Mercury Isotope Fractionation in the Subsurface of a Hg(II) Chloride-Contaminated Industrial Legacy Site

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ABSTRACT: To understand the transformations of mercury (Hg) species in the subsurface of a HgCl₂-contaminated former industrial site in southwest Germany, Hg isotope analysis was combined with an investigation of Hg forms by a four-step sequential extraction protocol (SEP) and pyrolytic thermodesorption. Data from two soil cores revealed that the initial HgCl₂ was partly reduced to metallic Hg(0) and that Hg forms of different mobility and oxidation state coexist in the subsurface. The most contaminated sample (K2-8, 802 mg kg⁻¹ Hg) had a bulk δ²⁰²Hg value of around −0.43 ± 0.06‰ (2SD), similar to published average values for industrial Hg sources. Other sample signatures varied significantly with depth and between SEP pools. The most Hg-rich samples contained mixtures of Hg(0) and Hg(II) phases, and the water-extractable, mobile Hg pool exhibited heavy δ²⁰²Hg values of up to +0.18‰. Sequential water extracts revealed slow dissolution kinetics of mobile Hg pools, continuously releasing isotopically heavy Hg into solution. This was further corroborated by heavy δ²⁰²Hg values of groundwater samples. Our results demonstrate that the Hg isotope signature of an industrial contamination source can be significantly altered during the transformations of Hg species in the subsurface, which complicates source tracing applications but offers the possibility of using Hg isotopes as process tracers in contaminated subsurface systems.

INTRODUCTION

Mercury (Hg) is recognized as a priority hazardous substance that, once released locally, can travel long distances and becomes a toxic global pollutant.¹ A global treaty (Minamata Convention on Mercury) to minimize new inputs of Hg into the environment has come into force in 2017.² However, past Hg contamination is frequently prone to remobilization, leading to emissions from legacy sites impacted by previous mining, ore processing, or industrial Hg applications. They are estimated to release 82 ± 13 Mg year⁻¹ to the atmosphere and 116 ± 49 Mg year⁻¹ Hg to the hydrosphere globally,¹ making them sources of secondary pollution to groundwater, soil, and the atmosphere for centuries. Re-emissions from land are estimated to contribute 21–40% of the yearly Hg emissions.¹ Localy, the risk associated with a Hg-contaminated site in terms of Hg toxicity and mobility strongly depends on the particular Hg species present.

The fractionation of stable Hg isotopes during kinetic and equilibrium species transformations has been well-documented.⁴ Several studies have demonstrated the potential of Hg isotope signatures for source⁵−¹¹ and process tracing¹²−¹⁴ based on the principle that mass-dependent (MDF) Hg isotope fractionation and mass-independent (MIF) Hg isotope fractionation during species transformations provide information about the geochemical history of environmental Hg pools. In samples with complex environmental matrices, one main limitation to process tracing is the isolation of different Hg species for isotopic measurement. This is especially challenging if samples are comprised of a mix of species each representing intermediates or end points of different species transformation processes. Sequential extraction protocols (SEP) present a commonly adopted method for separating Hg from environmental samples¹⁵−¹⁹ such as soils or sediments into operationally defined pools.²⁰,²¹ Few studies have combined such a separation with Hg isotope analysis, investigating the environ...

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mental impact of Hg mining and industrial contamination using different SEP protocols.

Here, we present a study of an industrial legacy site that differs from previous studies in several key respects. First, in contrast to sites influenced by mining of HgS or by atmospheric deposition of Hg, the industrial contamination source at our site is highly water-soluble Hg(II) chloride (HgCl₂). Second, samples from cores are assessed in terms of Hg forms and transformations in the subsurface rather than focusing on the topsoil. Third, Hg isotope measurements were conducted on four distinct SEP pools as well as on sequential water extracts that were compared to the results of analyses of groundwater samples. Due to the operational character of the results obtained by SEP and limitations of the selectivity of the extracts for Hg species identification, we additionally applied pyrolytic thermodesorption (PTD), which allows the detection of metallic Hg(0) and Hg(II) sulfides in solid samples. The HgCl₂ from industrial contamination (wood impregnation) entered the soil at our site for many decades followed by vertical transport into the subsurface. The investigated soil layers contain <1% organic carbon (OC) and consist of carbonatic silt (loess). Thus, the site is poor in the strongest bonding partners for Hg, reduced sulfur functional groups on organic matter, and sulfides. In summary, highly mobile Hg(II) from a well-defined source has been present in the OC-poor soil for decades and we expect that it has undergone Hg species transformations and associated Hg isotope fractionation during that time.

The main research questions of this study were (i) the extent to which Hg isotope signatures are variable and reflect the process transformation history in the subsurface of the site and (ii) the implications of the findings for source and process tracing at contaminated sites using Hg isotope signatures. To this end, Hg isotope fractionation was studied from three viewpoints: (1) between two soil cores taken at different locations, (2) between bulk soil samples from different depths, and (3) between operationally defined subpools of Hg within individual samples. In addition, SEP and PTD were used to constrain the Hg forms present in the samples and investigate transformations that the initial contaminant HgCl₂ has undergone in the subsurface of the site.

**MATERIALS AND METHODS**

**Field Methods.** Two soil cores were taken in June 2016 at a former industrial site (Bad Krozingen, southwest Germany). From 1904 to 1965, a HgCl₂ solution (0.66%) was used at this site for wood impregnation in a process called kyanization, accompanied by releases of approximately 10–20 t of Hg to the soil and groundwater. The site was converted to a residential area in the 1970s. After the discovery of soil contamination in the 1990s, the top 50 cm of the soil was replaced with uncontaminated material, but most of the released Hg still resides in the unsaturated zone and the underlying aquifer exhibiting a groundwater contamination plume that is ~1.3 km in length. A further introduction to the site and its contamination history is given in earlier publications.

Core K2 was located in the most contaminated area near the former kyanization hall and extends to a depth of 4.5 m. Core K3 (3.5 m) was located in the less contaminated former wood storage area. The groundwater table at the field site is at a depth of ~10 m; thus, both soil cores probed the unsaturated zone. The investigated soil layers contain <1% organic carbon (OC) and consist of homogeneous carbonatic silt (loess), overlaid by redeposited loessy soil material and building rubble. The cores were taken with a stainless steel pile driving device with a diameter of 5 cm, and 20 cm core sections were combined. The samples were gently homogenized on site using a stainless steel spatula and then split into two aliquots for (1) PTD and (2) digests/ex extricts for Hg concentration and Hg isotope analysis. Samples were stored in polypropylene vessels under cooled conditions. To minimize changes in Hg speciation (e.g., loss of elemental mercury and redox changes), no sieving, milling, or drying of the soil samples was conducted. The water content was determined on a separate aliquot, and all concentrations reported here are based on dry weight. Groundwater samples from three monitoring wells (groundwater table ~10 m below surface) near the investigated soil cores were sampled in September 2015. The samples were filtered (0.45 μm cellulose acetate membranes), preserved with 1% (v/v) BrCl in the field, and stored in glass vials with PTFE-lined lids prior to analysis.

**Extractions.** All extractions were carried out in 50 mL PP centrifuge tubes. Filtered solutions were stored in acid-washed borosilicate vials with PTFE-lined lids and analyzed within 2 weeks of extraction. For determination of the total amount of mercury, 12 mL of aqua regia (8 mL of concentrated HCl, 3 mL of concentrated HNO₃, and 1 mL of concentrated BrCl) was mixed with 1 g of wet soil, covered with perforated parafilm, and agitated overnight (18 ± 4 h) at 150 rpm on a horizontal shaker inside a fume hood and then diluted with 8 mL of H₂OMQ and centrifuged at 3992 g. followed by filtration through 0.45 μm prewetted PTFE filters. Reference material NIST-2711 (Montana Soil) was digested in parallel with the samples resulting in 97.7 ± 6.6% (SD, n = 4) recovery of the certified Hg content.

The samples with the highest Hg concentrations and their adjacent samples were chosen for the SEP, with K2-8 and K3-3 extracted in triplicate to assess the heterogeneity of the samples. The SEP was based on the method of Bloom et al. (F1, deionized water; F2, 0.01 M HCl/0.1 M CH₃COOH; F3, 1 M KOH; F4, 12 M HNO₃; F5, aqua regia), but the third step targeting organically bound Hg was omitted due to the low OC content of the samples. Step 2 was modified to accommodate for the high carbonate content of the samples by increasing the acid strength. To avoid an influence of residual chloride from the second step on the dissolution of Hg sulfides in nitric acid, HCl was replaced with HNO₃ in step 2. Moreover, the acid strength of the following step was decreased from 12 to 6 M HNO₃, following newer recommendations with respect to the extractability of Hg sulfides in HNO₃. Thus, the four extract solutions were H₂OMQ (F1), 0.5 M HNO₃ (F2), 6 M HNO₃ (F3), and aqua regia (F4). F1 targets water-extractable species such as HgCl₂ and Hg bound to dissolved organic matter (DOC). F2 is functionally defined as the “labile, bioavailable pool” and expected to extract weakly sorbed and carbonate-bound Hg species. F3 is expected to extract all mercury species except HgS. Finally, fraction F4 is targeted at sulfide-bound Hg (α-HgS and β-HgS). Solid to solution ratios were 1 g of sample per 25 mL of extractant solution. The vials containing soil and extractant solution were placed on an end-over-end shaker at 15 rpm overnight for 18 ± 4 h. The next day, samples were centrifuged at 2360–3000g and filtered (0.45 μm) into acid-washed borosilicate vials with PTFE-lined lids. Cellulose acetate filters (F1 and F2) were prewashed with the extractant.
solutions. PTFE filters (used for F3, F4, and aqua regia digests due to their high acid strength) were first wetted with ethanol and then flushed twice with H2O2 prior to use. For all F1–F3 samples, 1 vol % concentrated BrCl (prepared following the procedure of Bloom et al.36) was added to the supernatant after filtration to stabilize the Hg(II) in solution.

Sequential water extractions were conducted in seven consecutive steps with the most contaminated sample K2-8 identically to the F1 step. After equilibration and centrifugation, half of the solution was immediately acidified with a 1 vol % BrCl solution. The other half was purged with N2 for 30 min to drive out any Hg(0) before acidification. Dissolved organic carbon (DOC) was measured on 8 mL of the unacidified F1 extracts within 24 h of extraction for nonpurgeable organic carbon and total nitrogen on a Shimadzu (Kyoto, Japan) TOC-L instrument.

**Analyses.** For PTD, sample aliquots were continuously heated from room temperature to 700 °C at a rate of 0.5 °C s⁻¹ under a N2 gas flow (300 mL min⁻¹) and the Hg release curves were measured using atomic absorption spectrometry (AAS) as a function of temperature. They were compared to characteristic release curves of Hg reference compounds (see Figure S1).36 Mercury concentration analysis of the extracts was carried out by a cold vapor flow injection atomic absorption spectrometer (CV-AAS) (FIMS 100, PerkinElmer, Waltham, MA, USA). For Hg isotope measurements, a Nu Plasma II multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS) (Nu Instruments, Wrexham, UK), fitted with a HGX-200 cold vapor introduction system (Teledyne Cetac, Omaha, NE, USA), was used. Mass bias was corrected for using standard sample bracketing, and internal drift was corrected by NIST SRM 997 Tl standard correction for using standard sample bracketing, and internal drift was corrected by NIST SRM 997 Tl standard.

The overall precision for all presented data in this study (2SD; n = 52) was ±0.13 % for δ²⁰²⁰Hg and ±0.05 % for δ¹⁹⁹Hg, but the standard deviations for the individual sample measurements are reported on the basis of the “ETH Fluka” reproducibility of the respective session. No δ²⁰⁰Hg and δ²⁰⁴Hg anomalies were observed in the samples examined in this study.

**RESULTS AND DISCUSSION**

**Total Concentrations.** The total Hg content of all measured samples was above geogenic background levels (<0.1 μg g⁻¹).44 The depth profiles of both cores had pronounced Hg concentration maxima (Figure 1), but values in K2 ranged from 3.6 to 802 μg g⁻¹, while values in K3 reached a maximum of only 99.6 μg g⁻¹ (Figure 1 and Table S1). The depth of maximum concentrations differed as well, with ∼2.5 m in core K2 and <1 m in core K3, highlighting the heterogeneous distribution of Hg and the presence of contamination “hot spots” in the subsurface, consistent with previous studies at this site.29 While it is difficult to provide a clear explanation for why the Hg concentration maximum in core K2 occurred at this particular depth within a relatively homogeneous silty loess layer, one can conclude that downward transport of Hg from the soil surface must have taken place. On the basis of these results, the most contaminated samples of each core were chosen for further analyses.

**PTD Results.** All samples released Hg between 150 and 250 °C (Figure 2), but some also released Hg at lower and higher temperatures, indicating the presence of Hg(0) and Hg sulfides, respectively. Previous studies found that soils often exhibit Hg release around 250 °C and that several Hg standards decompose at this temperature. These include β-HgS, Hg(II) bound to humic acid or Fe(OH)₃, Hg(NO₃)₂, HgCl₂, and Hg₂Cl₂, the original Hg compound of the contamination at our site (Figure S1). Although we consider
Hg(II) adsorbed to mineral surfaces and/or Hg(II) bound to soil organic matter to be the most likely predominant Hg phases in our samples, Hg release in this temperature range was summarized under the term “matrix-bound Hg(II)”.

Release below and around 100 °C was observed only in the most highly contaminated samples of core K2 and was attributed to the presence of Hg(0) in the soil upon comparison to the PTD results of standard materials.

In the most contaminated sample K2-8, >50% of the total Hg was present as Hg(0) based on the PTD results. In core K3, only small amounts of Hg(0) were detected in K3-4, but one sample (K3-3) released some mercury at around 350 °C, hinting at the presence of α-HgS.

SEP Results. The samples chosen for sequential extraction were K2-5–K2-12 (180–340 cm) and K3-2–K3-5 (30–160 cm). In all samples, up to 4% of the total mercury was extracted in the F1 water leach (Figure 3, left panel) which represents the most mobile Hg pool. This value is comparable to those from previous studies of contaminated soil samples. In the most contaminated sample K2-8, >50% of the total Hg was present as Hg(0) based on the PTD results. In core K3, only small amounts of Hg(0) were detected in K3-4, but one sample (K3-3) released some mercury at around 350 °C, hinting at the presence of α-HgS.

The remaining Hg was distributed between steps F2 and F4 with large relative differences between the samples. In K2, >50% of total Hg was extracted in steps F2 and F3 from the most contaminated samples (K2-7 and K2-8), while F4 dominated the Hg budget in the less contaminated samples. In K3, most Hg (53–90%) was extracted in the last step, showing that it was bound more tightly to the soil and less mobile in core K3 than in core K2 (Table S2). The notable absence of high-temperature peaks characteristic of α-HgS, in all samples except for K3-3, compared with the large amount of mercury in F4 suggests that the residual Hg in F4 was likely metacinnabar (β-HgS) or Hg that could not be released from the soil matrix in previous extraction steps. The abundant presence of Hg(0) in the most contaminated samples of K2 provides clear evidence that Hg(II) reduction processes have taken place in the soil, but it further complicates a clear attribution of Hg species to extracts F1–F3, as Hg(0) is known to be not selective of one specific extract but rather partially extracted in each of them. The sample aliquot of K2-7 used in the SEP contained significantly more mercury (950 μg g⁻¹ Hg) than the one used for the aqua regia digest (624 μg g⁻¹ Hg), illustrating the heterogeneity of Hg concentrations in the soil. However, triplicate extractions performed on two samples (K2-8 and K3-3) confirmed the good reproducibility of the SEP results (Figure S4).
Integrating the findings of PTD and SEP on Hg forms in the two soil cores, we conclude that the original, highly mobile, and water-soluble HgCl₂ compound was transformed significantly in all measured samples, yielding >34% very stable Hg forms that were soluble in only aqua regia (F4) and presumably consist mostly of β-HgS or other Hg species that could not be released from the soil matrix in the previous extraction steps. Due to the presence of up to >50% Hg(0) in the most contaminated samples of K2 as revealed by PTD, it was difficult to constrain the Hg species extracted in F2 and F3. In addition to Hg(0), F2 and F3 may have extracted several sorbed or precipitated non-sulfide Hg species with volatilization temperatures of around 150–250 °C in PTD. The samples with the highest total Hg contents yielded the highest percentages of water-soluble Hg (F1). DOC has been documented to exert a strong influence on the distribution of mercury in aqueous systems. In the study presented here, however, no correlation \( R^2 = 0.18 \) between DOC and Hg in the F1 extracts was observed (Table S8). Thus, the F1 step has probably extracted highly water-soluble Hg(II) species (e.g., HgCl₂) as well as some Hg(0) as suggested by the results for the sequential water extracts discussed further below.

**Mercury Isotopes.** Hg isotope analysis has great potential to track transformations of a once-isotopically-homogeneous contamination source. As the industrial facility was shut down more than 50 years ago, there was no original HgCl₂ source material available for measurement. Therefore, the mean of Grigg et al.’s literature average of published data of Hg ore minerals, liquid Hg(0), and sediments contaminated by industrial sources was used as the source signature estimate: \(-0.52 ± 0.41\%e\) (1 SD; \( n = 57 \)) for \( \delta^{202}\text{Hg} \) and 0.00 ± 0.09\%e (1 SD; \( n = 48 \)) for \( \Delta^{199}\text{Hg} \) (see Table S7). The bulk soil \( \delta^{202}\text{Hg} \) values (MDF) in the most contaminated layers of core K2 were in good agreement with this estimated source signature, while the K2 samples with lower Hg concentrations above and below, as well as all samples from core K3, were isotopically heavier by up to ∼0.5\%e (Figure 1b). The range of \( \delta^{202}\text{Hg} \) (±0.09\%e, 2SD) in K2 was from −0.43\% (K2-8, 240–260 cm) to 0.10\% (K2-10, 280–300 cm), while that of K3 ranged from −0.12\% (K3-5, 140–160 cm) to 0.08\% (K3-3, 50–70 cm). Interestingly, a negative correlation between \( \delta^{202}\text{Hg} \) and Hg concentration \( R^2 = 0.87 \) was found for the hot spot samples of core K2 (Figure S2). Such a trend would be consistent with a preferential loss of light Hg isotopes during processes causing a decrease in Hg concentration and shifting the isotope signature of the remaining Hg in the soil toward more positive \( \delta^{202}\text{Hg} \) values (e.g., by volatilization), as further discussed below. The observed variations in \( \Delta^{199}\text{Hg} \) (MIF) were generally small and mostly within the analytical uncertainty in both cores, but with some negative values of up to −0.2\%e in K2 (Figure 1c). The three groundwater samples, taken from a depth of ∼10 m and thus far deeper than the investigated soil cores located in the unsaturated zone, exhibited highly elevated Hg concentrations (76–192 µg L⁻¹), similar to those from previous studies at the site in which inorganic Hg(II) was identified as the dominant Hg species in the groundwater, with only small amounts of DOM-bound Hg(II) and almost no detectable dissolved Hg(0). Large \( \delta^{202}\text{Hg} \) variations in the groundwater were observed from −0.13\%e to 0.75\%e (well B3, located closest to core K2), but all three were significantly heavier than the estimated source signature and the most contaminated soil samples (Figure 1). Thus, the groundwater data suggest that the isotope signature of Hg that is entering the aquifer via leaching from the contaminated soil layers above has been altered from the observed solid phase isotope signatures. However, the extent of isotopic variations and the apparent negative correlation between groundwater Hg concentrations and \( \delta^{202}\text{Hg} \) (Figure 1a,b) will need to be investigated in future studies.

The observed Hg isotope fractionation patterns in the subsurface did not only occur between different depths of the contaminated soil layers and the groundwater but were apparent between different soil extracts of individual soil samples too. While the most stable Hg forms (F4) generally displayed the lowest \( \delta^{202}\text{Hg} \) values, the Hg pools extracted in steps F2 and F3 were mostly heavier than the bulk soil value (Figure 2b and Figure S3). The water-soluble Hg pool (F1) showed a less regular pattern, with lighter-than-bulk signatures in the two uppermost and the lowest sample of K2, but displaying very heavy \( \delta^{202}\text{Hg} \) signatures in the most contaminated soil layers. In principle, stable isotope fractionation could be induced during a sequential extraction procedure due to incomplete extraction of a target pool, partial extraction of a nontarget pool, or secondary re-adsorption processes. However, because dissolution is a surface-controlled process proceeding along a moving reaction front, its potential to create an isotopically fractionated solution is very limited. For example, partial dissolution of HgS and incomplete extraction of NOM-bound Hg were found to cause no measurable Hg isotope fractionation. Moreover, our SEP with increasing acid strength from step 2 to 4 was designed to prevent re-adsorption processes. Therefore, the variations in \( \delta^{202}\text{Hg} \) between different extracted Hg pools of the contaminated soil samples of ∼0.5\%e in several samples provide clear evidence for in situ Hg isotope fractionation during Hg species transformation and/or partitioning processes in the subsurface.

These findings are in general agreement with those of previous studies at contaminated sites investigating Hg isotope ratios in soil extracts, though different extraction protocols were used. For example, significant variations in \( \delta^{202}\text{Hg} \) between extracted Hg pools of >2%e were reported in mining waste samples from a tailings pile in Wanshan, China (F1, water-extractable; F2, ammonium thiosulfate-extractable; F3, residual) and in tailings and calcines from New Idria, United States (F1, “stomach acid”-extractable; F2, 12 M HNO₃-extractable; F3, residual). Sediments impacted by the chloralkali and paper industry similarly exhibited differences of up to 1%e between the extracted pools (F1, nonsulfidic Hg; F2, sulfidic Hg). A recent study of contaminated soils and sediments downstream of an industrial facility in Switzerland (F1, water-soluble; F2, organic matter-bound; F3, residual Hg) however, did not detect resolvable Hg isotope variations between sequential extracts. We suggest that the extent of Hg isotope variations between different soil extracts is strongly dependent on Hg speciation at the site and especially on the spatial variability of the species that are present. Thus, the potential for in situ Hg isotope fractionation may be variable between different sites. At the site investigated here, the high mobility of the original HgCl₂ species as well as the documented formation of different Hg species, including elemental Hg(0), likely facilitated the extent of the observed Hg isotope fractionation in the subsurface.

**Sequential Water Extracts.** The most mobile Hg pool (F1 extract) from the most contaminated soil sample (K2-8) was further studied by a series of consecutive water extracts on the
same sample to investigate the slow release of Hg from the soil matrix. Even after seven extraction steps, dissolved Hg concentrations in the water leachates were still high [111 μg L⁻¹ in step 7 compared with 429 μg L⁻¹ in step 1 (Figure 4)], indicating that a single F1 extraction is clearly not sufficient to leach all water-extractable Hg from the sample. If the water-extractable pool were to consist of only dissolved HgCl₂, leach all water-extractable Hg from the sample. If the water extracts were all significantly higher than the bulk soil concentration (left, bulk soil total Hg of 802 μg g⁻¹) and the solution concentration in extract (right) with a Hg(0) solubility of ~0.06 mg L⁻¹ indicated as a pink dashed line. The size of the symbols in panel b corresponds to the relative pool sizes of the extracted Hg. F1D had an equilibration time that was longer than those of the other extracts and is colored pink as a potential outlier (see the text). The gray dashed line at ~0.43‰ (δ²⁰²Hg) indicates the bulk soil signature of K2-8.

**Figure 4.** (a) Hg concentrations and (b) δ²⁰²Hg values of seven consecutive sequential water extracts of sample K2-8 (F1A–F1G). The concentration axes in panel a refer to the extracted solid phase concentration (left, bulk soil total Hg of 802 μg g⁻¹) and the solution concentration in extract (right) with a Hg(0) solubility of ~0.06 mg L⁻¹ indicated as a pink dashed line. The size of the symbols in panel b corresponds to the relative pool sizes of the extracted Hg. F1D had an equilibration time that was longer than those of the other extracts and is colored pink as a potential outlier (see the text). The gray dashed line at ~0.43‰ (δ²⁰²Hg) indicates the bulk soil signature of K2-8.

Over extended periods of time. The sequential water extraction of sample K2-8 was initially planned for only three steps, but because the released Hg concentrations remained high, we decided to prolong the experiment to seven steps, even though there was a time delay of ~1 week between steps 3 and 4. We hypothesize that the anomalously heavy δ²⁰²Hg value in step 4 (Figure 4) might be an artifact caused by partial Hg(II) reduction and loss of gaseous Hg(0) from the PP centrifuge tubes during this period. The observed heavy δ²⁰²Hg signatures of the water extracts were in good agreement with the heavy Hg isotope signatures of the groundwater samples (Figure 1b), supporting the concept that isotopically heavy Hg is leaching into the aquifer that is fractionated relative to the original contamination source signature.

**Implications for Source and Process Tracing.** If the observed Hg isotope variations of ~0.5‰ in δ²⁰²Hg between bulk soil samples of different depths as well as between different soil extracts of individual samples were not caused by fractionation processes in the subsurface but instead by the mixing of isotopically distinct Hg sources, then one would expect to find correlations or a grouping of samples toward end-member values in a plot of δ²⁰²Hg versus 1/Hg, which was not observed (Figures S2 and S3). Even though temporal variation in the isotopic composition of the HgCl₂ source material used during the operation of the industrial facility (1904–1965) cannot be completely excluded, there is no evidence of it and existing knowledge about the site history does not suggest the presence of Hg contamination sources other than HgCl₂. This suggests that the observed Hg isotope variations were indeed caused by fractionation during Hg species transformation and/or partitioning processes in the subsurface. These have shifted the Hg isotope signatures in soil and groundwater samples away from the isotopic signature of the industrial HgCl₂ source. This finding has important implications for the application of Hg isotope ratios as tracers for Hg cycling at contaminated sites.

(1) In source tracing, it is assumed that isotopically distinct anthropogenic or background sources of Hg mix and their relative contributions can be quantified. When these end members are fractionated on a local scale such as in the study presented here, this is not feasible. Even the identification of a representative source signature for a particular contamination source may be challenging, especially in subsurface systems with high isotopic variability. Nevertheless, we stress that at our field site, contamination occurred in the form of highly soluble HgCl₂ and conditions in the subsurface (e.g., low organic matter content) favor the formation, coexistence, and transport of different Hg species. Due to these circumstances, our findings cannot be transferred to all Hg-contaminated sites. Other field systems might be well suited for source tracing applications, for example, systems in which the presence of very stable Hg forms such as HgS minerals limits the extent of species transformations or single Hg species dominate the Hg budget of the site. This has been demonstrated by many successful examples of this approach. However, we suggest that there are likely other Hg-contaminated sites with similar contamination history and soil characteristics to which the results presented here will be very relevant.

(2) One the other hand, our observed Hg isotope variations in the subsurface of a contaminated site highlight the potential of Hg isotope signatures to serve as tracers of past Hg species transformations, partitioning processes, and the mobility of the transformation products. This has barely been explored in
The Hg isotopic ratios in the water extracts (F1) exhibited a bulk sample and the other more mobile soil Hg pools were consistently enriched with light Hg isotopes relative to the stable and least mobile Hg pools (Figure 5). Within individual soil samples, the most contaminated soil layers, presumably by the preferential fractionation than larger and less reactive soil Hg pools. The most contaminated samples to lighter than bulk soil in high variability, ranging from much heavier than bulk soil in the most contaminated samples to lighter than bulk soil in lower-concentration samples. This observation can be explained by the concept that isotope signatures of smaller Hg soil pools with a relatively high reactivity and mobility are more easily altered to a measurable extent by Hg isotope fractionation than larger and less reactive soil Hg pools.

The ultimate goal of process tracing studies with Hg isotopes would be the unambiguous identification and quantification of specific Hg species transformation processes. However, this is challenging, not only because a complete separation of all relevant Hg species by extraction methods is difficult but also because overprinting of multiple signatures commonly occurs. In the field system presented here, a number of different fractionation processes may have contributed to the observed Hg isotope variations of our samples. During the ~100 years since the onset of soil contamination, numerous Hg species transformation, partitioning, and transport processes may have left an isotopic imprint on the studied samples and are evidenced by the SEP and PTD results. For example, reduction of Hg(II) to Hg(0) is detected by PTD. Sorption, complexation, and precipitation reactions of mostly Hg(II) species are seen by SEP, forming Hg pools with distinct properties and mobilities. Another potentially important process is the formation of gaseous Hg(0) species followed by volatilization to the atmosphere, as outgassing of volatile Hg species at the soil surface has been observed at the site in previous work but could not be assessed in this study.

But which reaction pathways have most likely generated the observed Hg isotope signatures in our field system? The answer to this question can only be approximated with the data set presented here. While remarkable MDF has taken place, little MIF was observed, which has been used as a clear identifier of photochemical processes in other studies. This is not surprising because our samples were not exposed to sunlight in the subsurface. Mass-dependent fractionation trends can be investigated from different perspectives, between samples or within subpools. It can be generalized that for kinetically controlled reduction, the δ$^{202}$Hg of the produced Hg(0) is lighter than the original Hg(II), independent of the pathway being biotic or abiotic nonphotochemical. (De)methylation shows similar trends. As for equilibrium isotope fractionation, studies of Hg(0) evaporation and Hg(II) complexation and sorption have found an enrichment of heavy Hg isotopes in the substrate with the product enriched with light mercury isotopes. Both mechanisms, albeit inducing opposite signals, may have influenced our water extracts, where Hg may volatilize [such as during the unplanned break between two of our sequential water extracts (see above)], pushing the samples toward heavier signatures. Hg may be equally well complexed to organic ligands, enriching the complexes with light isotopes. Another potential fractionation mechanism could be fast isotope exchange between Hg(0) and thiol-bound Hg(II) causing an enrichment of heavy Hg isotopes in the oxidized Hg(II) species as recently reported from laboratory experiments. Moreover, precipitation of β-HgS from Hg(II)-containing solutions has been shown to enrich the solid phase with light isotopes. This is consistent with the generally light F4 extracts of all samples. However, pool size effects may complicate the direct transfer of experimentally determined fractionation factors to natural systems. Taking into consideration all possible fractionation processes, we must conclude that no single process can be identified as being responsible for the signature of a particular Hg pool in our samples. While some processes can be ruled out (e.g., photochemical), the observed isotope signatures are not distinct enough to attribute observed values to individual processes. Nevertheless, our results reveal that Hg species transformations and/or partitioning processes associated with Hg isotope fractionation have occurred in the subsurface of the studied industrial site, but it is likely that a variety of different processes have been involved.

**Environmental Implications.** The observed Hg isotope signatures of our samples reveal that the industrial Hg source material was transformed not only chemically but also isotopically in the subsurface of the site. The extent of fractionation within two soil cores on one contaminated site is
remarkable when considering that these alterations have taken place without the influence of photochemistry, which is recognized as one of the strongest drivers of Hg isotope fractionation in natural samples. Several studies distinguish between isotopically different industrial products (such as strongly fractionated calcine and ore) and deposition pathways from the atmosphere or contamination sources. Here, geochemical transformations and isotope fractionation processes have taken place in situ, under environmental conditions in the subsurface of a contaminated site. The subsequent translocation of fractionated products due to the differing mobility of the fractionated subpools is reflected in the Hg isotope signatures of the groundwater, which will be investigated in more detail in future studies. The demonstration of in situ fractionation has important implications for source tracing studies in which caution should be applied when using Hg isotope data of surface samples as representative signatures for the entire contamination. In conclusion, our results demonstrate the need for a combination of high-resolution sampling to assess the fate and mobility of Hg at contaminated sites.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b00619. Tables S1–S8 and Figures S1–S4 (PDF)

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**Notes**

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