

Fate of Selenium in Soils at a Seleniferous Site Recorded by High Precision Se Isotope Measurements

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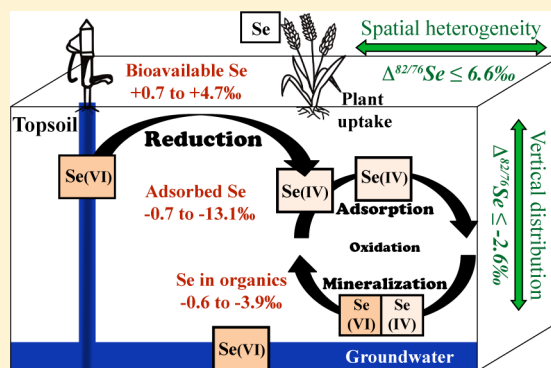
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Supporting Information

ABSTRACT: Selenium poisoning is a significant health problem in parts of Punjab, India, which is an area of intense agricultural productivity. To determine the complex soil dynamics that control distribution of Se in this area, we measured concentrations and $\delta^{82/76}\text{Se}$ of bulk Se and individual Se pools in four soil profiles. This was compared against $\delta^{82/76}\text{Se}$ of crops and groundwater used for irrigation. The isotopic composition of bulk Se and component Se pools reveal spatial heterogeneity. The bulk $\delta^{82/76}\text{Se}$ show progressively lower values with increasing soil depth indicating the preferential migration of isotopically lighter Se downward through the soil profile. The $\delta^{82/76}\text{Se}$ of water-soluble Se is isotopically heavier than $\delta^{82/76}\text{Se}$ of adsorbed Se, suggesting Se isotope fractionation by reduction prior to scavenging by reactive minerals in the soil. The organically bound Se is isotopically lighter than water-soluble Se and correlates with the C/N ratio at different soil depths. Thus, Se immobilization by redox cycling controls the biogeochemical Se cycle in the soil. Se isotope ratios help to trace biochemical processes of Se in agricultural seleniferous soils and provide an important assessment for better soil management mitigating Se concentrations of ecotoxicological levels.



INTRODUCTION

Selenium (Se) is an essential micronutrient with a narrow tolerance range before it becomes toxic at higher concentration. It has received considerable attention in human health and agricultural studies.^{1,2} The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) recommend a daily intake of Se for healthy adults of 25–35 μg .³ Higher exposure leads to selenosis, a disease that entails loss of hair and nails and potential dysfunction of the nervous system.⁴

Human intake of Se occurs mainly through drinking water and certain types of food. A key factor for uptake by crops and livestock from their surroundings is not only Se concentration but also the chemical speciation determining its bioavailability in soils.^{4,5} Selenium is a redox sensitive element (typically occurring in the –II, 0, +IV, and +VI oxidation states) with soil concentrations that vary strongly with geological conditions and geographic location.^{5,6} Under oxic and suboxic conditions in soils and groundwater, the oxidized forms Se(VI) (selenate; SeO_4^{2-}) and Se(IV) (hydroselenite and selenite; HSeO_3^{2-} and SeO_3^{2-}) are water-soluble and hence mobile and bioavailable. This contrasts with more reducing conditions where elemental

selenium, Se(0), and selenide Se(–II) are generally found in insoluble solid phases that are relatively resistant to oxidation.⁶

The Se found in soils is derived from different sources such as weathering of parent bedrocks and atmospheric deposition as well as anthropogenic activity.^{5,7} Seleniferous regions are highly localized but can be found in a variety of continental settings, with well-known examples from China, Ireland, Venezuela, and the USA.^{8–10} High concentrations above 100 mg kg^{-1} have been recorded in southwestern Ireland and in the San Joaquin Valley, USA, which are derived from weathering of carbonaceous shales.^{10,11} Selenium poisoning has been well-studied in the Daba region (Shaanxi) and the Enshi District (Hubei) in China.^{9,12,13} The source of Se was identified as weathering of Se-rich shales, volcanic tuff, and coal.^{12,13} Mobilization from rocks and sediments can be enhanced by agricultural practices such as irrigation.^{5,11} This problem was first documented in the 1980s in the San Joaquin Valley (USA),

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resulting in extensive studies investigating sources and environmental reworking of Se.^{11,14} Despite the fact that elevated Se concentrations are well-known in different regions across the world, the mechanisms by which Se species are released and migrate through soils remain poorly understood and contamination remains a problem.¹⁵

Localized Se poisoning of farm animals was first discovered in the 1980s in several villages in the northeastern part of Punjab, India that extends across 1000 ha of agricultural land. Soil Se concentrations range from 0.25 to 6.8 mg kg⁻¹ with a mean value of 3.6 mg kg⁻¹.^{16,17} A 2004 study¹⁸ showed that the dietary intake of Se is greater than 600 μg Se day⁻¹ in this region in excess of the recommended WHO limit.³ Analysis of human hairs and nails revealed 6 to 9 times higher Se contents compared to background levels.¹⁹

Dhillon and Dhillon²⁰ argued that these seleniferous soils initially developed from alluvial Se-rich sediments transported by seasonal rivulets from the adjoining Shiwalik Hills. Selenium contamination has been further accentuated through seasonal irrigation over the last 30 years.²¹ The authors calculated an annual amount of Se, introduced to the soil through irrigation, adding up to 1420 g Se per hectare based on a maximum permissible Se value of 20 μg L⁻¹ for irrigation water.²¹ The improved irrigation system led to a change in the cropping practice from wheat–maize to wheat–rice rotations resulting in up to 70% of rice–wheat cropping, 20% sugar cane, and 10% other crops at the current time.²⁰ Crops growing in this seleniferous region can accumulate Se to hazardous concentrations of 2 to 37 mg kg⁻¹.²⁰ Although concentrations of Se are well constrained for this soil, water, and crop system, to date little is known about the biogeochemical processes that control its distribution. Better knowledge about the fate of Se linked to biogeochemical reactions is crucial to predict its mechanistic cycle. It has been shown that stable isotope signatures can provide useful information about processes in environmental systems.²² The use of stable Se isotope ratios elucidates Se transformation during biogeochemical processes and can potentially serve as a process tracer in soils.

Selenium has six stable isotopes and its isotope ratios can help identify Se sources and the role of redox reactions in its biogeochemical cycle.^{14,23,24} Experimental studies have shown that kinetic isotope fractionation of Se can be linked to both abiotic and biotic redox processes.^{23–30} The abiotic reduction of Se is controlled by pH and E_h values, with iron(II)-minerals being the dominant potential reductants in soil.^{29–31} The interaction of Se(VI) with Fe(II)–Fe(III) double layered hydroxide (green rust) produces Se(IV), accompanied by isotopic fractionation of ca. 11‰.²⁹ A similar fractionation effect of up to 10‰ has been observed for Se(IV) reduction to Se(0) by Fe(II) sulfides.³⁰ In contrast, the biological reduction of Se oxyanions exhibits a larger variation in Se isotope fractionation of 0.3–7.5‰ [Se(VI) to Se(IV)] and 1.7–12‰ [Se(IV) to Se(0)] depending on the bacterial species and conditions.²⁷ Selenium isotope fractionation during reduction of Se(VI) or Se(IV) is much smaller in natural microbial communities than in pure bacterial cultures.²⁵ Volatilization of Se has revealed that methylated Se is 3–6‰ isotopically lighter relative to the Se oxyanion source.²⁸ Two studies have reported Se isotope ratios in environmental settings showing the interaction of hydrology and biogeochemistry in controlling Se distribution.^{14,24} To date, however, there have not been any stable Se isotope studies published that identify Se mobility and

cycling in soil, crop, and water systems, where elevated concentrations have led to toxicity in humans.

Our objectives are (1) to determine the stable Se isotope ratios of bulk soil, groundwater, and crop samples to constrain potential biogeochemical reworking and (2) to use the isotopic composition of different Se pools within the soil to provide additional information about the fate of Se in the soils. Assessing redox reactions is difficult when only looking at Se concentrations. While $\delta^{82/76}\text{Se}$ of bulk soil Se provides the general trend and distribution of Se, $\delta^{82/76}\text{Se}$ of sequentially extracted Se can potentially add mechanistic insights into complex Se reactions, especially those involving redox transformations between different phases. As mobile Se from irrigation water is the only bioavailable Se species currently entering the system, significant differences in isotopic ratios between Se in irrigation water and soil pools should suggest changes in redox state and geochemical properties. The detailed information on the scale of natural processes regulating the mobility and fate of Se in the Punjab soils provided by this study will be an important step to improve remediation strategies.

ANALYTICAL METHODS

Site Description and Sample Collection. The study area is located in the Northwest of India in the state of Punjab (31°8' N, 76°10' E; see Figure S1, Supporting Information). Our sampling campaign was performed in January 2012 (dry season) at four different locations across the seleniferous site where wheat (Sites A, B, and D) and sugar cane (Site C) are cropped annually (Table S1 and Figure S1, Supporting Information). We sampled four soil profiles (0–100 cm below the surface, divided in six soil-depth intervals) along with the aboveground biomass of juvenile wheat plants from the same sampling points (Sites A and B). Irrigation water was collected from selected wells situated as close as possible (within 10–100m) to the sampled soils [i.e., Site A comprises sample soil (SC-A) corresponding to tube well (TW-A)]. The depth of tubewells varies between 75 and 100 m into the deeper aquifer.²¹ All wells were purged before taking a sample by pumping out a minimum of three well volumes of groundwater, and samples were immediately filtered in the field. We also sampled sediments from the dry riverbed of the seasonal rivulet (CH samples) and reservoir water (SH-L) in the Shiwalik Hills to the north of the contaminated site.

Total Se Analysis and Sequential Extraction (SEP). Soil samples were digested in aqua regia for bulk Se analysis. To characterize operationally defined Se pools, the soils were sequentially extracted using a method modified from Chao and Sanzalone³² and described in detail by Schilling et al.³³ The four-step extraction was carried out in sequence, separating water-soluble (bioavailable) Se (=soil pool 1), adsorbed Se (=soil pool 2), organically bound Se (=soil pool 3), and residual Se (=soil pool 4). A more comprehensive description of the extraction method and extracted Se phases is provided in the Supporting Information.

Se Isotope Analysis. Sample preparation for Se isotope analysis is described in the Supporting Information. A double spike solution (⁷⁴Se/⁷⁷Se) was added to all samples (groundwater, soil, and plant digestions) before the sample purification step to correct for potential isotopic fractionation during analytical separation as well as instrumental mass bias during mass spectrometry.²⁴

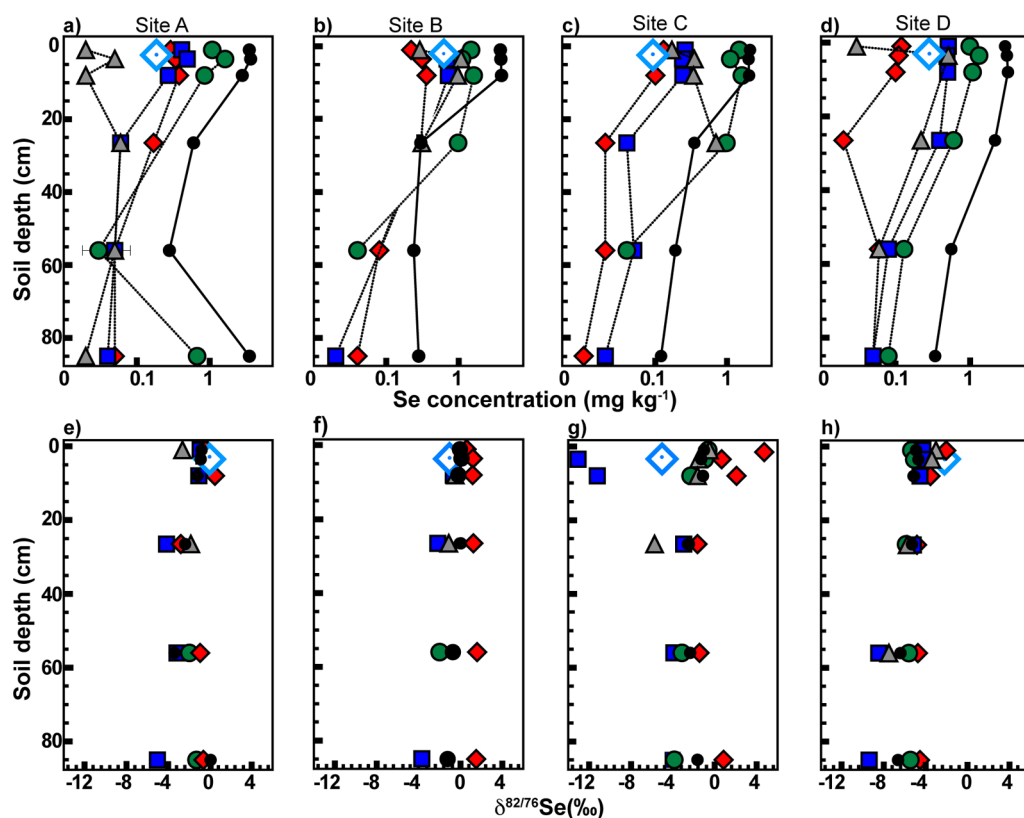


Figure 1. Soil depth trend of Se concentration (log scale) (a–d) and Se isotopic composition (e–h) for bulk Se and different Se soil pools. Black circle: bulk Se; red diamond: bioavailable Se; blue rectangle: adsorbed Se; green circle: organic Se; and gray triangle: residual Se. Soil samples represent the depth intervals: 0–2 cm, 2–5 cm, 5–11 cm, 11–42 cm, 42–70 cm, and 70–100 cm. Each point represents median depth for each interval. The single blue diamond in both sets of diagrams represents the Se concentration and Se isotopic composition of irrigation water. Error bars on $\delta^{82}\text{Se}$ values are within the size of the symbols.

Selenium isotope ratios were measured using a Nu Plasma multiple-collector ICP-MS coupled to an in-house built hydride generator at the University of Illinois, Urbana–Champaign, USA. All Se isotope data are reported using the standard δ notation relative to Se(IV) standard solution (NIST SRM 3149):

$$\delta^{82/76}\text{Se} = \left[\frac{(^{82/76}\text{Se}_{\text{sample}})}{(^{82/76}\text{Se}_{\text{standard}})} - 1 \right] \times 1000(\text{‰}) \quad (1)$$

The uncertainty (2σ) of $\delta^{82/76}\text{Se}$ was $\pm 0.15\text{‰}$ calculated from 2 times the root mean squared differences of 23 pairs of duplicate sample preparations, representing 21% of the complete data set. Long-term precision and accuracy are the values described by Schilling et al.³³ The Se concentrations were determined using the isotope dilution calculations from the measurements of spike isotopes. The uncertainty of Se concentration was $\pm 11.8\%$ based on 4 duplicate analysis of bulk soil Se.

The offset for Se isotope ratios between different Se soil pools or between different soil depths in the studied soil–water–plant systems is expressed as:

- (i) $\Delta^{82}\text{Se}_{\text{pool1} - \text{poolx}} = \delta^{82}\text{Se}_{\text{pool1}} - \delta^{82}\text{Se}_{\text{poolx}}$ (different Se pools)
- (ii) $\Delta^{82}\text{Se}_{100-0 \text{ cm}} = \delta^{82}\text{Se}_{100 \text{ cm}} - \delta^{82}\text{Se}_{0 \text{ cm}}$ (different soil depth)

$\Delta^{82/76}\text{Se}$ can then suggest specific reaction pathways, assuming that larger $\Delta^{82/76}\text{Se}$ is linked to reduction, whereas

smaller $\Delta^{82/76}\text{Se}$ may indicate adsorption, oxidation, or mineralization.²³ To interpret the observed differences in Se isotope ratios ($\Delta^{82}\text{Se}$) in terms of Se fluxes, we need to consider the magnitudes of isotope fractionation for specific processes, which are described by the enrichment factors, $\epsilon^{82}\text{Se}$ ($\epsilon \approx \delta_{\text{reactant}} - \delta_{\text{instantaneous product}}$). The reported enrichment factors in the Se isotope literature are experimentally derived values for different reaction mechanisms.^{23,27–30} Note that the Se isotopic ratios of reactant and the product pools represent only a snapshot of the soil reactions due to the complexity of the studied agricultural system that changes across wet and dry seasons.

Mass Balance Calculation. To assess the mass balance of the sequential extracts' Se isotope data, we used the mass balance calculation of our previous study.³³ There, we defined the calculated total isotopic ratio of all individual pools as

$$\delta^{82/76}\text{Se}_{\text{SEPCalc.}} = \sum_{f=1}^n [(\delta^{82/76}\text{Se}_i \times C(\text{Se})) / \sum_i C(\text{Se})] \quad (2)$$

where $\delta^{(82/76)}\text{Se}_i$ represents the $\delta^{82/76}\text{Se}$ values (‰) and $C(\text{Se})$ the relative fraction (%) of individual Se pools from the sequential extraction step i . Because it is not possible to perform these calculations when data for one or more steps are missing, we are able to estimate the isotope mass balance for only 13 of 24 samples from 4 soil profiles (Table S2, Supporting Information).

RESULTS AND DISCUSSION

Vertical Variation in Se Concentrations. Selenium concentrations are elevated in the topsoil and rapidly decrease with depth in the vertical soil profiles across the site (Figure 1a–d, Table S2, Supporting Information; excluding depth 70–100 cm at Site A). In the uppermost 3 intervals of the topsoils (0–11 cm), the bulk Se concentrations range from 2 to 3.9 mg kg⁻¹ (Table S2, Supporting Information) with significant differences between the four sampling sites (ANOVA $p \leq 0.0001$). This is consistent with previous results by Sharma et al.¹⁷ who reported topsoil Se concentrations of up to 6.5 mg kg⁻¹ and lateral heterogeneity on a scale of tens of meters to kilometers across the seleniferous zone. Se concentrations decrease significantly below a soil depth of 11 cm in all four profiles ($Se_{\text{bulk}} < 0.34$ mg kg⁻¹) (Figure 1a–d, Table S2, Supporting Information). For sequentially extracted Se pools, we observe a similar Se concentration pattern between the four sampling sites (excluding depth 70–100 cm at Site A). Selenium of soil pool 3 (organically bound Se) is the predominant pool in most soil depth intervals with a relative fraction of up to 80% of total sequentially extracted Se (ΣSe_{SEP}). The positive correlation between organic C and pool 3 ($0.53 > r^2 > 0.95$) strongly suggests that Se is mainly associated with organics (Table S2, Supporting Information). The water-soluble Se (soil pool 1) constitutes 2–27% of total sequentially extracted Se (ΣSe_{SEP}) and becomes proportionally greater with depth. Adsorbed Se (soil pool 2) is uniform in its proportion relative to ΣSe_{SEP} across the soil depths ($24.1 \pm 8.2\%$, 1σ , $n = 11$). The concentrations of Se in soil pool 4 are more variable and represent the most abundant fraction at shallow depths at sites B and C. Se in soil pool 4 is likely a mix of different Se species [Se(IV), Se(-II), Se(0)] associated with recalcitrant organic matter,³⁴ occluded in phyllosilicates³⁵ or ferruginous concretions, which were nonextractable by $KClO_3$.³³

Se Isotope Variability with Soil Depth. The $\delta^{82/76}Se$ values of bulk Se ($\delta^{82/76}Se_{\text{bulk}}$) cover a large range with the most positive value of +0.09‰ at site B (depth 2–5 cm) and the most negative value of -6.64‰ at site D (depth 70–100 cm) (Figure 1e–f, Table S2, Supporting Information). All soil profiles show increasingly more negative $\delta^{82/76}Se_{\text{bulk}}$ values with soil depth and decreasing Se concentrations (excluding depth 70–100 cm at Site A). The maximum Se isotopic variation within a soil profile is -2.6‰ ($\Delta^{82}Se_{100-0\text{ cm}}$) at site A. For the other soil profiles, $\Delta^{82}Se_{100-0\text{ cm}}$ values are -1.2‰ (site B), -1.6‰ (site C), and -1.7‰ (site D). Soils of sites A and D have more negative $\delta^{82/76}Se_{\text{bulk}}$ at the surface than the irrigation water. In contrast, soils at sites B and C have more positive $\delta^{82/76}Se_{\text{bulk}}$ in the uppermost depth intervals (0–11 cm) than the corresponding irrigation water (Figure 1e–f). The operationally defined Se pools track $\delta^{82/76}Se_{\text{bulk}}$ by becoming generally more negative with depth. However, some features are consistent across all soil profiles. The water-soluble Se consistently gives the highest $\delta^{82/76}Se$ values, which is slightly more positive or equal to the irrigation water in the uppermost part of the soil. The adsorbed Se (pool 2) shows the most negative $\delta^{82/76}Se$ in all profiles. The $\delta^{82/76}Se$ of soil pool 3 values range between 0.05‰ and -5.87‰ with more negative values typically seen in deeper soil horizons (<11 cm) compared to the topsoils (0–11 cm) and correlate closely with $\delta^{82/76}Se_{\text{bulk}}$ ($r^2 = 0.8$).

Mass Balance and Potential Limitations. The percent recoveries are calculated from the concentration sum of individual Se pools (sequential extraction) and compared to the bulk Se concentration (total digestion). The average recovery is $64 \pm 17\%$ (1σ , $n = 11$). The recovery is more than >70% for 5 samples, 50–60% for another 5 samples, and less than <40% for 1 sample. The precision of the sum of Se soil pools is 24%, calculated through error propagation from the uncertainties of the individual pools (11.8%). The samples SC-C H4 and SC-B H4 show an anomalously high recovery (>200%), probably due to low bulk Se concentration (<0.5 mg Se kg⁻¹) where soil heterogeneity has a larger impact on the percentage of Se recovery. These samples are therefore excluded from further data interpretation. An additional problem leading to poor recovery is the loss of suspended soil material from the initial sample weight during separation of supernatants after each sequential extraction step. While the concentration mass balance has relatively low recoveries, isotopic mass balance, calculated using the sum of individual Se pools compared to bulk $\delta^{82/76}Se$, is in good agreement for 8 of 13 samples within an uncertainty of 0.3‰ (Table S3, Supporting Information).

We acknowledge that three samples exhibit a poor isotopic mass balance and the interpretations of these data may not be secured because of the larger uncertainties of the pool sizes. Also, it seems that all of the samples systematically suffered low Se recoveries. Taking into account the low recovery of the sequentially extracted Se with potential loss of Se from one or more Se pools, we assert that Se concentrations and $\delta^{82/76}Se$ data for different Se soil pools are interpreted with caution. Despite variable recoveries for Se concentrations and $\delta^{82/76}Se$ data, we find consistencies across the four sites that we interpret to reflect primary environmental variability.

Se Isotope Fractionation Semiclosed/Open System Soils. In an ideal case, for soils in semiclosed or open systems,^{36,37} isotope dynamics are balanced and differences in isotope ratios between reactant and product(s) should approximately reflect the isotopic fractionation, $\Delta: \delta_{\text{reactant}} - \delta_{\text{product}}$. However, soils are complex systems with different chemical species involved in simultaneous and sequential reactions leading to multiple reaction products. Isotopic mixing as well as the kinetic isotope effects involved in individual reactions thus characterize the soil cycle. Here, we applied a simplified isotopic mixing-reduction model, modified from Fry,³⁶ assuming an open system (see Table S4, Supporting Information) to investigate irrigation input and redox reactions that both control the isotopic composition of Se in the soil pools. The parameters for our model are described in the Supporting Information. Since we know that reduction is the major reaction governing Se isotope fractionation, the mixing-reduction model focuses primarily on Se soil pool 1 (water-soluble) as the reactant and soil pool 2 (adsorbed) as the potential reduction product. Figure S2, Supporting Information, shows the model results, which describe how the isotopic composition and Se concentration of Se soil pool evolve throughout the soil column. The mixing-reduction model reproduces the observed isotope ratios for the adsorbed Se soil pools fairly well. Further work should expand on this by developing a more rigorous reactive transport model of the system.

In order to assess the potential flux of Se through plants by processes such as uptake and volatilization, we consider that (i) $\delta^{82/76}Se$ of Se pool 1 is the reactant for uptake by crops and (ii)

the $\delta^{82/76}\text{Se}$ value of the crops represents the intermediate pool between uptake and volatilization. The extent of the reaction f expressed as $f = (\delta_{\text{reactant}} - \delta_{\text{product}})/\epsilon$ describes the potential pool size for the reaction of Se volatilization.

Se Isotope Variation in Adsorbed and Water-Soluble Pools. The elevated concentration of Se in the topsoil relative to deeper layers suggests immobilization of Se through reduction, adsorption, and/or sequestration in organic matter. As highly mobile Se(VI) is the only Se species in the irrigation water (Table S3, Supporting Information), biological assimilation or Se reduction could lead to gradual Se accumulation in the topsoils. Isotopes can be used to distinguish between these processes, since assimilation produces only minor and positive shifts in $\delta^{82/76}\text{Se}$ toward the plant material as reaction product ($\epsilon^{82}\text{Se} = 1.5\text{--}3.9\text{‰}$),²³ while reduction from Se(VI) to Se(IV) tends to produce much larger isotopic fractionation by an enrichment of more positive $\delta^{82/76}\text{Se}$ in the remaining reactant ($\epsilon^{82}\text{Se} = 4\text{--}11\text{‰}$).^{24–26,29}

The average $\delta^{82/76}\text{Se}_{\text{pool1}}$ values (water-soluble Se) have equal (Sites A and D) or elevated $\delta^{82/76}\text{Se}$ values (Sites B and C) relative to the irrigation water (Figure 2, Table S3 and S5,

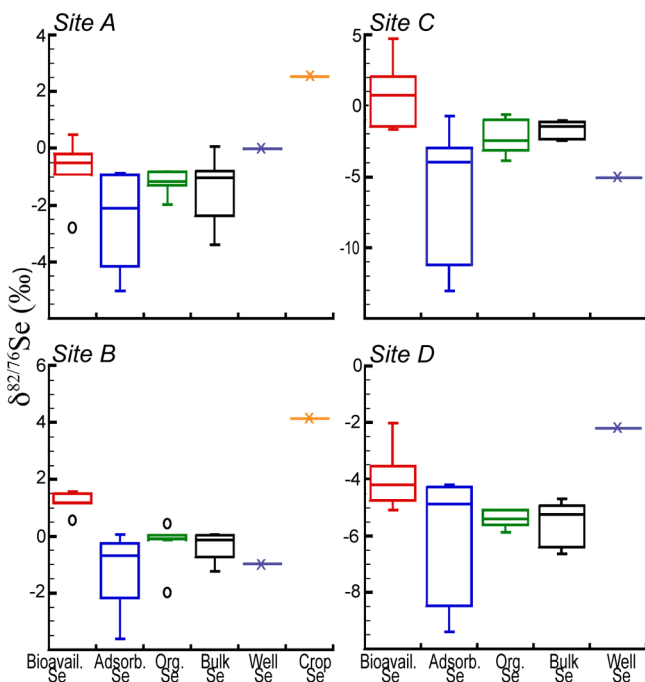


Figure 2. Mean isotopic composition and variance of combined samples (including all depths) of the Se-pools, bulk Se, irrigation water, and crops for each soil profile. The lines within the boxes give the mean $\delta^{82/76}\text{Se}$, the upper and lower limits of the boxes are the 25th percentile and 75th percentile of the variance, and the whiskers indicate the maximum and minimum values.

Supporting Information). The corresponding $\delta^{82/76}\text{Se}_{\text{pool2}}$ values for adsorbed Se show depletion in ^{82}Se compared to water-soluble Se. Especially, the topsoil of site C shows a significant enrichment of lighter Se isotopes in the adsorbed Se(IV) pool ($\delta^{82/76}\text{Se}$ of -13.1‰ and -11.22‰) relative to depth-corresponding water-soluble Se ($\delta^{82/76}\text{Se}$ $+0.66\text{‰}$ and $+2.08\text{‰}$). The $\Delta^{82/76}\text{Se}_{\text{pool1-pool2}}$ of $\sim 13\text{‰}$ is consistent with redox reactions resulting in large isotope fractionation. When Se(VI) of irrigation water is reduced in the topsoils, the water-soluble Se pool exhibits elevated $\delta^{82/76}\text{Se}$. Accordingly, the expected reaction product as adsorbed Se(IV) is enriched in

lighter isotopes.^{23,24,29} Using the mixing-reduction model, the fraction of reduction from the water-soluble Se pool varies between 7% (Site A) and 58% (Site C) in the topsoil (0–11 cm).

The largest offset ($\Delta^{82/76}\text{Se}_{\text{pool1-pool2}}$) between water-soluble Se and adsorbed Se at Site C corresponds to Se isotope effects observed in laboratory experiments for both microbial and abiotic Se reduction ($\epsilon^{82}\text{Se} = 5.8\text{‰}$ to 12‰).^{27,29} Microbial processing at this seleniferous site is confirmed by previous studies that have identified and characterized several facultative anaerobic bacteria, which have the capability to metabolize Se(VI) or Se(IV).^{38,39} Decomposing plant material in the soils provides an ample carbon source for bacterial Se reduction and enhances locally reducing conditions.⁴⁰ Likewise, the observed $\Delta^{82/76}\text{Se}_{\text{pool1-pool2}}$ values can be explained by abiotic reduction of mobile Se(VI) mediated by the presence of dissolved Fe(II) or Fe(II)-bearing minerals. At the time of our sampling during the wheat rotation, the soil conditions were oxic. The topsoil can alternate from oxic to suboxic during the wet season and rice cropping, which potentially leads to reductive dissolution of Fe(III) or formation of Fe(III)–Fe(II) mineral phases.^{31,41,42} Under these conditions, the formation of green rust as a known reductant of Se(VI) is likely and can result in the enrichment of heavier Se isotopes in the water-soluble Se pool.^{29,41}

The presence of nearly 30% of $\Sigma\text{Se}_{\text{SEP}}$ as phosphate-extractable adsorbed Se in the soils suggests the formation of Se(IV) surface complexes during adsorption onto different minerals (i.e., (hydro)oxides, carbonates).⁴³ Adsorption of Se(IV) in soils can prevent further reduction to Se(0) or Se(–II) by inner-sphere complexation.⁴⁴ Since adsorption of Se induces only minor isotopic fractionation ($<1\text{‰}$), the $\Delta^{82/76}\text{Se}_{\text{pool1-pool2}}$ differences must thus be derived from the previous reduction step.^{23,30} Therefore, differences in $\delta^{82/76}\text{Se}$ values for water-soluble and adsorbed Se supports reduction from Se(VI) to Se(IV).

Se Isotope Variations in Soil Organic Matter. Higher C/N ratios (10 to 14.5) and more positive $\delta^{82/76}\text{Se}_{\text{pool3}}$ in the topsoil relative to deeper horizons suggest a transformation of Se in soil organic matter. A positive correlation exists between $\delta^{82/76}\text{Se}_{\text{pool3}}$ and the carbon–nitrogen ratio (C/N) that decreases with increasing soil depth (Figure 3 and Table S2, Supporting Information). Similar changes in C/N ratio with soil depth have been observed elsewhere and are interpreted to

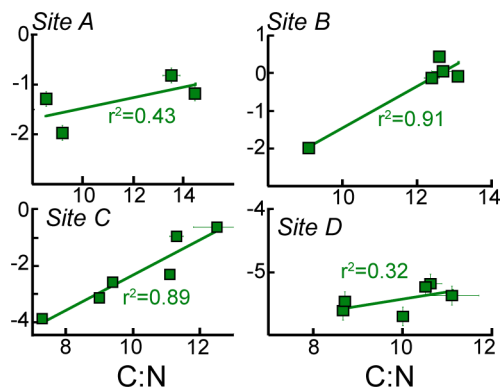


Figure 3. Relationship between C/N ratio and Se isotopic composition of Se soil pool 3 (organically bound, ‰) for the soil profiles. Error bars for $\delta^{82/76}\text{Se}$ represent 2σ of $\pm 0.15\text{‰}$ from duplicate samples analysis. The error for C/N ratio is based on standard deviation of duplicate sample measurements.

represent an increase in processing and decomposition of organic matter.^{45,46} Dissolved Se can be immobilized directly by complexation with organic matter⁴⁷ for which isotopic fractionation is minimal.²³ However, release of Se from organic compounds may potentially governs Se isotope fractionation.²³ For that reason, we hypothesize that variation in $\delta^{82/76}\text{Se}_{\text{pool3}}$ between the topsoils and deeper horizons is the result of organic matter decomposition leading to release of Se. In such a scenario, isotopically lighter Se would be preferentially released leading to an isotopic shift throughout the soil profile as isotopically lighter Se leaches down to deeper soil horizons. However, further investigations are required to determine the magnitude of Se isotope fractionation during complexation of Se with organic matter or decomposition of organic-associated Se.

Effect of Se Mobility on Se Isotope Compositions. The downward transport of dissolved mobile Se species is unlikely to play any major role in determining the Se isotopic composition of the deeper soils. Due to intensive irrigation and rainfall (wet season), dissolved Se leaches down toward the aquifer.¹⁶ An experimental study, using soil samples from the field site, suggested that a vertical transport of dissolved Se from the topsoil (0–11 cm) to deeper soil horizons of up to 13.0 g Se per hectare is likely for an annual rainfall scenario of 1000 mm.¹⁶ However, this accounts for less than 1% of the Se soil budget for an annual Se input of 1420 g per hectare. In addition, Se reduction in topsoils should render the percolating Se(VI) isotopically heavy.^{23,24,27,29} However, $\delta^{82/76}\text{Se}_{\text{bulk}}$ values show gradual enrichments of lighter Se isotopes ($\Delta^{82}\text{Se}_{\text{bulk100-0 cm}} -1.2\text{‰}$ to -2.6‰) (Figure 1e–h). Also, isotopically light Se(IV) is less likely to percolate due to its higher adsorption potential and gives rise to enrichment of lighter isotopes in the deeper soils.

The oxidation of reduced Se species along with mineralization of Se from organic matter may lead to a preferential transport of lighter Se isotopes. Microbial and chemical oxidation mobilize reduced Se species, such as adsorbed Se(IV) or organically bound Se.^{48,49} It seems that oxidation of Se(IV) involves a small kinetic isotope effect favoring lighter isotopes.²³ A recently conducted laboratory experiment revealed that abiotic Se(IV) oxidation to Se(VI) by manganese oxide induces an isotopic fractionation ($\epsilon^{82}\text{Se}$) of 1‰ (unpublished data). This process can generate isotopically light Se(VI) by oxidation of Se(IV).

Alternatively, mineralization of Se from organic matter can preferentially remove lighter isotopes. As discussed above, the correlation of $\delta^{82/76}\text{Se}$ and C/N ratio (Figure 3) suggests that mineralization leads to the transport of isotopically lighter Se in highly decomposed organic matter in deeper soil horizons.

In summary, our Se concentration and $\delta^{82/76}\text{Se}$ data suggest that:

- (i) Reaction sequences of reduction of water-soluble Se(VI) (derived from seasonal irrigation) and adsorption of Se efficiently immobilize Se in topsoils.
- (ii) Reduction, assimilation into organic matter, adsorption, and complexation lead to gradual immobilization of Se in the soil. Oxidative leaching of Se and mineralization of organic matter increase Se mobility.
- (iii) A correlation between C/N and Se isotopes in organic matter ($\delta^{82/76}\text{Se}_{\text{pool3}}$) indicates increased reworking of organics (mineralization) that further changes isotope ratios with depth.

Agricultural Controls on Se Distribution. The spatial distribution and $\delta^{82/76}\text{Se}$ values are highly heterogeneous over small distances across the study site. This corroborates the two studies, which have reported large variations in soil Se concentration across the same region.^{16,17} Our results clearly show spatial variation in $\delta^{82/76}\text{Se}$ of both bulk Se and individual soil Se (ANOVA $p < 0.001$).

Effect of Irrigation Water. Seasonal irrigation is apparently a major input of Se to the soil. Selenium in the groundwater occurs exclusively as Se(VI), and its concentrations are 1.8 to 13.6 times higher than the WHO recommended limits for drinking water³ with a median value of $294 \mu\text{g L}^{-1}$ (Table S5, Supporting Information). Due to the high intensity of irrigation during rice cropping, the addition of Se to the soil is seven times higher than for other crops.^{17,21} $\delta^{82/76}\text{Se}$ values of the irrigation water vary between -0.01‰ and -5.06‰ tending toward lighter Se compositions with decreasing Se concentration. As shown for other isotope systems (i.e., Cr, U),^{50,51} aquifers have heterogeneous isotopic distribution caused by differing reaction rates, source, and recharge. Since Se isotopic variability is also reflected in the Se isotopic composition of the bulk soil, the most likely scenario is Se groundwater recharge due to leaching of Se from the soil. In fact, the input of Se by irrigation also influences $\delta^{82/76}\text{Se}$ of the water-soluble Se due to isotopic mixing. A rough correlation exists (to within an order of magnitude) between the concentration of water-soluble soil Se and the amount of Se(VI) in the well waters (Table S3, Supporting Information). This observation supports previous assertions that the accumulation of Se is presumably due to irrigation at this site.^{16,20}

Effect of Cropping System. Selenium can be removed from the system by the harvesting of the rice–wheat and sugar cane.^{20,21} Measured Se concentrations in the crops in this study (Table S2, Supporting Information) vary between 43 and 320 mg kg^{-1} Se, which is in agreement with the published data by Sharma et al.¹⁷ Previous phytoremediation field experiments over 2–3 years of wheat–rice rotations reported an effectiveness of about 3–6% Se removal from the topsoil (upper 15 cm) by crop uptake per year.²⁰

Our Se isotope data for the crops are puzzling because, instead of observing slightly lower $\delta^{82/76}\text{Se}$ values compared to water-soluble Se, we determined a significant enrichment of heavier Se isotopes in the wheat plants ($\delta^{82/76}\text{Se}_{\text{crops}} = +2.54\text{‰}$ and $+4.14\text{‰}$) (Figure 2). The enrichment of heavier Se isotopes relative to water-soluble Se ($\Delta^{82}\text{Se}_{\text{water-soluble-plant}} = 2.5\text{‰}$ and 3.2‰) is a considerably larger isotopic discrimination than we expect for Se uptake and assimilation. Despite limited data on Se isotope fractionation associated with assimilation by higher plants, the Se isotopic composition of plants from a wetland study showed little variation in isotope ratios ($<1\text{‰}$) compared to the soil solution.¹⁴

On the other hand, plants are able to transform Se into volatile Se compounds that then exit the seleniferous system into the atmosphere.^{52,53} A previous study reported enrichment factors for biovolatilization of Se(VI) (-2.7‰ to -3‰),²⁸ which supports loss of isotopically lighter volatilized Se. However, the difference we observe between crops and water-soluble Se (pool 1) ($\Delta^{82/76}\text{Se}_{\text{pool1-crops}} = -2.5\text{‰}$ to -3.2‰) would require a large proportion of Se being volatilized driving the plant matter to the positive $\delta^{82/76}\text{Se}$ values of $+2.54\text{‰}$ and $+4.14\text{‰}$. A contribution of 64% and 89% of Se volatilization would be necessary to shift above-ground biomass toward such heavy Se isotopic compositions,

but as observed for an *in situ* study, the rice–wheat crop rotation generates a maximum of 2.0 g ha⁻¹ year⁻¹ volatile Se,²⁰ which corresponds to <1% of Se in the crop biomass. Although Se isotopic fractionation by volatilization in higher plants has not yet been studied, the amount of Se removed by volatilization is presumably small and thus insufficient to produce the observed heavy Se isotope signature in the crops. Therefore, we assume that alternative processes, such as translocation of Se in the aboveground crop material, may fractionate Se isotopes. But since Se isotope effects in plants are not fully understood, we cannot provide clear evidence for such a Se isotope-fractionating mechanism.

Different agricultural practices may also lead to spatial variations in $\delta^{82/76}\text{Se}$ values that leave its record in the soils. Sampling sites A, B, and D were dominated by wheat harvesting while soil C was taken from a sugar cane plantation. Distinctive profiles for Se speciation (Figure 1a–d) and $\delta^{82/76}\text{Se}$ with depth (Figure 1e–h) may reflect the different crops grown in these soils. It is not currently possible to explain the exact relationships governing Se isotope fractionations without a more detailed understanding of isotope partitioning in and between both soils and plants during growth of the respective crops. It appears that Se(VI) reduction coupled to adsorption of Se(IV) in the root systems of sugar cane may promote a pronounced isotopic shift in adsorbed Se ($\delta^{82/76}\text{Se}_{\text{pool2}} = -11.2\text{‰}$ and -13.06‰) as compared to the soils beneath wheat plants ($\delta^{82/76}\text{Se}_{\text{pool2}} > -4.5\text{‰}$). It has been shown that processes at the root soil interface lead to redox transformation of Se depending on the plant species.⁵⁴ For instance, wheat plants have the ability to obtain Se from adsorbed phase.⁵⁴ Thus, Se isotope ratios may be sensitive to the agricultural practices employed, as well as the type of crops.

Origin and Transport of Contaminant Se. The elevated Se in the aquifers at the studied seleniferous site could be related to weathering of parent bedrocks or caused by input from a more distant source. To assess the importance of an input source, we measured the Se concentration and isotopic composition of sediments from a riverbed that seasonally delivers runoff water from the east of the seleniferous site as well as uncontaminated soil and groundwater (Figure S1, Supporting Information). The uncontaminated groundwater taken 20 km north of the seleniferous site contains only 0.7 $\mu\text{g L}^{-1}$ Se (Figure S1, Supporting Information). Assuming the control groundwater represents the Se background signal (CTW; $\delta^{82/76}\text{Se} = 0.23\text{‰}$), the seleniferous well water is significantly enriched in lighter Se isotopes compared to nearby uncontaminated waters. The $\delta^{82/76}\text{Se}$ values of the control soil ($\delta^{82/76}\text{Se}_{\text{bulk}} = +0.62\text{‰}$)³² and the sediment from the riverbed ($\delta^{82/76}\text{Se}_{\text{bulk}} = -0.6\text{‰}$) are likely to reflect the soil Se isotopic background. In general, lower $\delta^{82/76}\text{Se}$ of the bulk seleniferous soils compared to the soil background likely arises from the addition of lighter Se isotopes by irrigation water and redox processes. Nevertheless, we cannot quantitatively determine the effects of the Se isotope background on the Se isotopic heterogeneity at the seleniferous site.

The concentrations of Se in the sandy sediments from the riverbed are consistently extremely low (average 0.08 mg kg⁻¹). $\delta^{82/76}\text{Se}_{\text{bulk}}$ values (-0.1‰) do not differ significantly with depth within the sediments taken from the riverbed (Table S3, Supporting Information). Although the reservoir water (SH-L) in the Shiwaliks has a negative $\delta^{82/76}\text{Se}$ value of -3.6‰ similar to the seleniferous well water (Sites C and D), the Se concentration of 0.13 $\mu\text{g L}^{-1}$ is very low (Table S3, Supporting

Information). We are thus unable to clearly identify a riverine input that is sufficiently rich in Se or variable in $\delta^{82/76}\text{Se}$ to explain our observations at the seleniferous site.

Environmental Implications. Stable Se isotopes reveal how Se is distributed between irrigation waters, soils, and crops in a seleniferous agricultural site in Punjab, India. Topsoil (0–11 cm) efficiently retains Se, which rapidly drops to low levels below an 11 cm depth. Water-soluble Se(VI) is immobilized by reduction to form Se(IV) that readily adsorbs onto minerals and organic matter. The distinctive negative $\delta^{82/76}\text{Se}$ seen in phosphate extracted Se (believed to represent the adsorbed component) is not seen in the extraction of Se pool 3 that captures Se(0) or Se(-II), suggesting that Se is probably not further reduced beyond Se(IV). The removal of Se by reduction drives the residual water-soluble Se toward more positive $\delta^{82/76}\text{Se}$ values relative to adsorbed Se.

Without an active remediation strategy (i.e., phytoremediation, redox manipulation), Se will persist in the topsoils because of continuous input of Se from irrigation water and its immobilization into adsorbed and organic fractions in response to redox reactions. Recycling through oxidation and mineralization is difficult to constrain without further knowledge of isotope effects during these processes, but all isotope differences measured so far can be explained by redox Se cycling. Our work has important implications for the interpretation of lighter Se stable isotope values in soils compared to the potential soil background. The original source of Se at this contaminated site remains elusive but is likely to be an unidentified geological source.

■ ASSOCIATED CONTENT

§ Supporting Information

Details about analytical methods (sample preparation); tables reporting sample location and declaration (Table S1), the soil characteristics, concentration, and $\delta^{82/76}\text{Se}$ of bulk Se and individual Se pools (Table S2), isotope mass balance calculation (Table S3), and physiochemical properties and Se of the groundwater samples (Table S4). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b00477.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Girling, C. A. Selenium in agriculture and the environment. *Agric., Ecosyst. Environ.* **1984**, *11*, 37–65.
- (2) Rayman, M. P. Food-chain selenium and human health: emphasis on intake. *Br. J. Nutr.* **2008**, *100*, 254–268.
- (3) World Health Organization (WHO). FAO. IAEA. *Human Vitamin and Mineral Requirements*; FAO: Rome, 2002; pp 1–303.
- (4) Spallholz, J. E. On the nature of selenium toxicity and carcinostatic activity. *Free Radical Biol. Med.* **1994**, *17*, 45–64.
- (5) Winkel, L. H. E.; Johnson, A. C.; Lenz, M.; Grundl, T.; Leupin, O. X.; Amini, M.; Charlet, L. Environmental selenium research: From

microscopic processes to global understanding. *Environ. Sci. Technol.* **2012**, *46*, 571–579.

(6) Zawislanski; Zavarin. Nature and rates of selenium transformations: A laboratory study of Kesterson Reservoir soils. *Soil Sci. Soc. Am. J.* **1996**, *60*, 791–800.

(7) Fordyce, F. Selenium deficiency and toxicity in the environment. In *British Geological Survey*; Elsevier, London, 2005; Chapter 15, pp 373–415.

(8) Seiler, R. L., et al. *Irrigation-induced contamination of water, sediment, and biota in the Western United States. Synthesis of Data from the National Irrigation Water Quality Program*; USGS, Professional paper 1655; USGS: Denver, CO, 2003.

(9) Zhu, J. M.; Wang, N.; Li, S.; Li, L.; Su, H.; Liu, C. X. Distribution and transport of selenium in Yutangba, China: Impact of human activities. *Sci. Total Environ.* **2008**, *392*, 252–261.

(10) Rogers, P.; Arora, S. P.; Fleming, G. A.; Crinion, R.; McLaughlin, J. G. Selenium Toxicity in Farm-Animals - Treatment and Prevention. *Irish Vet. J.* **1990**, *43*, 151–153.

(11) (a) Presser, T. S.; Ohlendorf, H. M. Biogeochemical cycling of selenium in the San Joaquin Valley, California, USA. *Environ. Manage.* **1987**, *11*, 805–821. (b) Yang, G. Q.; Wang, S. Z.; Zhou, R. H.; Sun, S. Z. Endemic selenium intoxication of humans in China. *Am. J. Clin. Nutr.* **1983**, *37*, 872–881.

(12) Kunli, L.; Lirong, X.; Jian'an, T.; Douhu, W.; Lianhua, X. Selenium source in the selenosis area of the Daba region, South Qinling Mountain, China. *Environ. Geol.* **2004**, *45*, 426–432.

(13) Wu, L. Review of 15 years of research on ecotoxicology and remediation of land contaminated by agricultural drainage sediment rich in selenium. *Ecotoxicol. Environ. Saf.* **2004**, *57*, 257–269.

(14) Herbel, M. J.; Johnson, T. M.; Tanji, K. K.; Gao, S.; Bullen, T. D. Selenium stable isotope ratios in California agricultural drainage water management systems. *J. Environ. Qual.* **2002**, *31*, 1146–1156.

(15) Bailey, R. T.; Hunter, W. J.; Gates, T. K. The influence of nitrate on selenium of irrigated agricultural groundwater systems. *Soil Sci. Soc. Am. J.* **2012**, *41*, 783–792.

(16) Dhillon, S. K.; Dhillon, K. S.; Kohli, A.; Kera, K. L. Evaluation of leaching and runoff losses of selenium from seleniferous soils through simulated rainfall. *J. Plant Nutr. Soil Sci.* **2008**, *171*, 187–192.

(17) Sharma, N.; Prakash, R.; Srivastava, A.; Sadana, U. S.; Acharya, R.; Prakash, N. T.; Reddy, A. V. R. Profile of selenium in soil and crops in seleniferous area of Punjab, India by neutron activation analysis. *J. Radioanal. Nucl. Chem.* **2009**, *281*, 59–62.

(18) Hira, C. K.; Patal, K.; Dhillon, K. S. Dietary selenium intake by men and women in high and low selenium areas of Punjab. *Public Health Nutr.* **2004**, *7*, 39–43.

(19) Ohta, Y.; Nakano, A.; Matsumoto, M.; Hoshi, M. Comparison of selenium content in human hair from different individuals in different countries by ^{76}Se (n,γ) $^{77\text{m}}\text{Se}$ reaction. *J. Radioanal. Nucl. Chem.* **1987**, *114*, 75–82.

(20) Dhillon, S. K.; Dhillon, K. S. Phytoremediation of selenium-contaminated soils: the efficiency of different cropping systems. *Soil Use Manage.* **2009**, *25*, 441–453.

(21) Dhillon, K. S.; Dhillon, S. K. Quality of underground water and its contribution towards selenium enrichment of the soil–plant system for a seleniferous region of northwest India. *J. Hydrol.* **2003**, *272*, 120–130.

(22) Wiederhold, J. G. Metal stable isotope signatures as tracers in environmental geochemistry. *Environ. Sci. Technol.* **2015**, *49*, 2606–2624.

(23) Johnson, T. M. A review of mass-dependent fractionation of selenium isotopes and implications for other heavy stable isotopes. *Chem. Geol.* **2004**, *204*, 201–214.

(24) Clark, S. K.; Johnson, T. M. Selenium stable isotope investigation into selenium biogeochemical cycling in a lacustrine environment: Sweitzer Lake, Colorado. *J. Environ. Qual.* **2010**, *39*, 2200–2210.

(25) Ellis, A. S.; Johnson, T. M.; Herbel, M. J.; Bullen, T. D. Stable isotope fractionation of selenium by natural microbial consortia. *Chem. Geol.* **2003**, *195*, 119–129.

(26) Rees, C. E.; Thode, H. G. Selenium isotope effects in the reduction of sodium selenite and of sodium selenate. *Can. J. Chem.* **1966**, *44*, 419–427.

(27) Herbel, M. J.; Johnson, T. M.; Oremland, R. S.; Bullen, T. D. Fractionation of selenium isotopes during bacterial respiratory reduction of selenium oxyanions. *Geochim. Cosmochim. Acta* **2000**, *64*, 3701–3709.

(28) Schilling, K.; Johnson, T. M.; Wilcke, W. Isotope fractionation of selenium during fungal biomethylation by *Alternaria alternata*. *Environ. Sci. Technol.* **2011**, *45*, 2670–2676.

(29) Johnson, T. M.; Bullen, T. D. Selenium isotope fractionation during reduction by Fe(II)-Fe(III) hydroxide-sulfate (green rust). *Geochim. Cosmochim. Acta* **2003**, *67*, 413–419.

(30) Mitchell, K.; Couture, R.-M.; Johnson, T. M.; Mason, P. R. D.; Van Cappellen, P. Selenium sorption and isotope fractionation: Iron(III) oxides versus iron(II) sulfides. *Chem. Geol.* **2013**, *342*, 21–28.

(31) Charlet, L.; Scheinost, A. C.; Tournassat, C.; Greneche, J. M.; Géhin, A.; Fernández-Martínez, A.; Coudert, S.; Tisserand, D.; Brendle, J. Electron transfer at the mineral/water interface: Selenium reduction by ferrous iron sorbed on clay. *Geochim. Cosmochim. Acta* **2007**, *71*, 5731–5749.

(32) Chao, T. T.; Sanzolone, R. F. Fractionation of soil selenium by sequential partial dissolution. *Soil Sci. Soc. Am. J.* **1989**, *53*, 385–392.

(33) Schilling, K.; Johnson, T. M.; Mason, P. R. D. A sequential extraction technique for mass-balanced stable selenium isotope analysis of soil samples. *Chem. Geol.* **2014**, *381*, 125–130.

(34) Kulp, T. R.; Pratt, L. M. Speciation and weathering of selenium in Upper Cretaceous calc and shale from South Dakota and Wyoming, USA. *Geochim. Cosmochim. Acta* **2004**, *68*, 3687–3701.

(35) Shand, C. A.; Balsam, M.; Hillier, S. J.; Hudson, G.; Newman, G.; Arthur, J. R.; Nicol, F. Aqua regia extractable selenium contents of some Scottish topsoils measured by ICP-MS and the relationship with mineral and organic soil components. *J. Sci. Food Agric.* **2010**, *90*, 972–980.

(36) Fry, B. *A Supply/Demand Model for Open System Fractionation; Stable Isotope Ecology*; Springer: New York, USA, 2007.

(37) Kendall, C.; Caldwell, E. A. *Fundamentals of isotope geochemistry; Isotope Tracers in Catchment*; Elsevier: Amsterdam, The Netherlands, 1998; p 63.

(38) Prakash, N. T.; Sharma, N.; Prakash, R.; Acharya, R. Removal of selenium from Se enriched natural soils by a consortium of *Bacillus* isolates. *Bull. Environ. Contam. Toxicol.* **2010**, *85*, 214–218.

(39) Bajaj, M.; Schmidt, S.; Winter, J. Formation of Se(0) nanoparticles by *Duganella* sp. and *Agrobacterium* sp. isolated from Se-laden soil of North-East Punjab, India. *Microb. Cell Fact.* **2012**, *11*, 64–78.

(40) Gao, S.; Tanji, K. K.; Peters, D. W.; Herbel, M. J. Water selenium speciation and sediment fractionation in a California flow-through wetland system. *J. Environ. Qual.* **2000**, *29*, 1275–1283.

(41) Myneni, S. C. B.; Tokunaga, T. K.; Brown, G. E., Jr. Abiotic selenium redox transformation in the presence of Fe(II,III) oxides. *Science* **1997**, *278*, 1106–1109.

(42) Favre, F.; Tessier, D.; Abdelmoula, M.; Genin, J. M.; Gates, W. P.; Boivin, P. Iron reduction and changes in cation change capacity in intermittently waterlogged soil. *Eur. J. Soil Sci.* **2002**, *53*, 175–183.

(43) Singh, M.; Singh, N.; Relan, P. S. Adsorption and desorption of selenite and selenate selenium on different soils. *Soil Sci.* **1981**, *132*, 134–141.

(44) Neal, R. H.; Sposito, G. Selenate adsorption on alluvial soils. *Soil Sci. Soc. Am. J.* **1989**, *53*, 70–74.

(45) Coppin, F.; Chabroulet, C.; Martin-Garin, A. Selenite interactions with some particulate organic and mineral fractions isolated from a natural grassland soil. *Eur. J. Soil Sci.* **2009**, *60*, 369–376.

(46) Jenkinson, D. S.; Poulton, P. R.; Bryant, C. The turnover of organic carbon in subsoils. Part 1. Natural and bomb radiocarbon in soil profiles from the Rothamsted long-term field experiments. *Eur. J. Soil Sci.* **2008**, *59*, 391–399.

- (47) Gustafsson, J. P.; Johnson, L. Selenium retention in the organic matter of Swedish forest soils. *J. Soil Sci.* **1992**, *43*, 461–472.
- (48) Dowdle, P. R.; Oremland, R. S. Microbial oxidation of elemental selenium in soil slurries and bacterial cultures. *Environ. Sci. Technol.* **1998**, *32*, 3749–3755.
- (49) Losi, M. E.; Frankenberger, W. T., Jr. Microbial oxidation and solubilization of precipitated elemental selenium in soil. *J. Environ. Qual.* **1998**, *27*, 836–843.
- (50) Raddatz, A. L.; Johnson, T. M.; McLing, T. L. Cr stable isotopes in Snake River Plain aquifer groundwater: Evidence for natural reduction of dissolved Cr(VI). *Environ. Sci. Technol.* **2011**, *45*, 502–507.
- (51) Basu, A.; Brown, S. T.; Christensen, J. N.; DePaolo, D. J.; Reimus, P. W.; Heikoop, J. M.; Woldegabriel, G.; Simmons, A. M.; House, B. M.; Hartmann, M.; Maher, K. Isotopic and geochemical tracers for U(VI) reduction and U mobility at an in situ recovery mine. *Environ. Sci. Technol.* **2015**, *49*, 5939–5947.
- (52) Zayed, A. M.; Terry, N. Selenium volatilization in roots and shoots: Effect of shoot removal and sulfate level. *J. Plant Physiol.* **1994**, *143*, 8–14.
- (53) de Souza, M. P.; Mel Lytle, C.; Mulholland, M. M.; Otte, M. L.; Terry, N. Selenium assimilation and volatilization from dimethylselenoniopropionate by Indian mustard. *Plant Physiol.* **2000**, *122*, 1281–1288.
- (54) Blaylock, M. J.; James, B. R. Redox transformations and plant uptake of selenium resulting from soil-root interactions. *Plant Soil* **1994**, *158*, 1–12.