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A sequential extraction technique for mass-balanced stable selenium isotope analysis of soil samples

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ABSTRACT

The sequential extraction (SEP) of selenium to determine the isotopic composition of different Se pools in soils is an important tool for tracking biogeochemical cycling of Se in soils. An appropriate Se extraction method for the stable Se isotope analysis was established and tested for different Se pools in soils in terms of reproducibility and recovery. Soil samples ($n = 4$) from a seleniferous field site in Punjab, India and two standard reference materials were chosen because of their relatively high total Se concentration of 2 to 4.5 mg kg^{-1}. Additionally, a soil sample with low Se content was utilized to verify the procedure and reproducibility. The isotopic mass-balance calculation gives consistent δ^{82}Se values for the sum of the extracted pools, relative to δ^{82}Se of the bulk soils. For instance, in one studied soil (soil-1), the sum of δ^{82}Se of all individual Se-pools ($\delta^{82}\text{Se}_{\text{mass-balance}} = 3.35‰$) is in good agreement with the analysis of δ^{82}Se of the bulk soil ($\delta^{82}\text{Se}_{\text{bulk}}$ of 3.56‰). We observed up to a 2‰ range in δ^{82}Se among the different Se soil-pools (soil-3). Our Se isotopic results of Se soil pools provide more detailed observations of the Se linked processes in soils compared to bulk δ^{82}Se. Thus, quantitative Se isotopic analysis of different Se pools can be an important tool for predicting the environmental cycling of selenium in soils or sediments.

1. Introduction

Selenium is an essential trace element for animal metabolism and has received much attention in connection to human nutrition (Levander, 1987). However, selenium becomes toxic to humans and animals at higher amounts, and as a micronutrient Se has a very narrow range between deficiency and excess. The World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO) and International Atomic Energy Agency (IAEA) recommend a daily intake of selenium for healthy adults of 25–35 μg Se day^{-1} (WHO–FAO–IAEA, 1996). Toxic Se exposure (through ingestion of food and water) results for an intake of more than 400 μg Se day^{-1}.

Selenium enters into the food chain primarily via plant uptake from Se-containing soils. In general, the concentration of selenium in soils is variable and ranges from 0.1 to 2 mg kg^{-1} with a worldwide mean concentration of 0.4 mg kg^{-1} (Berrow and Ure, 1989). Local variations of Se concentration in soils are mainly controlled by the geological background and soil type. Igneous rocks, sandstones, and limestones are typically low in Se (<0.1 mg kg^{-1}) while shales are usually Se-rich (>0.6 mg kg^{-1}).

In natural settings, Se occurs in four oxidation states (Se(VI), Se(IV), Se(0), and Se(−II)) and its chemical behavior and mobility are strongly linked to the prevalent redox conditions. Factors such as pH, soil water content and soil texture, also control the distribution and availability of Se (Gissel-Nielsen et al., 1984; Jayaweera and Biggar, 1996). In oxidized environments, such as groundwater and aerated soils, hexavalent selenium, abbreviated Se(VI), occurs as selenate (SeO_4^{2-}). Under mildly reducing (suboxic) conditions, tetravalent Se, abbreviated Se(IV), occurs as selenite (SeO_3^{2-}), and hydroselenite (HSeO_3^{-}) These Se-oxyanions are highly soluble and hence mobile and bioavailable. Under reducing conditions, elemental selenium (Se^0) and selenide (HSe^- and Se(−II)) are the main species. Se^0 is insoluble and generally resistant to oxidation, whereas selenide binds to organic matter and readily combines with metals to form precipitates.

Soils are compositionally heterogeneous natural systems, in which selenium is present in various Se species in a diverse matrix (Fernandez-Martinez and Charlet, 2009). Sequential extraction is an established technique to identify and quantify different selenium pools (i.e., Chao and Sanzolone, 1989; Martens and Suarez, 1997; Bujdoš et al., 2000; Kulp and Pratt, 2004; Hagarova et al., 2005), but has been developed for many different purposes and with no consistent methodology. The procedures for quantifying selenium pools vary from a single-step (Hagarova et al., 2005) or three-step method (Bujdoš et al., 2000), up to a seven-step extraction (Piatak et al., 2006). The wide variety of

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reagents used for the extraction of different Se compounds makes a comparison between techniques difficult. However, the soluble and ligand-exchangeable Se is extracted using a phosphate buffer in all published methods because phosphate competes with Se(IV) for the same binding sites in soils (Barrow and Whelan, 1989). Various extractants are used to access Se associated with metal oxides and organic matter, including NaOH (Hagarova et al., 2005; Nakamaru et al., 2005; Keskinen et al., 2009), concentrated HCl (Chao and Sanzolone, 1989) and $K_2O_8S_2$ (Martens and Suarez, 1997). To date, no validated procedure has been developed for stable isotope ratio measurements of Se in different soil fractions.

Stable Se isotope ratios are useful indicators of processes that control Se distribution and speciation. Selenium has six naturally occurring stable isotopes with the masses ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se and ^{82}Se. Various biotic and abiotic environmental processes involving redox transformations of Se fractionate stable Se isotopes (Johnson and Bullen, 2004). During partial reduction of Se(IV) or Se(VI), the reaction products are enriched in lighter isotopes whereas higher mass isotopes become enriched in the reactant pool as reduction proceeds. In a closed system, the degree of isotope fractionation can be used to quantify the extent of reduction. Johnson and Bullen, 2003 showed that abiotic reduction of Se(VI) to Se(IV) by green rust created an Se isotope fractionation of approximately 11‰. More recently, the Se isotopic fractionation of up 10‰ was indicated for chemical Se(IV) reduction by iron sulfides (Mitchell et al., 2013). Besides abiotic Se reduction, experimental studies have determined isotopic fractionation for microbial reduction of selenoxyanions to Se(0), Se(−II) or methylselenides (Herbel et al., 2000; Schilling et al., 2011a). The $\varepsilon^{82/76}$Se values ($\approx \delta^{82/76}\text{Se}_{\text{product flux}} - \delta^{82/76}\text{Se}_{\text{reactant}}$; notation defined below) range from −0.3 to −7.5‰ as well as −1.7 to −12‰ for the reduction of Se(IV) to Se(0) and −3‰ to −6‰ for methylation. These laboratory studies provide a framework to interpret Se isotope variability and hence Se cycling in the modern environment and are useful for interpreting biogeochemical processes in the geological record (Mitchell et al., 2012).

Selenium in meteorites and igneous rocks (i.e., basalt and peridotite) exhibits δ^{82}Se values in a narrow range close to 0‰ (Rouxel et al., 2002) reflecting the lack of fractionation due to the absence of redox reactions. In contrast, hydrothermal sulfides, some terrestrial and marine sediments as well as soils show significant local variations in δ^{82}Se of up to 25‰ (Rouxel et al., 2002; Schilling et al., 2011b; Wen and Carignan, 2011; Mitchell et al., 2012; Zhu et al., 2014). The most extreme variability in δ^{82}Se has been observed in Se-rich supergene alteration zones in China (Wen et al., 2007; Zhu et al., 2014). To date, studies that have used Se stable isotopes to track the biogeochemical Se cycle in modern Se-rich environments have found much more limited isotopic variability (Herbel et al., 2002; Clark and Johnson, 2010).

Suitable sequential extraction methods in combination with stable isotope measurements have been successfully established with a few other non-traditional isotope systems such as calcium (Hindshaw et al., 2011) iron (Emmanuel et al., 2005; Wiederhold et al., 2007) and lead (Bäckström et al., 2004). Previous studies of Se (Herbel et al., 2002; Clark and Johnson, 2010; Schilling et al., 2011b) have not yet successfully measured the isotopic composition of individual Se pools with an accurate mass balance for soils.

The main objective of our study was to establish a reliable method to characterize δ^{82}Se in different Se-pools in soils. We show the reproducibility of our technique for both Se concentrations and δ^{82}Se measurements by comparing the sum of individual Se-pools with standard bulk measurements of the same soils. We argue that this approach will enable the use of stable Se isotopes to address more detailed problems related to the biogeochemical cycling of Se (i.e. (de)sorption, assimilation, reduction) in soils than is possible with bulk measurements alone. We hypothesize that Se isotopes of operationally defined Se pools in soils can be applied for risk assessment of Se contaminated environments (e.g., areas in China, India and Ireland).

2. Methods

2.1. Site description

The study area is located in the north-west of India in the state of Punjab (31°8′ N, 76°10′ E) (Fig. 1). The contaminated site is part of the Kandi region, Punjab, which is a sub-mountainous zone that stretches in a narrow belt along the northeast and comprises the Shivalik Range. Seasonal streams cause flash floods during the monsoon season. Thereby weathered rock fragments from the Shivalik are transported and deposited in low-lying areas, such as the seleniferous field site considered here. As reported by Dhillon and Dhillon (2014), Se concentrations of rocks from the upper Shivalik Formation (e.g., sandstones, shales) vary between 11 and 287 μg Se kg^{-1}. The Se concentrations in our studied seleniferous soils varied between 2 and 4.5 mg kg^{-1} with a lower value of 0.5 mg kg^{-1} for the low Se control soil.

2.2. Total digestion

All samples were air-dried, sieved and ground with an agate mortar. Each soil sample (1.0 g) was weighed into 30 mL PTFE vessels and 12 mL of aqua regia was added. The vessels were closed tightly and the samples were digested at 100 °C overnight followed by drying down to obtain a residue of approximately 500 μL to which 3 mL of 0.1 M HCl were added. Two standard reference materials, Montana soil (NIST SRM-2711a) from the U.S. National Institute of Standards and Technology (Gaithersburg, MD, USA) and the green river shale (SGR-1) from U.S. Geological Survey (USGS, Reston, VA, USA), were used to assess the accuracy of the bulk measurements. All samples and reference materials were prepared in duplicate.

2.3. Sequential extraction (SEP)

Different Se soil pools were sequentially extracted using a modified version of the method established by Chao and Sanzolone (1989). Our revised method does not include an extraction step using 4 M HCl after several tests showing that this Se pool (Se in carbonates, acid-volatile sulfides) is negligible in our soil samples. Soil samples (2.0 g) were placed into 50 mL centrifuge tubes. For the first extraction step (step 1 = bioavailable Se), 25 mL of 0.5 M KCl were added and shaken on a horizontal shaker at room temperature for 1 h. Afterward the samples were centrifuged at 5000 rpm (Eppendorf Centrifuge 5810R) for 30 min and the supernatant was decanted. The sample aliquots were laced with 25 mL of 0.1 M KH_2PO_4-buffer (Trace Select®, Sigma Aldrich) to extract Se(IV) adsorbed onto oxide minerals (step 2 = adsorbed Se pool). For the third step (step 3 = organically-bound Se), 0.5 g of potassium chlorate (ACS reagent ≥ 99%, Sigma Aldrich) and of 10 mL of concentrated HCl (Merck Suprapur) were added to the soil residues for 30 min with occasional shaking to recover sulfide complexes as well as organic matter from the soils. After the reaction 10 mL of DI water was added and the solution was centrifuged to separate the supernatant. The supernatants from all extraction steps were filtered through 0.45 μm membrane filters (mdi-Membrane Technologies). The SEP for every soil sample was performed in duplicate. Se blank tests were prepared to estimate the procedural Se content for total soil digestion and SEP.

2.4. Sample preparation for isotope measurements

Prior to purification steps (HG-purification and anion exchange chromatography), the samples were spiked with enriched stable isotopes of ^{74}Se and ^{77}Se with an adjusted ^{74}Se/^{77}Se ratio of about 1:1. For the total digestion (bulk soil), samples of Se pool-1 (bioavailable Se) and Se-pool-4 (residual Se) the purification was carried out in 2-steps by hydride generation and anion exchange as described by Clark and Johnson (2008) and Schilling et al. (2011b). Use of the