE TERRESTRIAL ECOSYSTEM?

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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 2^n isomers (n is number of chiral carbons/stereogenic centers in molecule) with different optical activities. Nineteen out of 209 PCBs congeners, or organochlorine pesticides such as α-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, 2, 3]. The chirality of biologically active compounds is of special relevance due to 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]. It has been shown, for instance, that PCB 88, 139, and 197 (the racemic mixture and their respective enantiomers) have different biological activities [5].

Since soil serves as a sink of the atmospheric pollution and preserves the long-term record of contamination, it has also been a subject of numerous investigations focused on the fate of chiral compounds. Enantiomeric fractions of various chiral pollutants were measured at a variety of sampling sites. Deviations from racemic mixtures were observed in both directions but racemic mixtures (e.g. EF=0.5) were also found at many cases [6-13]. The activity of the microbial community and its degradation preferences were suggested to be responsible for fluctuation in the enantiomeric ratios in soils, but the factors influencing this fluctuation were never addressed in detail.

Material and methods

Soil sampling and characterization
Soils were sampled, manipulated and stored in accordance with the ISO standard [14-17]. For microbiological analysis, fresh soils were sieved (< 2 mm) and stored at 4°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 [18]: soil pH values (pH(H_2O) and pH(KCl)), total organic carbon (TOC), total soil nitrogen (N_{tot}), $CaCO_3$ content, humic compounds (HCs) in 0.1M 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_{bio}),

extractable carbon (C_{ext}), basal respiration (BR) , substrate-induced (SIR) respiration , and biomass-specific respiration rate (qCO_2).

Sample preparation
Each dry soil sample (5g) was extracted with dichloromethane (DCM) in a Büchi System B-811 automatic extractor. A glass column (30 cm length, 1 cm I.D.) filled with 5 g of silica gel (activated overnight at $150^{o}C$, and modified with sulfuric acid) was used for a clean-up. PCBs and OCPs were eluted using DCM : *n*-hexane (1:1) mixture (30 mL), and samples were concentrated under a gentle stream of nitrogen to the final volume of 1 mL.

GC-GC-MS-MS
The method developed by Bucheli & Brändli [6] for quantification of atropisomeric PCBs was adapted to include several chiral organochlorine pesticides. The enantiomeric fraction (EF) is used as a descriptor of enantiomeric signatures.

Results and discussion
To study factors controlling enantioselective processes in soil, we had to distinguish significant EF shifts from the racemic value (EF=0.5) from non-significant deviations of the analytical technique. A stochastic definition of racemic samples can be derived from the relative standard deviation of EF quantification. Bucheli and Brandli [6], for instance, found that apparative 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 non-racemic.

Soil samples (n=112) from various sampling sites in the Czech Republic (n=60), former Yugoslavia (n=33), and Sultanate Oman (n=19) covering a range of soil types and contamination levels were collected in a pilot study. There was no significant difference in the variability of the EFs determined in the three regions, and measured values were also similar to the ones published previously. Air samples were collected at all soil sampling sites using the passive air samplers, and analyzed for selected chiral PCBs and OCPs. Irrespective of the sample origin, EF values in air did not differ significantly from the racemic mixture, and they ranged from 0.49 to 0.51. Effective mixing of the air layers prevented us from detection of any imprints of the local soil sources.

To overcome this problem, a hatlike sampling device developed by Meijer [19] was employed. This sampler was positioned about 3 cm above the soil surface and the air samples were collected for 24 hours. Enantiomeric shift in the same direction as the one observed in soil was found in the air samples in all cases. The extent of this deviation in air samples was the same or lower than the one measured in corresponding soil sample.

In search for the factors affecting the enantioselective behaviour of chiral compounds, three PCB congeners were selected to be model compounds for a detailed study. 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, α, β, γ, δ-HCHs, *o,p'*- and *p,p'*-DDE, DDD and DDT). All samples with Σ PCBs > 50 $\mu g.kg^{-1}$ manifested EFs very close to the racemic mixture (ranging from 0.47 to 0.52), while non-racemic EFs appeared to be increasingly probable in soils with relatively low PCB concentrations where some concentration–related trends occurred. Samples with significant EF shifts for PCB 132 had lower TOC content while those of PCB 95 and PCB 149 were found in carbon rich soils. The overall correlation between EF and TOC was hard to interpret when applied to the whole data set. 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.
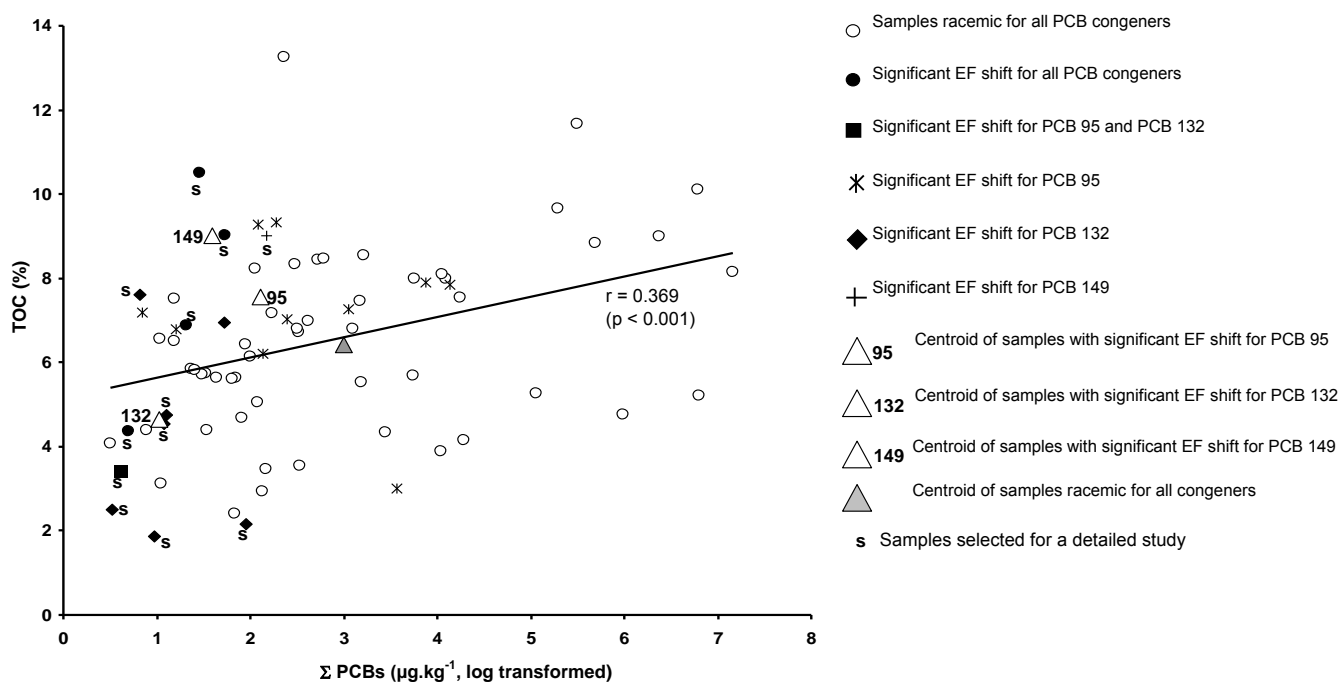
Figure 1. Scatter plot localization of the soil samples in a space given by TOC content and PCB concentration.

Reduced but well documented set of 12 soils with low levels of PCB contamination and a range of EFs was identified among the original collection. The abiotic and biotic characteristics derived from the long-term bio-monitoring 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. To assess soil conditions that facilitate enantioselective behavior, soils were aggregated in two groups (A: soils, where EF of one PCB congener was significantly shifted from racemic value; B: soils with significant EF shifts for 2-3 PCB congeners). Group B samples were more carbon rich than group A samples but they also contained significantly more humic (HA) and fulvic (FA) acids and total nitrogen (N_{tot}). These differences were accompanied by significantly increased values of all key biotic variables.

A relationship between parameters predicting the soil degradation potential and the actual EF of the individual compound in soil is not so trivial, however, 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.

Even though laboratory studies may not reflect exactly what is happening in the real environment, they are still very important for revealing mechanisms. While 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, the long-term experiments with naturally contaminated soils including native bacterial cultures are currently in progress in our laboratory.

References

1. Kallenborn, R. and Hühnerfuss, H. *Chiral environmental pollutants: trace analysis and ecotoxicology*. 2001: Springer-Verlag, Berlin, Heidelberg.
2. Muller, T.A. and Kohler, H.P.E., *Appl. Microbiol. Biotechnol.*, 2004. 64(3): 300-16.
3. Borga, K. and Bidleman, T.F., *Environ. Sci. Technol.*, 2005. 39(10): 3464-73.
4. Blanch, G.P., Glausch, A., Schurig, V., Serrano, R., and Gonzalez, M.J., *J. High Res. Chromatog.*, 1996. 19(7): 392-6.
5. Rodman, L.E., Shedlofsky, S.I., Mannschreck, A., Puttmann, M., Swim, A.T., and Robertson, L.W., *Biochem. Pharm.*, 1991. 41(6-7): 915-22.
6. Bucheli, T.D. and Brandli, R.C., *J. Chromatogr. A*, 2006. 1110(1-2): 156-64.
7. Finizio, A., Bidleman, T.F., and Szeto, S.Y., *Chemosphere*, 1998. 36(2): 345-55.
8. Leone, A.D., Amato, S., and Falconer, R.L., *Environ. Sci. Technol.*, 2001. 35(23): 4592-6.
9. Wiberg, K., Harner, T., Wideman, J.L., and Bidleman, T.F., *Chemosphere*, 2001. 45(6-7): 843-8.
10. Bidleman, T.F. and Leone, A.D., *Environ. Pollut.*, 2004. 128(1-2): 49-57.
11. Harrad, S., Jamshidi, A., and Robson, M., *Organohal. comp.*, 2004. 66: 460-6.
12. Robson, M. and Harrad, S., *Environ. Sci. Technol.*, 2004. 38(6): 1662-6.
13. Kurt-Karakus, P.B., Bidleman, T.F., and Jones, K.C., *Environ. Sci. Technol.*, 2005. 39(22): 8671-7.
14. ISO10381-6, *Guidance on the collection, handling and storage of soil for the assassment of aerobic microbial processes in the laboratory*, in *Soil quality - Sampling*. 1993, International Organization for Standardization: Geneve.
15. ISO14240-1, *Subtrate-induced respiration method*, in *Soil quality - Determination of soil microbial biomass*. 1997, International Organization for Standardization: Geneve.
16. ISO16072 *Soil quality - Laboratory methods for determination of microbial soil respiration*. 2002, Geneve: International Organization for Standardization.
17. ISO14240-2 *Part 2: Fumigation-extraction method, in Soil quality - Determination of soil microbial biomass*. 1997, Geneve: International Organization for Standardization.
18. Foerster, J.C., *Soil physical analysis*, in *Methods in soil microbiology and biochemistry*. 1995, Academic Press: London. 105-21.
19. Meijer, S.N., Shoeib, M., Jantunen, L.M.M., Jones, K.C., and Harner, T., *Environ. Sci. Technol.*, 2003. 37(7): 1292-9.
20. Liu, Z.F. and Lee, C., *Mar. Chem.*, 2007. 105(3-4): 240-57.