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Combined isotope and enantiomer analysis to assess the fate of phenoxy acids in a heterogeneous geologic setting at an old landfill

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ABSTRACT

Phenoxy acid herbicides and their potential metabolites represent industrial or agricultural waste that impacts groundwater and surface waters through leaching from old landfills throughout the world. Fate assessment of dichlorprop and its putative metabolite 4-CPP (2-(4-chlorophenoxy)propionic acid) is frequently obstructed by inconclusive evidence from redox conditions, heterogeneous geologic settings (e.g. clay till) and ambiguous parent–daughter relationships (i.e. 4-CPP may be daughter product or impurity of dichlorprop). For the first time, a combination of four methods was tested to assess transformation of phenoxy acids at a contaminated landfill (Risby site): analysis of (i) parent and daughter compound concentrations, (ii) enantiomer ratios (iii) compound-specific isotope analysis and (iv) enantiomer-specific isotope analysis. Additionally, water isotopes and chloride were used as conservative tracers to delineate two distinct groundwater flow paths in the clay till. Metabolite concentrations and isotope ratios of chlorinated ethenes demonstrated dechlorination activity in the area with highest leachate concentrations (hotspot) indicating favorable conditions also for dechlorination of dichlorprop to 4-CPP and further to phenoxypropionic acid. Combined evidence from concentrations, enantiomer ratios and isotope ratios of dichlorprop and 4-CPP confirmed their dechlorination in the hotspot and gave evidence for further degradation of 4-CPP downgradient of the hotspot. A combination of 4-CPP enantiomer and isotope analysis indicated different enantioselectivity and isotope fractionation, i.e. different modes of 4-CPP degradation, at different locations. This combined information was beyond the reach of any of the methods applied alone demonstrating the power of the new combined approach.

1. Introduction

Hundreds of thousands of landfill sites throughout the world may leach pollutants with long-term and local-to-regional impact on groundwater and surface waters (Kjeldsen et al., 2002; Schwarzenbach et al., 2010). Often former landfill sites contain substances whose use has subsequently been banned or restricted, such as phenoxy acid herbicides like dichlorprop

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(2-(2,4-dichlorophenoxy)propionic acid, CAS RN 120-36-5) (Baun et al., 2004). Moreover, phenoxy acids and impurities in their production and/or transformation products such as 4-CPP (2-(4-chlorophenoxy)propionic acid, CAS RN 3307-39-9) (Reitzel et al., 2004) are commonly found in groundwater (Malaguerra et al., 2012). Thus, a proper understanding of the fate of phenoxy acids is crucial for risk assessment at landfill sites and for groundwater quality protection and management in general.

In addition to chemical variability of landfill contaminants, leachate indicators in groundwater samples collected beneath landfills may reflect a complex spatial distribution of the deposited waste at old landfill sites, including secondary sources of the same chemical (Kjeldsen and Christophersen, 2001). In landfill leachate plumes in such complex pattern, fate assessment of different xenobiotic organic compounds is complicated (Baun et al., 2003; Eganhouse et al., 2001; Rügge et al., 1995). Heterogeneous underlying geologic settings such as fractured rock or clay sediments can also complicate the spreading of contaminants. In Scandinavia and parts of North America clay till is a common geologic setting. This is glacially deposited clay with very low permeability and with preferential groundwater flow paths through vertical fractures and mostly horizontally interbedded sand lenses (e.g. Gerber et al., 2001; Hendry et al., 2004; McKay et al., 1998).

In addition to spatial complexity, physiochemical properties of the contaminants, degradability of the contaminants and redox conditions at the site may govern the attenuation of different contaminants. Physical attenuation such as sorption/desorption of xenobiotics in clay till settings (Stringfellow et al., 2011) may affect their availability for microorganisms. Microbial attenuation, e.g. reductive dechlorination of chlorinated hydrocarbons such as chlorinated ethenes is primarily expected under reducing, anaerobic conditions. The depletion of electron acceptors alone cannot be used as proof of degradation of xenobiotic compounds in a landfill leachate plume, because it is mostly driven by the turnover of general organic matter rather than the specific contaminants. The use of normalization with a conservative compound such as chloride may also fail, because of more than one chloride source in the landfill or in the plume (Bjerg et al., 1995; Tuxen et al., 2003). Thus, in avoiding the challenges of uncertainty over chloride concentrations in the source, the fate assessment of xenobiotics requires information that is specific of their transformation (Bjerg et al., 2011), such as parent-to-daughter compound concentration ratios, enantiomer ratios of chiral compounds and compound-specific isotope analysis (CSIA) (Eganhouse et al., 2001; Reitzel et al., 2004; Richnow et al., 2003).

Phenoxy acid herbicides are generally expected to be recalcitrant under strongly anaerobic conditions (Rügge et al., 1999), but prone to biodegradation under nitrate reducing or aerobic conditions (see review by Buss et al., 2006; Reitzel et al., 2004). Presence of 4-CPP might indicate dechlorination of dichlorprop (Fig. 1) in the same way as less chlorinated ethenes give evidence of dechlorination of more chlorinated ethenes. However, the presence of metabolites may be ambiguous, since proven or suggested degradation products can be present as an impurity in the production of the source compound, which is the case for dichlorprop and 4-CPP (Reitzel et al., 2004; Tuxen et al., 2003). 4-CPP might be further dechlorinated, transforming to a non-chlorinated compound. This pathway was indicated by an abiotic chemical reaction under laboratory conditions with reductive electron transfer (Azzena and Pittalis, 2011). Reductive dechlorination of chlorobenzenes resembling dichlorprop and 4-CPP (without the propionic acid entity) has been proven (Liang et al., 2011; Nelson et al., 2011).

Phenoxy acids are chiral compounds consisting of two isomeric molecules that differ only in the spatial orientation of their atoms (Fig. 1). Their enantioselective microbial degradation has been shown in laboratory conditions (Heron and Christensen, 1992; Reitzel, 2005) and in the field (Harrison et al., 1998; Reitzel, 2005; Williams et al., 2003; Zipper et al., 1998, 1999) and was found to be dependent on redox conditions (Harrison et al., 2003; Reitzel et al., 2004). (S)-4-CPP-preferential enzymes and deracemizing enzymes (i.e. enzymes which can invert the spatial orientation of one 4-CPP enantiomer in a mixture with equal quantities of each enantiomer) have been identified by Kato et al. (2010). Both processes result in the preferential occurrence of one enantiomer form. Compared to metabolic analysis, where the same metabolite can derive from different parent compounds, enantiomer analysis has the advantage that it is compound-specific. The use of enantioselective concentration measurements fails, however, if an enzyme happens to degrade both enantiomers equally well or if both (S)- and (R)-preferential enzymes are present.

Compound-specific isotope analysis (CSIA) is a fate assessment tool with an increasing number of applications in recent fate assessment studies of different contaminant groups (Elsner, 2010; Thullner et al., 2012). CSIA measures the stable isotope ratios of a compound (e.g. $^{13}C/^{12}C$, $^{2}H/^{1}H$, $^{15}N/^{14}N$, etc.) at natural abundance. Isotope ratios, such as $R_{sample} = {}^{13}C/{}^{12}C$, are expressed in the delta notation relative to that of an international reference $R_{reference}$.

$$\delta^{13}C = \frac{R_{sample} - R_{reference}}{R_{reference}} \tag{1}$$

where a difference of, e.g., $-0.028 = -28‰$ indicates that the sample contains 28 per mille less $^{13}C/^{12}C$ than the reference.

During a biochemical reaction, molecules with light isotopes tend to be degraded faster than molecules possessing a heavy isotope in the reactive position (because the bond is slightly weaker), leading to an enrichment of heavy isotopes in the remaining substrate ($\delta^{13}C$ becomes more positive) (Bigeleisen and Wolfsberg, 1958; Wolfsberg et al., 2010). This enables the detection of *in situ* biodegradation of organic contaminants. Furthermore, if the isotope enrichment factor (ε) of a specific compound is determined in laboratory experiments based on the Rayleigh equation, it can be used to estimate the extent of biodegradation of that compound from changes in isotope ratios of field samples (Hunkeler et al., 2008).

The carbon-based CSIA has recently been extended to assess microbial degradation of different pesticides (Badea et al., 2009; Penning et al., 2010; Reinnicke et al., 2012) and the method was very recently optimized for phenoxy acids (Reinnicke et al., 2010). CSIA has been widely used for degradation assessment of chlorinated ethenes and monoaromatic petroleum hydrocarbons such as benzene, toluene, ethylbenzene, m-,o- and p-xylene (BTEX) on different typical contaminated sites, and the literature on their natural attenuation and isotope fractionation is extensive (Thullner et al., 2012). Richnow et al. (2003) used CSIA to examine the fate of