Hazardous concentrations of selenium in soil and groundwater in North-West India

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ABSTRACT

Soil and groundwater samples were collected for bulk elemental analyses in particular for selenium (Se) concentrations from six agricultural sites located in states of Punjab and Haryana in North-West India. Toxic concentrations of Se ($45–341\,\mu g\,L^{-1}$) were present in groundwater (76 m deep) of Jainpur and Barwa villages in Punjab. Selenium enrichments were also found in top soil layers (0–15 cm) of Jainpur ($2.3–11.6\,mg\,kg^{-1}$) and Barwa ($3.1\,mg\,kg^{-1}$). Mineralogical analyses confirmed silicates and phyllosilicates as main components of these soils, also reflected by the high content of SiO_2 (40–62 wt.%), Al_2O_3 (9–21 wt.%) and K_2O (2.2–3.2 wt.%). Prevailing intensive irrigation practices in Punjab with Se enriched groundwater may be the cause of Se accumulation in soils. Sequential extraction revealed >50% Se bioavailability in Jainpur soils. Appearance of selenite was observed in some of the batch assays with soil slurries under reducing conditions. Although safe Se concentrations were found in Hisar, Haryana, yet high levels of As, Mo and U present in groundwater indicated its unsuitability for drinking purposes. Detailed biogeochemical studies of Se in sediments or groundwater of Punjab are not available so far; intensive investigations should be started for better understanding of the problem of Se toxicity.

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1. Introduction

Selenium (Se) is required as micronutrient for several physiological and biochemical processes mainly as a component of selenoproteins or of amino-acids such as selenomethionine and selenocysteine. The recommended daily dietary allowance for selenium is $55\,\mu g\,d^{-1}$ for persons above 14 years and the tolerable upper intake level (TUL) is $400\,\mu g\,d^{-1}$. Exceeding TUL can lead to chronic or acute selenosis [1,2]. Selenium enters the food chain mainly through the soil–plant-system. Although it is still unclear if Se is essential for plant growth the high Se level in some food and forage plants are toxic for animals and humans [3–5].

In natural environments, four inorganic Se species are known. These are selenate (SeO_4^{2-}), selenite (SeO_3^{2-}), elemental Se (Se^0) and selenide (Se^{2-}). The Se oxy-anions are among the trace elements common in soils. The oxy-anions are soluble in water and especially the SeO_4^{2-} is leaching into the aqueous phase [6]. Generally, Se (IV) (selenite) has a much higher affinity to adsorb onto soil particles compared to Se (VI) (selenate) [7]. As a consequence,

Se (VI) is more mobile and easily available to plants especially under oxidizing, neutral to alkaline conditions, where it is the dominant species [6]. Se^0 mainly occurs under reducing conditions, is insoluble and exhibits no or very little toxicity. Se^{2-} is a highly toxic gas; however it is rapidly oxidized to non-toxic elemental Se in the presence of air [8,9]. Considerable enrichments of Se can be found in carbon shales ($\geq 600\,mg\,kg^{-1}$, phosphatic rocks ($\leq 300\,mg\,kg^{-1}$) or coal ($\leq 6500\,mg\,kg^{-1}$) [4,9–11]. Selenium concentrations in soils are generally low with $0.01–2\,mg\,kg^{-1}$ (average: $0.4\,mg\,kg^{-1}$) but soils with highly elevated Se concentrations ($>2–5000\,mg\,kg^{-1}$), called seleniferous soils, are widely distributed throughout the world (USA: up to $28\,mg\,kg^{-1}$; Ireland: up to $1200\,mg\,kg^{-1}$, China: up to $59\,mg\,kg^{-1}$ [3,6,9,11]. The Se concentration in soils is mainly determined by the Se content of the parent rock, the topography and the climate, but introduction of seleniferous erosion material, poor drainage of soils, irrigation with Se containing water, usage of phosphate fertilizer, influence of mining operations or volcanic eruptions and combustion of coal and petroleum can considerably enhance the soil Se content [6,9,12,13]. The mobility and bioavailability of Se depends on processes such as adsorption, precipitation and transformation which are heavily influenced by the pH-value, redox conditions, amount of competing ions, Se-speciation, soil mineralogy, organic matter content, microbiology, plant species, etc. [3,6,9,14]. Therefore, knowledge of total Se concentrations in soil is not sufficient in order to predict possible health threats

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related to food and forage grown on seleniferous soils. An easy and frequently used way to assess bioavailability of Se in soils is to determine the water-soluble content e.g., by several sequential extractions schemes developed and adopted over the years [10,15,16]. Several regions throughout the world are known to suffer from Se intoxication due to consumption of food and forage grown on seleniferous soils, e.g., in Australia, China, India and the USA [10,17,18]. In India both, Se deficient (0.025–0.71 mg kg^{-1}) and seleniferous soils (1–20 mg kg^{-1}) have been recognized [9]. Selenium came into the scientific focus in India in the mid seventies after an animal disease locally called 'Degnala' was found to be associated with high intake of Se in fodder grown on alkali soils of Haryana [19]. But since the last two decades the presence of high concentration of selenium and it's toxicity to farm animals has been mainly reported for the Northeastern parts of Punjab, namely in Nawanshahr (renamed as Shahid Bhagat Singh (SBS) nagar) and Hoshiarpur districts [18,20–23]. Acceptable limits for Se in forage plants by the EPA of USA are 2–5 mg kg^{-1}. An acute long-term toxic effect of Se for animals is likely to occur when the Se concentration is above 5 mg kg^{-1} in forage. The presence of up to 69.5 μg L^{-1} Se in groundwater, 6.5 mg kg^{-1} Se in soil and 3–670 mg kg^{-1} Se in plants in locations at or near to present study area had been reported previously [18,23–25]. A study conducted two decades ago, reported that Se toxic sites ranging from 4 to 6 ha were spread over an area occupying >1000 ha in North-Eastern Punjab [18,24].

In the present study, the elemental composition of soil and groundwater as well as the speciation of Se in the topsoil was determined in samples collected from North-Eastern Punjab and Haryana. Apart from some reports [18,24], data on ground water quality of the selected study area is very scarce, although the region is well known for high levels of Se in soils and crops. The aim of the present study was to assess the influence of long-term irrigation with Se-burdened groundwater on the total concentration and speciation of Se in the top soil profile. Another aim was to estimate the potential microbial reduction of Se (VI) in the soils, which is thought to occur primarily via bacterial dissimilatory reduction [26–28] and alters the speciation/mobility of Se. Understanding the influence of long-term irrigation as well as speciation control mechanisms on the Se cycling will help to assess the fate of additional Se from irrigation water within the soil profile and to predict a possible health threat in the area.

2. Study area

The study area is located in the North-West of India. Out of six sampling sites, four sites were situated in the state of Punjab, one in the state of Haryana and one in Chandigarh, the capital of both states. In Punjab, samples were taken in Jainpur and Barwa villages in the SBS Nagar district (formerly known as Nawanshahr, 33.12°N, 76.13°E) and in the village of Simbli which is part of the Hoshiarpur district (31.12°N, 76.13°E) and situated approximately 3 km away from the other two villages. Both districts are situated in the North-Eastern part of Punjab. The map of study area in Punjab can be found in a report by Dhillon and Dhillon [18]. Total rainfall in that area varies from 631 to 1325 mm a^{-1} and about 80% of the yearly rainfall of approximately 1000 mm occurs in the rainy season, mainly from July to September [24,29]. The soils of the area are mostly loamy and alkaline in reaction and are considered to be formed from the alluvium deposited by Indus rivers system [18].

All sampling sites were fertile fields growing two seasonal crops (rice or maize and wheat) per year. There are some seasonal rivulet formations but farmers at the sampling sites depend mainly upon groundwater for irrigation purposes.

The sampling sites in Punjab were selected based on the known distribution of seleniferous soils and on related health problems

Fig. 1. A strong white patch (outlined as rectangle) in between a wheat field at Jainpur village (site 1).

that have been reported in previous studies [18,20,30] or on current complaints about selenosis from farmers, especially in Jainpur village. Feeding of forage grown on these seleniferous soils, which often show white chlorosis (Fig. 1), has evoked characteristic signs of selenosis in livestock, such as cracks in the hoofs, peeling-off of horns and loss of hair, premature abortions and even occasional death ([18,20]; personal communication with farmers). Meanwhile the local population has also been affected after regular consumption of cereals grown in the area, showing visible symptoms like cracked and discoloured nails (Fig. 2). This happened, although state authorities recommended to food grain procurement agencies in 2003/2004, to collect the grains from seleniferous soil separately and mix them with grains that were harvested from non-seleniferous fields to bring Se level within health safe limits before marketing.

Apart from three seleniferous sites (Jainpur 1, Barwa, Simbli), one field thought to be free of contamination (based on non visible symptoms of Se toxicity) by local farmers (Jainpur 2) was chosen in Punjab. However, this non-seleniferous site has been irrigated with Se burdened groundwater for some time. One sample was taken in the district of Hisar (29.5°N; 75.45°E), Haryana, from grounds of Guru Jambeshwar University of Science and Technology, Hisar. An earlier study by Sharma [19] has also reported increased Se concentrations in soils from Hisar (up to 6 mg kg^{-1}). The area is semi-arid and is located just 30 km North-East of the Thar Desert. The annual average rainfall is only 450 mm and agriculture is supported mainly by canals and irrigation with groundwater. A further sample was collected from the outskirts of Chandigarh (30.75°N; 76.78°E), which is a union territory of India as well as the capital city of both Punjab and Haryana. The average rainfall there is 1101 mm [31]. Chandigarh is located near the foothills of the Shivalik mountain range of the Himalaya but no seleniferous soils or Se toxicity symptoms have been reported from this area. Therefore, it was considered as control site.

3. Material and methods

3.1. Sampling

Soil samples were taken in January 2010 from different depths by manual drilling with a soil auger and stored in plastic bags at 4 °C till further analysis to minimise the effect of bio-geochemical activities and of evaporation on initial elemental composition. Groundwater was sampled from existing tube wells which were

Fig. 2. A local from Jainpur village showing deformed hand and foot nails due to selenosis.

regularly used by farmers for irrigation. In order to avoid stagnant water from the wells, water was pumped for 10 min before sampling. All water samples were acidified with conc. HNO_3 and stored in plastic bottles at 4 °C till further analysis. An acidified sample of Millipore water was taken as laboratory control. No water sample from Simbli village could be collected due to failure of electricity at the time of sampling. Further details of soil and water sampling are listed in Table 1.

3.2. Soil and water analyses

Dissolved concentrations of Fe, Mn, Se and other trace elements of water samples from five wells (Table 1) and one blank sample (Mili-Q) were analysed using high-resolution ICP-MS (Element 2, Thermo Fisher, Bremen, Germany). The accuracy ($\leq 12\%$) was tested by including the certified standard HPS CRM-TMDW (High Purity Standards, USA) into the measurements. Concentrations of dissolved F^-, Cl^- and SO_4^{2-} in ground water from Hisar were measured by ion chromatography (Dionex ICS-1000, IonPac AS4A-SC) using a $Na_2CO_3/NaHCO_3$-Eluent with a flow rate of $2\,mL\,min^{-1}$.

The bulk elemental composition of soil samples was determined with energy dispersive X-ray fluorescence analysis (Epsilon 5, PANalytical) using bulk powder samples in spectro cups, sealed with a 6 μm Mylar film. A tungsten X-ray tube was used as radiation source, whereas a Ge-detector was used for detection and quantification. In order to optimize the fluorescence measurements each sample was analysed by consecutively using BRAKLA – polarization targets (Al_2O_3) and secondary targets (CaF_2, Fe, Ge, KBr, Zr, Mo, Ag, CsI). Selenium was determined using excitation by the

Zr-secondary target, running the W-tube at 100 kV/6 mA for a measuring time of 500 s per sample. The detection limit of Se was calculated to be at ∼1 mg/kg. The bulk mineral composition of the soil samples was analysed by means of X-ray diffraction (XRD) analysis (Kristalloflex D500, Siemens, Germany) at 40 kV and 25 mA. CuKα1-radiation was used at angles between 3° and 63°.

3.3. Selenium extraction

In order to evaluate the mobility and bioavailability of Se in the top soil of the six sampling sites, the extraction scheme of Martens and Suarez [16] was modified: water soluble Se was extracted by shaking 1 g sample material twice with 25 mL of Mili-Q water for 1 h. For the estimation of adsorbed Se, 25 mL of 0.1 M K_2HPO_4/KH_2PO_4 solution (pH 7) was added twice to the Mili-Q extracted sediment and shaken for 2 h, respectively. Afterwards the samples were washed with 10 mL of Mili-Q water for 10 min. After shaking, sediment and solution were separated by centrifugation (40,000 rpm, 15 min). The respective supernatants of each extraction step were mixed. The Se concentration was determined using flow injection atomic absorption spectroscopy (FIAS 400, Analyst 200, Perkin Elmer) after pre-reduction of Se (VI) to Se (IV) with HCl (1:1) (80 °C, 20 min). During the measurements HCl (10%, p.a.) was used as carrier solution and $NaBH_4$ (0.2% in 0.05% NaOH) as reductant. The Se content of the remaining sediment, which may include organically associated Se, elemental Se, sesquioxide complexed Se, etc. was determined by ICP-MS (X-Serie, Thermo Scientific) after microwave digestion. Two mL Mili-Q water, 5 mL HNO_3 (65%, suprapur), 1 mL H_2O_2 (30%, suprapur) and 0.5 mL HF (40%, supra-

Table 1
Description of sampling sites.

Sample	Sample details	Composite soil (drilling depth) (cm)	Water (well depth) (m)
I	Jainpur village (SBS Nagar district, Punjab)	0–15 60–91 91–152	76
II	Jainpur village (SBS Nagar district, Punjab) located ca. 300 m away from location 1	0–15 60–91 91–152	76
III	Barwa village (SBS Nagar district, Punjab) located ca. 2 km away from location 1	0–15 60–91 91–183	76
IV	Simbli village (Hoshiarpur district, Punjab) located ca. 3 km away from location 1 as well as location 3	0–15 60–91 91–152	No water sample
V	Hisar (Haryana) located ca. 225 km North East from Chandigarh	0–30 30–60 60–90 90–120	16.5
VI	Chandigarh outskirts (capital of both Punjab & Haryana) located ca. 90 km away from location 1	0–15 60–91 91–152	60

Table 2
Concentration of dissolved elements and potentially toxic trace metals in $\mu g\,L^{-1}$ measured by HR-ICP-MS. Included are threshold and guideline values recommended by WHO [32].

Site	Se	Fe	Mn	P	Ni	Cu
Jainpur 1	324	2.3	103	16	0.6	3.1
Jainpur 2	341	1.3	82	1.2	0.2	1.3
Barwa	45	1.8	9.1	3.4	0.2	0.3
Hisar	3.2	30	8.2	55	0.8	0.5
Chandigarh	0.9	26.6	276	1.9	5.5	9.2
WHO (2006)	10	–	400	–	70	2000

Site	Zn	As	Mo	Cd	Pb	U
Jainpur 1	124	1.5	3.7	1.2	2.8	17.3
Jainpur 2	60	1.3	4.7	0.1	2.4	16.2
Barwa	32	1.3	2.9	0.1	0.1	16.1
Hisar	37	14.9	169	0.7	0.8	37.0
Chandigarh	259	0.8	0.7	0.3	0.8	2.9
WHO (2006)	–	10	70	3	10	15

pur) were added successively to 0.2 mg of each sample and heated with a microwave system (Start 1500, MLS) for sample digestion. The accuracy (<7.7%) was checked by including a certified water reference standard (CRM-TMWD, High Purity Standards, USA) into the ICP-MS measurement. As a reference for analysis, Chandigarh soil was taken as the Se free control.

3.4. Batch assay for selenate reduction

To investigate Se (VI) reduction by the indigenous microbial flora of soil, duplicate batch assays consisting of top soil (0–15 cm) and bottom soil (equally mixed soil samples from all depths below 15 cm) from each sampling site were carried out in closed serum bottles. Basal salt medium (BSM) after Ike et al. [8], autoclaved for 15 min at 121 °C, was used in order to produce a 10% soil slurry of top and bottom soils, respectively. A sterilized and concentrated solution of sodium selenate was prepared and added to each serum bottle to give a final concentration of 150 mg L^{-1} Se (VI) in the slurry. Similarly, a concentrated sodium acetate solution was added into each bottle to reach a final concentration of 400 mg L^{-1} acetate as an electron source for selenium reduction. On the 31st day of incubation 188 mg L^{-1} of glucose was added. Additionally, sterilized top and bottom soil slurries from each site, containing selenate and acetate/glucose, were incubated as control samples. Serum bottles with sterile BSM plus selenate and acetate or glucose without addition of soil were also incubated as controls. All samples were incubated at 37 °C in a shaker at atmospheric pressure under anaerobic conditions by exchanging the gas atmosphere of the serum bottles with nitrogen. Liquid samples (1.5–2 mL) from these assays were withdrawn with a syringe and centrifuged at 12,500 rpm for 7–8 min in an Eppendorf Microfuge (Eppendorf, Hamburg). The supernatant was used for further analyses. Selenite concentrations in the samples were determined with an ion exchange chromatograph (Dionex ICS-90) employing an AS9-HC 4 mm × 250 mm (IonPac®) analytical column and using 9 mM Na$_2$CO$_3$ as eluent and H$_2$SO$_4$ acid as regenerate. All chemicals used were of analytical grade and were purchased from Merck/VWR (Darmstadt), Fluka (Taufkirchen) or Carl Roth (Karlsruhe), Germany.

4. Results

4.1. Hydrochemistry

Dissolved Se concentrations varied considerably between all sites. In Jainpur 1, Jainpur 2 and Barwa dissolved Se concen-

trations by far exceeded the current WHO guideline value for drinking water of 10 $\mu g\,Se\,L^{-1}$ or 20 $\mu g\,L^{-1}$ for irrigation water [32], whereas, in Hisar and in Chandigarh the concentrations were relatively low (Table 2). The dissolved concentrations of redox indicative elements such as Fe or Mn were relatively low in all samples except for those from Chandigarh (Mn: 276 $\mu g\,L^{-1}$), indicating that oxidizing conditions were prevailing in the aquifers. This result was in line with relatively high dissolved U concentrations (16.1–37.0 $\mu g\,L^{-1}$), with exception of the sample from Chandigarh (U: 2.9 $\mu g\,L^{-1}$), which typically occur under oxidizing conditions. In Punjab and Chandigarh, concentrations of As (<1.5 $\mu g\,L^{-1}$) and various potentially toxic trace metals were generally low (Table 2). However in the groundwater of Hisar, noteworthy high concentrations of As, Mo and U accompanied by high concentrations of $SO_4{}^{2-}$ (500 mg L^{-1}), Cl$^-$ (240 mg L^{-1}) and F$^-$ (14.3 mg L^{-1}) were observed (not shown in Table 2). These elevated concentrations could be evoked by a combination of weathering of granite or sulfidic material with anthropogenic influence (Hisar is famous for its steel, electroplating and power industry). No negative effects of fertilizers on the groundwater can be deduced as P concentrations were generally low (<55 $\mu g\,L^{-1}$) in all samples (Table 2).

4.2. Mineralogical and geochemical composition

The bulk composition of major minerals was comparable at the different sites. Identified minerals included quartz, albite, kaolinite, illite, chlorite, montmorillonite and calcite in most of the samples. Clear Se enrichments were found in the soils of Jainpur 1 (1.3–11.6 mg kg^{-1}), Jainpur 2 (2.2 mg kg^{-1}) and Barwa (3.1, 1.4 mg kg^{-1}). As indicated by the mineralogical analyses, those soils were mainly composed of silicates and phyllosilicates, which was also reflected by the high content of SiO$_2$ (40–62 wt.%), Al$_2$O$_3$ (9–21 wt.%) and K$_2$O (2.2–3.2 wt.%). The content of Fe$_2$O$_3$ in the range of 3.6–7.5 wt.% (Table 3) was sufficient to assume that Fe oxy-hydroxides, which are important adsorbers of Se (IV) in soils [7], were present even though they could not have been identified by XRD-measurements.

4.3. Selenium speciation

The results of the different samples were highly variable especially when comparing seleniferous (Jainpur, Barwa) with non-seleniferous soils (Chandigarh, Hisar; Fig. 3). In the seleniferous soils including Simbli a considerable proportion of Se was water soluble (12.4–35.1%) or adsorbed (6.0–18.0%), whereas in the soils

Table 3
Concentration of Se, As, Fe_2O_3, Al_2O_3, SiO_2, K_2O and Rb measured with energy dispersive XRF in the soil samples.

Sample	Depth (cm)	Se (mg kg^{-1})	As (mg kg^{-1})	Fe_2O_3 (wt.%)	Al_2O_3 (wt.%)	SiO_2 (wt.%)	K_2O (wt.%)
Jainpur 1	0–15	11.6	9.6	5.4	16	56	3.1
	60–91	1.3	16.5	7.5	21	60	3.2
	91–152	1.5	20.3	7.5	14	44	3.2
Jainpur 2	0–15	2.3	10.7	5.4	13	46	3.2
	60–91	<1	10.2	4.5	11	41	3.1
	91–152	<1	12.1	6.6	20	62	3.3
Barwa	0–15	3.1	11.2	5.2	12	39	2.8
	60–91	1.4	26.1	7.6	18	61	2.8
	91–183	<1	11.2	6.5	17	57	2.8
Simbli	0–15	<1	7.2	3.9	10	46	2.8
	60–91	<1	18.2	6.1	15	54	3.0
	91–152	<1	13.9	5.8	11	43	2.7
Hisar	0–30	<1	6.6	3.6	10	44	2.5
	30–60	<1	7.9	4.1	16	62	2.8
	60–90	<1	9.1	4.7	16	60	3.0
	90–120	<1	7.7	4.3	16	62	2.9
Chandigarh	0–15	<1	6.1	3.7	11	51	2.2
	60–91	<1	7.1	3.7	9	42	2.2
	91–152	<1	10.2	5.8	12	40	3.0

of Hisar and Chandigarh more than 90% of Se could be determined in the residual fraction. In Jainpur 1, the sample with the highest total Se content (Table 3), more than 50% of the Se was only weakly associated with the soil matrix.

4.4. Batch assay for selenate reduction

Incubation of 10% soil slurries with sodium selenate over 88 days resulted in the occurrence of Se (IV) in the slurries due to Se (VI) reduction (Fig. 4). The presence of Se (IV) was observed only in assays with inocula from the top layer (0–15 cm) of soils and almost no Se (IV) was noticed in assays with bottom soils as inocula. In Jainpur 2, no Se (IV) was observed over a period of 88 days in samples even from the top soil. Furthermore, signs of any reduction activity were not detectable in all Chandigarh soil samples (results not shown) and in sterilized Se (VI) containing basal salt medium incubated without addition of any soil (results not shown). In assays from Jainpur 1, Simbli, Barwa and Hisar the top soil Se (IV) concentration increased over a certain period of time until it started to decrease again to concentrations of zero or near zero (Fig. 4). The sharp decrease might be due to further reduction of selenite to elemental Se or selenide, which could not be determined analytically with the ion chromatograph. The highest selenite concentration of 10.9 mg L^{-1} was observed in the top soil slurry from Jainpur 1 (Fig. 4a).

Fig. 3. Percentage of water soluble, adsorbed and residual selenium in the top soil of sampled sites.

5. Discussion

5.1. Groundwater quality

The Se concentrations in groundwater of Barwa and especially of Jainpur (<341 µg L^{-1}; Table 2) were much higher as reported in most of the previous studies. Dhillon and Dhillon [18,24] for instance, found concentrations up to 70 µg Se L^{-1} in groundwater of the area at or near the present study sites. All water samples in the present study were taken from deep tube wells and contained remarkably high Se concentrations, especially in Jainpur village. The cause of high Se concentrations in the groundwater of this area is still unclear. It was suggested [24,29] that high dissolved Se concentrations in the groundwater could be present due to leaching of Se from seleniferous top soils by rainfall and long-term drainage. In our study, the degree of leaching, however, which was indicated by the Se content in the soil below 15 cm, is probably not sufficient to explain the extremely high Se concentrations in the groundwater at a depth of 76 m. The observed concentrations by far exceeded the Se limits prescribed by the WHO [32]. Till now, the presence of selenium in groundwater is assumed to be natural as no anthropogenic activity which could release Se at the study area is known. The causes behind Se enrichment in groundwater are not confirmed and require further hydro-, geo- and bio-chemical studies. In Hisar, Se concentration in the groundwater was relatively low (3.2 µg L^{-1}) but due to As, Mo and U concentrations above WHO [32] guidelines (Table 2), it is not suitable either for drinking water purposes. No previous study is available indicating As, Mo and U levels in Hisar groundwater.

5.2. Source and impact of Se accumulation in top soils

Seleniferous soils in Punjab are thought to have developed due to deposition of Se-rich material transported through flood water from the nearby hills of the Shivalik range [24]. In the study area of North-Eastern Punjab, groundwater is the only source of water for irrigation ([24]; personal communication with farmers). A considerable enrichment of Se above background levels in the top soil was only detected in Jainpur (11.6, 2.3 mg kg^{-1}) and Barwa (3.1 mg kg^{-1}; Table 3) where Se concentrations in the irrigation water were high (45–341 µg L^{-1}). In Hisar and Chandigarh, where dissolved Se concentrations in the groundwater were low (<3.2 µg L^{-1}), the Se content throughout the whole soil profile was below detection limit. The low Se content in the lower soil horizons of Jainpur 2 (>15 cm) and Barwa, in the range of typ-
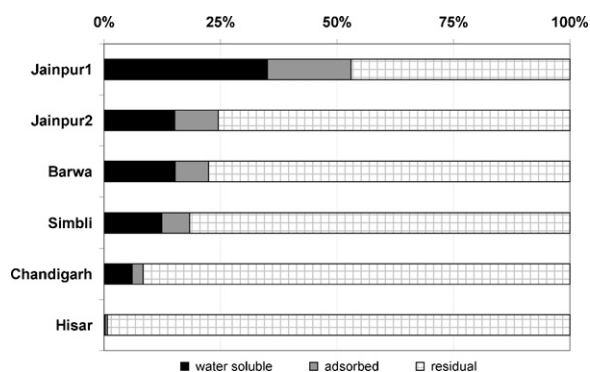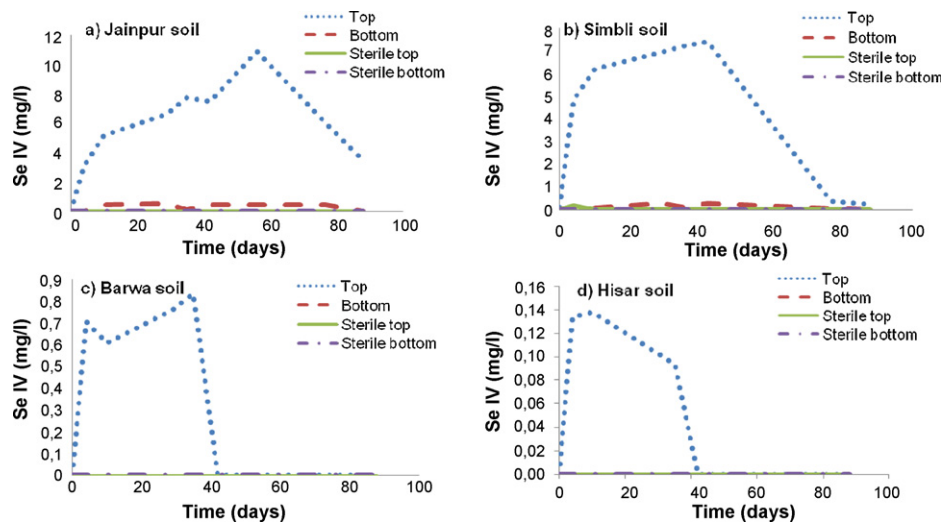
Fig. 4. Selenate reduction to selenite by indigenous bacteria in soil slurries from Jainpur 1 (a), Simbli (b), Barwa (c) and Hisar (d).

ical non-seleniferous soils, indicated that irrigation with Se rich groundwater was probably the main cause for the occurrence of seleniferous soils in this area. Since a large proportion of Se can be extracted from the soil either by water or by a phosphate solution (Fig. 3), the Se which is introduced by irrigation is likely to be retained in the top soil mainly through a loose adsorption onto Fe oxy-hydroxides and clay minerals present in soil (Table 3). Recently, Dhillon and Dhillon [30] proposed that additional enrichment of Se or its recycling might also occur due to the decay of Se rich organic residues of the crops which are left on the fields after harvesting as an organic fertilizer, an assumption that has to be further investigated.

Jainpur 1 and Jainpur 2 are just located 300 m away from each other but differ considerably in their solid Se content (Table 3). This, in addition to the low solid Se content at the study site of Simbli, a village where seleniferous soils can be found [22] highlights the fact that the Se content of the top soils is distributed patchily. The much lower Se content in the top soil of Jainpur 2 compared to Jainpur 1, even though irrigation water with an almost identical Se concentration (Table 2) was used, could be explained by a different crop rotation and the respective need for irrigation water. On the Jainpur 1 field, rice–wheat crop rotation is practiced and in the second field, maize and wheat are grown. For former rotation $\sim$2000 L water ha^{-1} yr^{-1} are required as compared to $\sim$600 L water ha^{-1} yr^{-1} for later rotation. Both crop rotations led to an additional yearly Se input of 6.5 kg ha^{-1} and 2.0 kg ha^{-1}, respectively [18]. This high annual input could explain the twice as high Se content found in the top soil of Jainpur 1 in this study as compared to previous studies carried out in this area (4.5 mg kg^{-1}; [18,20]). It also indicates that irrigation may be the major source of Se accumulation in top layers of soils and not only the depositions of material transported with rivulets from hills of Shivalik range [24]. In the field of Jainpur 1, some plants showed stronger chlorosis symptoms than others (outlined in Fig. 1). This might be due to variations in adsorption and desorption capacities of soil which in turn could be influenced by the distribution of ions during tilling and by the extent of bioavailability of Se to respective plants. All this can control the absorption of selenium and the nutrient up-take of plants. Dhillon and Dhillon [18] observed a roughly three times decrease in selenium concentration in the sugarcane leaves at maturity as compared to the selenium amount that was observed at the growth stage of 60 day. Wheat plants exhibited a varying degree of toxicity symptoms in shoots, roots and leaves of plants. Thus, the type of soil as well as maturity and physiological properties of respective

plants could result in varying distribution of toxicity symptoms in the same field. Apart from the high Se content in the top soil and in plant species, the high Se concentration in the crops produced on fields with seleniferous soil depends mainly upon the speciation of the Se [14]. A considerable proportion (18–53%) of Se in the seleniferous top soils is either water-soluble or adsorbed (Fig. 3) and thus considered to be available for plants [33]. As a consequence, crop cultivation especially on field Jainpur 1, where >50% of Se presumably was bioavailable, could lead to a high enrichment of Se in the plant tissue. Water soluble selenium compounds can easily be released from the soil matrix during summer monsoon, when heavy rain falls recharge the groundwater. This could lead to leaching of some Se into deeper soil horizons which might have occurred in the soils of Jainpur 1 and Barwa, where deeper soil horizons also have a relatively high Se content ($\sim$1.4, Table 3). On the other hand, evaporation during the dry season could lead to an upward transport of Se within the soil profile and, therefore, counteracting leaching. Ongoing intensive irrigation especially in regions with rice–wheat rotation, with Se-rich groundwater indicates that Se leaching in future might even increase and will eventually endanger shallow groundwater aquifers.

5.3. Microbial influence on Se speciation

During soil incubations, a comparatively high selenite concentration observed in Jainpur 1 top soil slurry (Fig. 4) might be due to the presence of selenate reducing bacteria in the soil that were resistant against Se-toxicity. Soil from Jainpur 1 contained a high concentration of Se (Table 3, Fig. 3), of which 53% was bioavailable, either solubilised in water or adsorbed onto soil particles. Interestingly, the surface soil sample of Barwa, which also had a relatively high Se concentration (Fig. 3), apparently did not contain much selenate reducing activity (Fig. 4c). However, selenate reducing bacteria were very active in the assay with top soil slurry from Simbli village (Fig. 4b), although the total Se concentration in the Simbli top soil layer was below detection limit (Table 3). This indicated the presence of different activities of indigenous bacteria which may have developed after adaptation due to bioavailability of Se during certain period of time. The order of bioavailability of selenium species in soil is selenate > selenomethionine > selenocysteine > selenite > elemental selenium > selenide [9]. Se transportation is strictly limited under reducing conditions due to the strong adsorption tendency of selenite [7]. In the Hisar soil assay (Fig. 3d), bacterial selenate

reduction activity was minimal which may have been the result of a very slow adaption and population enrichment of indigenous Se reducing bacterial communities due to absence of considerable initial concentration of total and bioavailable Se in Hisar soils (Fig. 3, Table 3). In the tested soil samples selenite reduction proceeded more rapidly than its occurrence, indicating that the activity of selenite reducing bacteria was faster than that of selenate reducing microbes. This phenomenon was also confirmed by Ike et al. [8].

6. Conclusions

Concentrations of Se in the groundwater of Jainpur and Barwa by far exceeded the WHO guidelines for Se in drinking or irrigation water which makes the water unsuitable for these purposes. High to low Se concentrations were found in soil samples from different sites in Jainpur indicating that Se concentrations were spread sporadically even within a small area. It is concluded that usage of groundwater as irrigation water is the main cause for high Se contents in the top soils of Punjab and that crop rotations may have been the reason for the differences in concentrations. Therefore, it could be assumed that ongoing irrigation with Se burdened groundwater will lead to further Se enrichment over the next decades. Selenium reducing soil microbes seem to gain resistance to high Se concentrations, for example in Jainpur 1 top soils and will therefore be able to influence the speciation and the mobility of Se in the soil. In order to better understand the Se cycling in the soils of Punjab and to be able to estimate the development of Se in the soil–plant and soil–groundwater system the influence of crop rotation including respective water and fertilizer requirements as well as the influence of the dry and rainy seasons on the speciation and mobility of introduced Se and has to be evaluated in future. This knowledge is required to predict Se health related risks for animals and humans living in the area and to introduce possible mitigation measures. During Se investigations, high levels of As, Mo, U were also found in Hisar groundwater and make it unsuitable for drinking purposes.

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