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Highland barley grain and soil surveys reveal the widespread deficiency of dietary selenium intake of Tibetan adults living along Yalung Zangpo River

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Objective: In order to assess selenium (Se) flux through the soil-plant-human chain in Tibet plateau and explore the reason why local Tibetan adult residents from large scale agricultural production areas in Tibet lacked daily Se intake.

Methods: A total of 210 intact highland barley plants and their corresponding cultivated topsoil samples were collected in fields of 14 agricultural counties along Yalung Zangpo River and quantitative dietary data were collected from a cross-sectional survey using a cultural-specific food frequency questionnaire that contained all local Tibetan foods in 2020.

Results: The mean value of The estimated daily Se dietary intake by each participant was 17.1 ± 1.9 μg/day/adult, the Se concentration in topsoil and highland barley grain were 0.128 ± 0.015 mg/kg and 0.017 ± 0.003 mg/kg, respectively. Although highland barley was the first contributor of dietary Se in local adult residents (34.2%), the dietary Se intake provided by highland barley only about 10% of the EAR value (50 μg/day/adult) currently. A significantly positive relationship was determined between soil total Se content (STSe), available Se content (SASe) and highland barley grain Se content (GSe). The amount of Se in food system depends on a number of soil properties (TOC, pH, clay content, Fe/Mn/Al oxides), climate variables (MAP, MAT) and terrain factor (altitude).

Conclusion: To sum up, it can be inferred that the insufficient dietary Se intake of Tibetan adult population living along Yalung Zangbo River is mainly caused by the low Se content in highland barley grain, which was result from the low Se content in cultivated soil. In order to enable adult participants in the present study to achieve recommended dietary Se-intake levels, agronomic fortification with selenised fertilizers applied to highland barley could be a great solution. It is necessary to combine the influencing factors, and comprehensively consider the spatial variation of local soil properties, climatic and topographic conditions, and planting systems.

KEYWORDS

selenium flux, selenium deficiency, highland barley, Tibetan adult population, Tibet Autonomous Region

Introduction

Selenium (Se) is essential for a number of enzymes that perform important metabolic functions necessary for good health (Brayman, 2000). Se enters the food systems through plants, which take it up from the soil (Gerald, 2001). Se deficiency has therefore been identified in parts of the world notable for their low soil content of biologically available Se (Natasha et al., 2018). Low amounts of Se in soils results in a generalized deficiency of the element throughout the food system, being low in the plants grown on those soils as well as the livestock and people fed by those plant foods (Lokeshappa et al., 2012; Fordyce, 2013). As a consequence, people in many countries do not appear to consume adequate amounts of Se to support the maximal expression of the Se enzymes (Gerald, 2001). Less-overt selenium deficiency can have adverse consequences for immune function (Kiremidjian-Schumacher et al., 1994), viral infection (Taylor et al., 1997), reproduction (Oldereid et al., 1998), mood (Hawkes and Hornbostel, 1996), thyroid function (Olivieri et al., 1995), cardiovascular disease (Suadicani et al., 1992), and other oxidative-stress or inflammatory conditions (Peretz et al., 1992). Two diseases have been associated with severe endemic Se deficiency in humans: a juvenile cardiomyopathy (Keshan disease, KD), and a chondrodystrophy (Kaschin-Beck disease, KBD). Each occurs in rural areas of China and Russia (eastern Siberia) in food systems with exceedingly low Se supplies in soil and poor uptake in the plants (Levander, 1986; World Health Organization, 1996). A long belt of mountainous terrain extending from the north-east to south-west portions of mainland China was found with low soil Se concentration, and mainly distributed throughout the provinces of Heilongjiang, Jilin, Liaoning, Hebei, Henan, Yunan, Guizhou, Sichuan, Tibet, Shanxi, and Shandong provinces (Tan et al., 1994). Interestingly, the incidences of KD and KBD disease, both of which are considered to be endemic diseases caused primarily by Se deficiency, overlap this fairly broad belt where the soil is extremely deficient in Se (Yang et al., 2007).

Currently, the prevalence rate of KD and KBD has decreased greatly in most parts of China with the socioeconomic development, but it is still active and mainly confined to the southwest part of China, especially in the Tibetan Plateau including the Tibet Autonomous Prefecture, Qinghai Province, and the west part of Sichuan Province (Winkel et al., 2012). A survey of 3,382 soil samples at depths of 0–25 cm of China mainland revealed that the lowest soil Se concentration was found in Tibet (Liu et al., 2020), which was a high-risk area for endemic diseases induced by Se deficiencies in China (Zhang et al., 2011). The mean Se concentrations of cultivated soil samples were 0.10 mg/kg in KBD areas in Tibet, which was significantly lower than the average of China (0.29 mg/kg; Li et al., 2009). The principal characteristics of the endemic zone in Tibet are dark brown and black soils with very low in bioavailable Se as water-soluble element fractions (Tan et al., 1994). Low Se translocation from soil to crops leaded to low Se concentration in crops. The Se concentration of highland barley, the staple food in Tibet (accounting for 75.6% of food consumption), was only 4.02 ± 2.4 μg/kg in KBD affect-areas and 13.99 μg/kg in non-KBD affect-areas in Tibet (Guo et al., 2017), which was is significantly lower than the limit of Se in foodstuffs (0.3 mg/kg; USDA, 2006), even lower than the threshold values of Se deficiency in grains (0.025 mg/kg; Tan, 1990). According to a latest study on daily Se intakes among residents in KBD endemic areas of Lhasa municipality in Tibet, Se intake through staple food was 8.30 μg/d and 76.1% of total daily Se intake was contributed to the consumption of purchased rice and flour; Se intake through local produced cereals was only 3.98 μg/d (Chen et al., 2015), which was significantly lower than the average daily intake of grain selenium of residents in 10 other provinces in China (10.68 μg/d; Li et al., 2014).

The relationship between the distribution of KBD and agricultural production structure in Tibet showed that 48.48% of the endemic affected counties were in the agricultural areas of Tibet (Yang et al., 2003). Low Se intake by inhabitants from these areas is caused by insufficient Se flux through the soil-plant-human chain (Navarro-Alarcon and Cabrera-Vique, 2008). The soil-plant system is instrumental to human nutrition and forms the basis of the "food chain" in which there is Se cycling, resulting in an ecologically sound and sustainable flow of Se (Yang et al., 2007). One problem that exists today is much less information is available on Se in West China, especially Tibet, which covers a large part of China. To fully understand the status of Se in the environment of China, more investigations should be conducted in this region (Ullah et al., 2019). Therefore, the Se content and translocation of food chain in agricultural counties of Tibet should be deeply studied. Perhaps primarily, Tibetan individuals need to be aware of the baseline intake in their country or region and whether that Se intake is adequate or not. Furthermore, though full knowledge of all the relevant factors in any particular set of circumstances can never be achieved, advisory bodies are obliged to do their best to make appropriate public-health recommendations. Therefore, the objectives of the present study were as follows: (i) assessing the dietary Se intake status of Tibetan adults in 14 agricultural counties along Yalung Zangpo River in Tibet and the contribution of highland barley to local residents' dietary Se; (ii) analyzing the relationships among soil Se, highland barley grain Se and dietary Se, and explore the root cause of dietary Se deficiency in Tibetan residents; (iii) analyzing of key environmental factors affecting soil Se, highland barley grain Se and dietary Se. Our results could provide a basis for understanding patterns of Se deficiency in Tibetan adult population, and for identifying areas where particular interventions might be most appropriate because of the poor local Se status of staple crops.

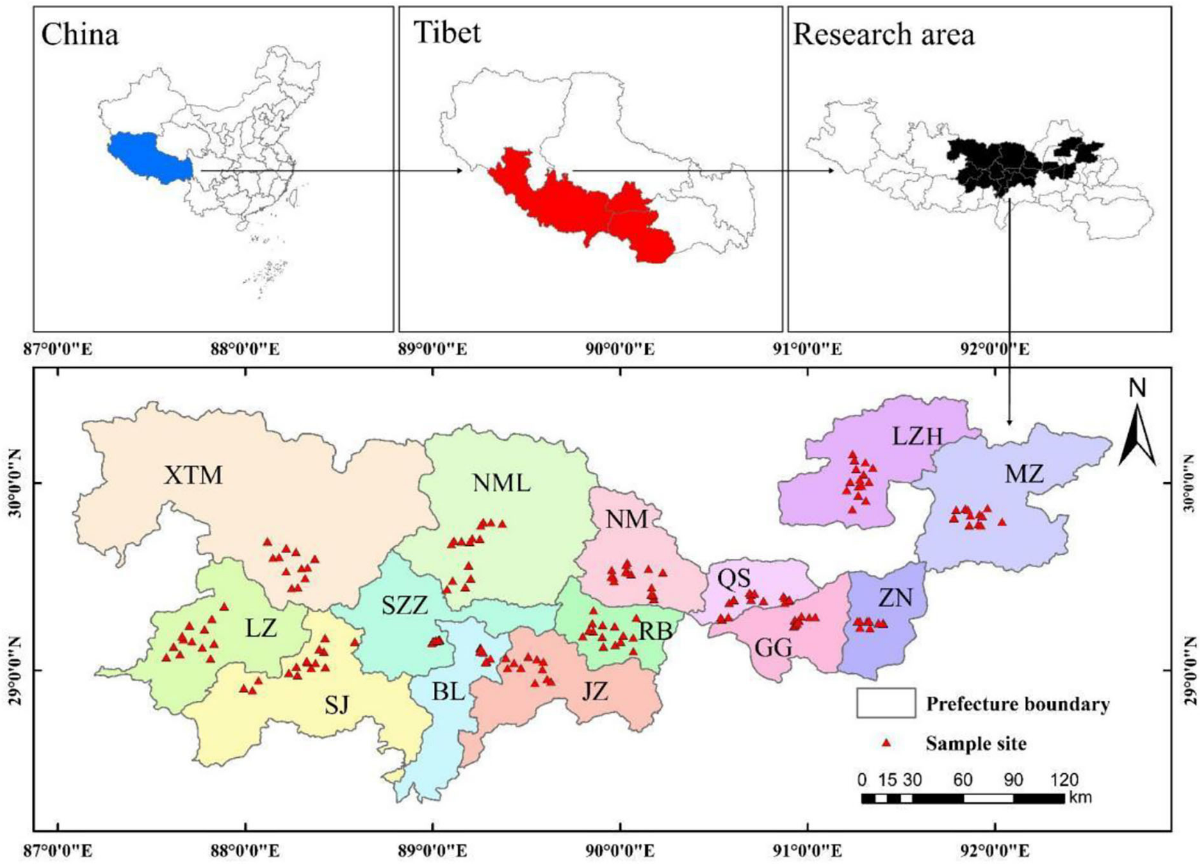

FIGURE 1
A sketch map showing the study area and sampling sites.

Materials and methods

Study sites and sampling

In August 2020, a total of 210 intact highland barley plants and their corresponding cultivated topsoil (0–20 cm) samples were collected in fields of 14 agricultural counties (Medrogongkar, MZ; Chushur, QS; Nyemo, NM; Lhundup, LZH; Danang, ZN; Gonggar, GG; Sangzhuzi, SZZ; Namling, NML; Gyantse, JZ; Sakya, SJ; Lhatse, LZ; Thongmon, XTM; Panam, BL, and Rinpun, RB) along Yalung Zangpo River (Figure 1). Geographic location and altitude were synchronously recorded at each sampling site. Mean annual precipitation (MAP), mean annual temperature (MAT) of each sampling site were collected from 1-km monthly mean temperature dataset for china (1901–2020; Peng, 2019) and 1-km monthly mean temperature dataset for china (1901–2020; Peng, 2020), respectively.

Dietary survey

A validated cultural-specific food frequency questionnaire that contained all local Tibetan foods, such as tsampa (flour obtained from roasted highland barley grains), Tibetan sweet tea (local black tea mixed with sweetened milk powder), yak buttered tea (salt cream tea), yak meat, and chang (wine made from highland barley in a unique local manner) combined with a 2-day, non-consecutive 24 h recall, was used to assess the types and frequency of food consumed (Supplementary Table 1). The detailed information of FFQ was shown in Supplementary Table 2. For each county, 20 urban residents and 20 rural residents were selected by simple random sampling, and different genders and ages were considered. A total of 552 participants were interviewed face-to-face by trained professional interviewers. After assessing the types and frequency of food consumed, the daily intake amount of each food item per person was calculated and dietary Se intakes were calculated by multiplying the Se content in the Chinese

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Food Composition Table (Yang, 2009; Standard Edition, version 6, 2019) by the daily intake of each food. The nutritional contribution rate of highland barley was determined by dividing the total Se intake contributed from highland barley group by the total Se intake (for each separately) from all food groups, and then multiplying it by 100. The gap between dietary Se intake and dietary Se intake thresholds for endemic [gap20 (%)] was calculated as the difference of dietary Se minus 20 divided by 20 then multiplying it by 100; The gap between dietary Se intake and recommended dietary intake of Se in China [gap50 (%)] was calculated as the difference of dietary Se minus 50 divided by 50 then multiplying it by 100. A detailed description of dietary survey can be found in our previous published work (Zhou et al., 2021, 2022).

Sample and preparation

Soil samples were cleaned of vegetation and other debris, air dried, and then ground into particles that could pass through 60-mesh and 100-mesh sieves for soil physicochemical properties and total Se determination separately. Then, soil samples were selected by coning and quartering, after which they were stored in an airtight plastic container for acid digestion. Grain samples were rinsed with deionized water three times, oven-dried at 55°C for 72 h, ground with a stainless steel grinder (FW-100, Taisite Instrument Co., Ltd., Tianjin, China), and then stored in an airtight plastic container at 25°C for acid digestion.

Chemical determination of soil Se and soil physicochemical properties

All treated samples were acid-digested (4 HNO_3:1 $HClO_4$, v/v) and analyzed for total Se *via* hydride generation atomic fluorescence spectrometry (HG-AFS) with a detection limit of 0.01 μg/L. The determination of total Se in soil (STSe) and highland barley grains (G-Se) referred to national standards on the determination of Se in foods (GB/T5009.93-2003) and soil (NY/T1104-2006). Soil available Se (SASe) was extracted with 0.1 and 1 M phosphate-buffer solution (K_2HPO_4-KH_2PO_4), both at pH 4.4 and 7.0. In the extraction, a 10-g soil sample and 50 ml of buffer solution were shaken for 4 h in a 100-ml tube, then centrifuged (15 min, 3,000 ×g), after which the supernatant was filtered (Whatman Grade 589, Black ribbon) into a 100-mL plastic storage bottle (Keskinen et al., 2009). Quality control was maintained by standard reference materials (SRM), reagent blanks and duplicated samples. GBW08503b wheat (Institute of Geophysical and Geochemical Exploration, Beijing, China) and GBW08302 Tibetan soil (Research Center for Eco-Environmental Sciences, CAS, Beijing, China) were used as the SRM for grain and soil samples separately. Fifteen percent of the samples in each batch were randomly employed as replicates. The results showed good agreement between the measured values and the certified reference values (RSD <5%; Wang J. et al., 2017). After the soil was air-dried and passed through a 200-mesh sieve, a 50-mg aliquot was digested with a mixture of HF, HNO_3, and $HClO_4$ at a ratio of 5:5:1 and heated at 180°C until the solution became transparent. X-ray fluorescence spectroscopy (XRF) was used to detect Al_2O_3, Fe_2O_3, MnO, Zn, Cu in the obtained solutions. The detection limit of Fe was <0.1 μg/L, the detection limit of Al, Zn, Cu, Mn was 0.1 μg/L (Yang et al., 2020). The pH of soil in water (1:2.5, w/v) was measured using a pH meter (PHS-3C).The organic matter of soil was determined by the potassium dichromate-sulfuric acid titration. Granularity analysis was conducted by the laser particle analyzer (Mastersizer 2000, Malvern Instruments Co., Ltd., UK).

Statistical analysis

All Se concentration data of soil and highland barley grain collected in 14 agricultural counties along Yalung Zangpo River, including climate and edaphic characteristics, were subjected to Box-Cox transformation to meet the assumptions of normality by R programming software. For Se intake data of local Tibetan adults, Shapiro-Wilk test was applied to detect possible non-normal distributions of the variables. When the statistical distribution was not normal, a logarithmic transformation of the variable was performed. All the statistics (mean, standard deviation, maximum, minimum, coefficient of variation) and one-way analysis of variance (ANOVA), were calculated using SPSS software (v.25.0, IBM Corp., Armonk, NY, United States) and plotting was performed on Origin 2021b (Origin lab, Northampton, Massachusetts, United States). Pearson correlation analysis was performed to explore the relationships between STSe, SASe, and G-Se. A $p < 0.05$ was considered for assessing significant statistical effects.

STSe, SASe, and G-Se were selected as data of species. Four climatic and topographical factors [mean annual precipitation (MAP), mean annual temperature (MAT), and altitude (Alt), and 10 soil factors (pH, TOC, clay, sand, silt, Fe, Al, Mn, Zn, Cu)] were used as environmental data. Redundancy analysis (RDA) was performed using Canoco 5.0 (Microcomputer Power, United States) to evaluate the effects of environmental factors on the Se concentration on the soil-highland barley system. Prior to RDA, the significance of the effect of each variable was assessed using a Monte Carlopermutation test.

Ethical approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the Chinese

Center for Disease Control and Prevention (CDC) of Tibet Autonomous Region. All participants signed an informed consent before participation.

Results

Dietary Se intake of Tibetan adults living in 14 agricultural counties along Yalung Zangpo River

The estimated daily Se dietary intake by each Tibetan adult living along Yarlung Zangbo River varied from 11.9 to 25.7 μg/day/adult, with a mean value of 17.1 ± 1.9 μg/day/adult and a coefficient of variation of 11% (Figures 2B, 3 and Supplementary Table 3). The cumulative proportion of dietary selenium intake in the range of 7.5–20 μg/day/adult reached 63.63%, of which the largest proportion was in the range of 12.5–14 μg/day/adult (15.78%; Figure 2A). All participants from 14 agricultural counties had insufficient Se intake below the estimated average requirement (EAR) value recommend by Chinese Nutrition Society (50 μg/day/adult; Figure 3), 71.4% (10 of 14 counties) participants had significantly insufficient Se intake below the endemic threshold value (20 μg/day/adult; Figure 3). The average dietary Se intake of six counties on the North Bank of Yarlung Zangbo River (XTM, MZ, LZH, NM, NML, QS) was 15.5 ± 1.4 μg/day/adult, which was lower than that of other 8 counties on the south bank (17.3 ± 1.9 μg/day/adult; $p < 0.05$; Supplementary Figure 1D and Supplementary Table 3). Tibetan adults living in RB county had the highest dietary Se intake (25.7 ± 3.6 μg/day/adult), while those living in MZ county had the lowest dietary Se intake (11.9 ± 1.7 μg/day/adult; Figure 3 and Supplementary Table 3).

Contribution of highland barley to dietary Se intake

The percentage contributions of each food group to dietary Se intake was shown in Figure 4. The top five food sources of Se were highland barley (34.2%), meat (13.0%), rice (12.4%), eggs (12.2%), and cultural-specific beverages (7.8%). Generally, highland barley contributed more than one third of dietary Se intake of Tibetan adults living along Yarlung Zangbo River. However, the dietary Se intake provided by highland barley only 10% of the EAR value (50 μg/day/adult; Figure 5A). Moreover, the dietary Se intake provided by highland barley showed significant differences among different counties ($p < 0.05$). More than 40% Se intake of the local adults living in NML, JZ, GG counties was provided by highland barley, the residents of BL county and SZZ county seemed to rely less on highland barley for dietary Se, which provides <30% of their dietary Se intake (Figure 5A). Only dietary Se intake of RB county participants was 28.14% more than the endemic threshold value (20 μg/day/adult), however it was 48.74% lower than the EAR value (50 μg/day/adult; Figure 5B). Figure 5B also shown there was a significant gap between daily Se intake of Tibetan adults living along Yarlung Zangbo River and the endemic threshold value (20 μg/day/adult), the EAR value (50 μg/day/adult), almost above 25 and 65%, respectively (Figure 5B).

Variation of Se concentration of soil and highland barley grain in 14 agricultural counties along Yalung Zangpo River

Concentration of total selenium in cultivated topsoil samples (0–20 cm) from 14 agricultural counties along Yarlung Zangbo River varied from 0.041 to 0.229 mg/kg (Figure 6A), with an arithmetic average of 0.128 ± 0.015 mg/kg and a coefficient of variation of 13% (Supplementary Table 3). Soil classification based on various investigations considering the relation between soil Se level and human health was given in Tan et al. (2002), namely, Se-deficient (<0.125 mg/kg), Se-marginal (0.125–0.175 mg/kg), Se-sufficient (0.175–0.4 mg/kg), and Se-rich (>0.4 mg/kg). As shown in Supplementary Figure 1A and Supplementary Table 3, the 14 agricultural counties along Yarlung Zangbo River were predominantly Se-deficient (50% of total area), followed by Se marginal (21.4%), Se-sufficient(28.6%), and no Se-rich areas. From the perspective of spatial distribution, the selenium content of soil on the North Bank of Yarlung Zangbo River is significantly lower than that on the south bank ($p < 0.01$; Supplementary Figures 1A,B). Se content in highland barley grain collected from 14 agricultural counties along Yarlung Zangbo River varied from 0.004 to 0.029 mg/kg, with a coefficient of variation of 20% and an average Se content of 0.017 ± 0.003 mg/kg (Figure 6B, Supplementary Figure 1C, and Supplementary Table 3). According to the standard proposed by World Health Organization (1996) for the Se-deficiency limit in grains (0.05 mg/kg), the Se concentration of grains collected from seven counties were classified as Se-deficiency grain (Figure 6B). The Se concentrations in highland barley grain grown in NM and XTM counties were lower than the Se-deficiency standard in grains proposed by Tan (1990; 0.025 mg/kg). Different from soil Se content and dietary Se intake, there was no significant difference between the grain Se concentration in North Bank of Yarlung Zangbo River and that in the South Bank ($p > 0.05$; Supplementary Figure 1C and Supplementary Table 3).

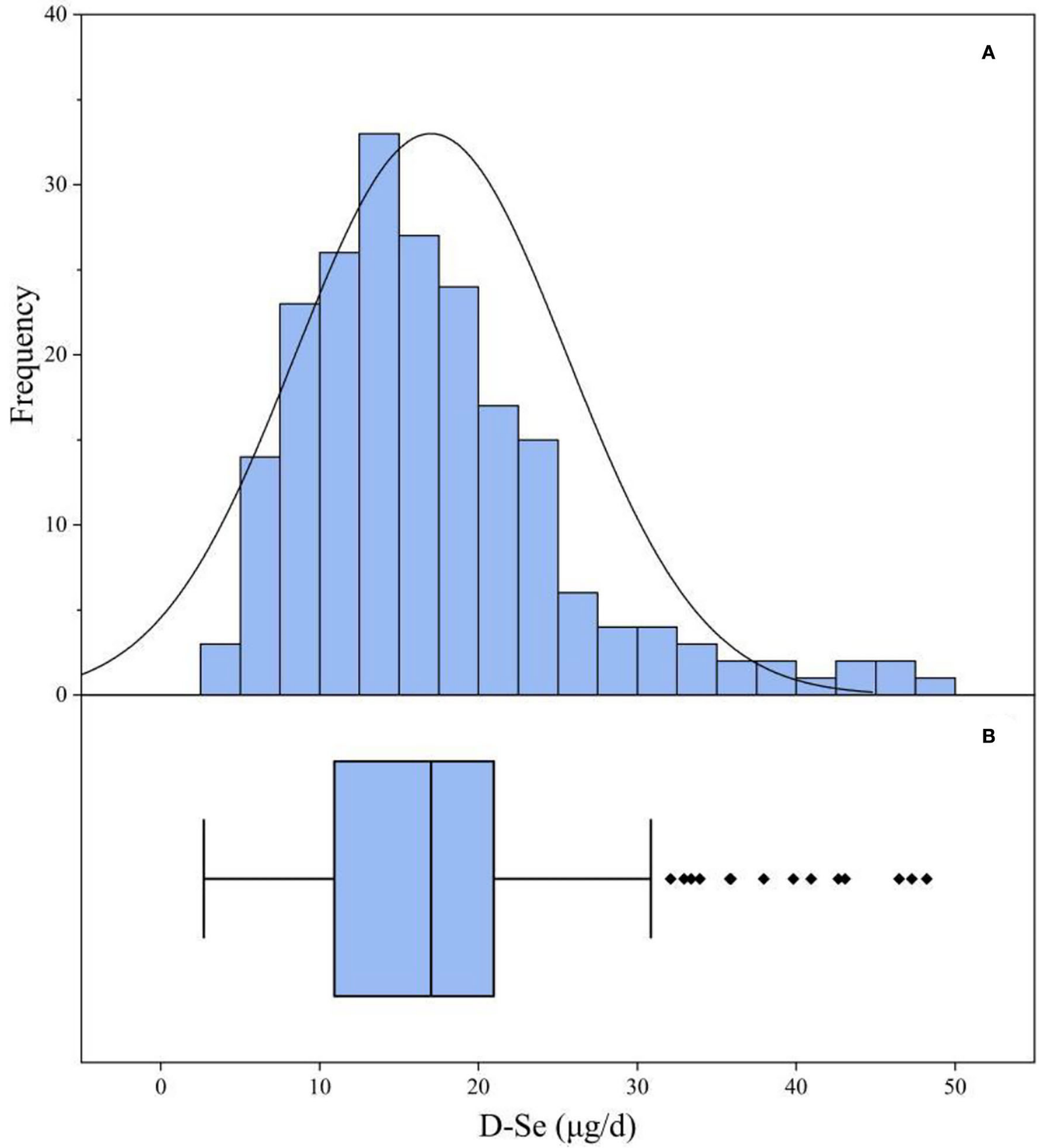

FIGURE 2
The statistical distribution of dietary Se intakes of Tibetan adults living in 14 agricultural counties along Yalung Zangpo River: **(A)** histogram; **(B)** boxplot. Box length represents the interquartile range (25th to 75th percentiles) and contains the mean value.

Relationships among soil-Se, grain-Se, dietary-Se, highland barley-Se, and their influencing factors

Figure 7 shows the correlation between soil total selenium content (STSe), soil available selenium content (SASe), and barley grain selenium content (GSe) in 14 counties along Yalung Zangpo River. The results showed that there was a significant positive correlation between STSe and SASe (r = 0.612; p < 0.001), STSe and G-Se (r = 0.191; p = 0.005), SASe and G-Se (r = 0.6425; p < 0.001).

We used redundancy analysis (RDA) sequence map to explore the influence of environmental factors on soil total Se content (STSe), soil available selenium content (SASe) and barley grain selenium content (G-Se). Before the RDA analysis, the detrended correspondence analysis (DCA) was performed

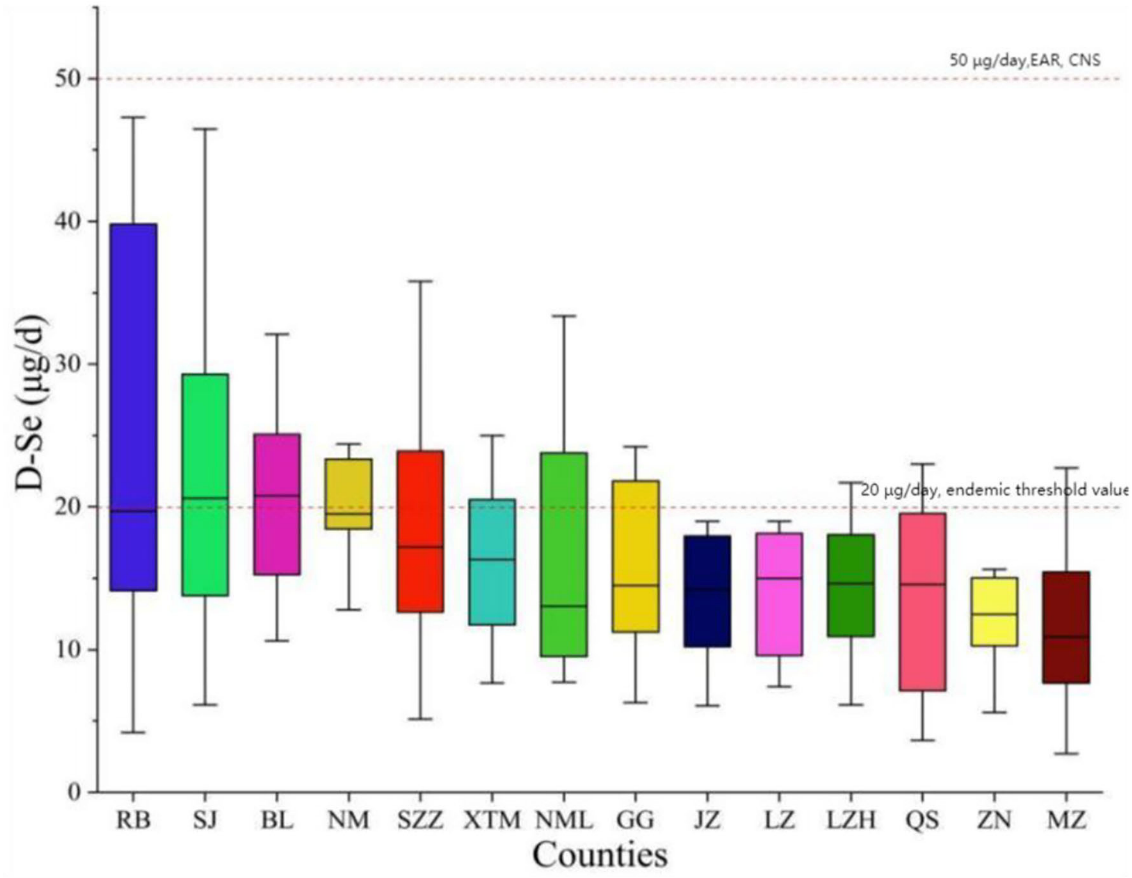

FIGURE 3
Dietary Se intake (*D-Se*) of Tibetan adults living in 14 agricultural counties along Yalung Zangpo River.

on the species data (STSe, SASe, G-Se) and the four sorting axes were all <3.0, then the RDA method could be applied. The cumulative explanation rate of environmental factors on the first two axes of the species data was 42.01%. Therefore, the first two ranking axes can be used for redundancy analysis to reflect the correlation between environmental factors and the species data (Table 1). According to the contribution rates of various environmental factors to the data of the three species, the main environmental factors affecting STSe were TOC, Fe, clay, MAP, and Zn, with the contribution rates of 41.0, 21.3, 10.0, 6.6, and 5.2%, respectively. The top five environmental factors with high contribution rate to SASe were clay (52.5%), Alt (19.2%), pH (10.2%), Fe (7.8%), and MAP (4.1%), respectively. Highland barley grain Se concentration GSe was mainly influenced by clay (14.3%), TOC (10.8%), Cu (15.5%), silt (10.3%), and pH (8.4%; Table 2). The Angle of vectors in the RDA sequence diagram can reflect the correlation between species data and environmental factors. If the angle is <90°, the correlation is positive; if the angle is >90°, the correlation is negative; if the angle is equal to 90°, there is no relationship between species data and environmental factors. It can be seen from Figure 8 that STSe, SASe were positively correlated with sand, Zn, Mn, silt, MAT, Cu and negatively correlated with Alt, Al, MAP, clay, pH, TOC, Fe. GSe was positively correlated with sand, Al, MAP, Zn, Mn, and negatively correlated with MAT, Cu, clay, pH, TOC, Fe.

Discussion

Widespread Se deficiency in food system of Tibetan adults living in 14 agricultural counties along Yalung Zangpo River

The present study showed widespread Se deficiency in "soil-highland barley-dietary intake" system of Tibetan adults living in 14 agricultural counties along Yalung Zangpo River. The calculated daily Se dietary intake by each participant varied from 11.993 to 25.699 μg/day (Figure 2), with an mean value of 17.063 ± 1.89 μg/day, which was far less than the estimated

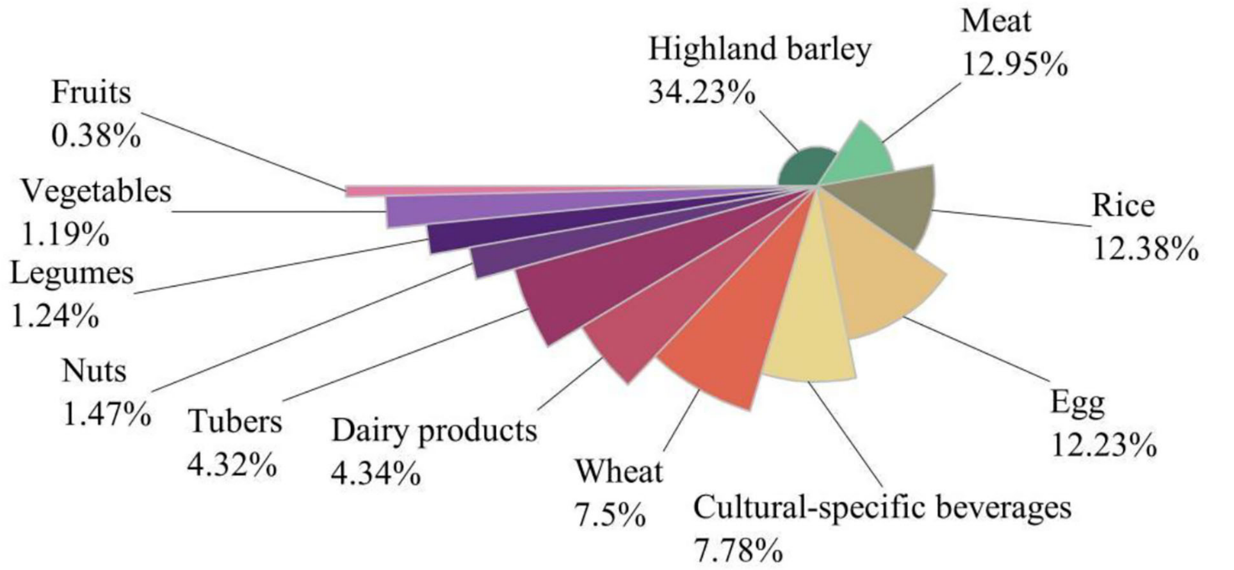

FIGURE 4
Food contributions to dietary Se intake of Tibetan adults living in 14 agricultural counties along Yalung Zangpo River.

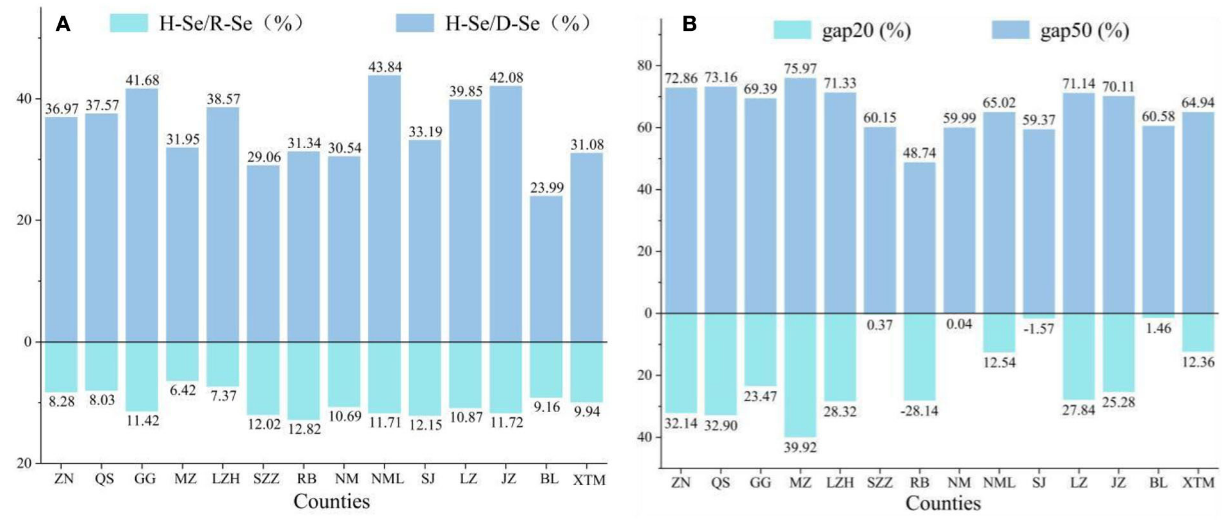

FIGURE 5
The contribution of highland barley to dietary selenium intake of Tibetan adults **(A)** and Gap between dietary selenium intake and recommended amount in Tibetan adults **(B)**. H-Se/D-Se (%): ratio between dietary selenium intake provided by highland barley and total dietary selenium intake of Tibetan adults; H-Se/D-Se (%): ratio between dietary selenium intake provided by highland barley and recommend amount of selenium intake (50 µg/adult/day); gap20 (%): gap between dietary selenium intake and dietary selenium intake thresholds for endemic (20 µg/adult/day); gap50 (%): gap between dietary selenium intake and recommended dietary intake of selenium in China (50 µg/adult/day).

average requirement (EAR) value (50 µg/day) recommended by Chinese Nutrition Society (Chinese Nutrition Society, 2014), and was just close to the recommended minimum daily Se intake for protection against KD in endemic areas [i.e., 17 µg/day (Yang and Xia, 1995) and 20 µg/day (Ryan, 2016)]. Also, this value was smaller than the daily dietary Se intake by the Chinese population living in Se-deficient areas (27.58 µg/day; Dinh et al., 2018), e.g., Suzhou in Jiangsu Province (43.9 µg/day; Gao et al., 2011), Qujing of Yunnan Province (25.9 µg/day; Zhang et al., 2012), and Qinghai Province (28.7 µg/day; Yuan et al., 1996). Se content in highland barley grain collected from 14 agricultural counties along Yarlung Zangbo

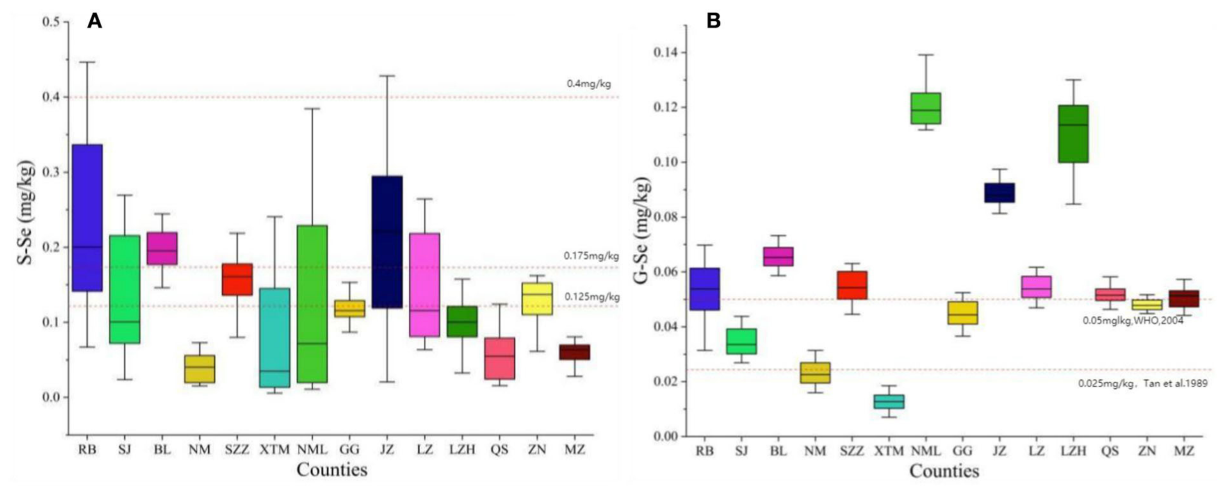

FIGURE 6
Selenium concentration of soil **(A)** and highland barley grain **(B)** in 14 agricultural counties along Yalung Zangpo River.

River varied from 0.004 to 0.029 mg/kg (Figure 2), with an average value of 0.017 ± 0.003 mg/kg, a little higher than that of KBD-affect areas in Tibetan plateau (0.009 mg/kg; Wang J. et al., 2017); however, it is significantly lower than the limit of Se in foodstuffs (0.3 mg/kg; USDA, 2006), even lower than the threshold values of Se deficiency in grains (0.025 mg/kg; Tan, 1990). Concentration of total selenium in cultivated topsoil samples (0–20 cm) from 14 agricultural counties along Yarlung Zangbo River varied from 0.041 to 0.229 mg/kg (Figure 2), with an arithmetic average of 0.128 ± 0.015 mg/kg. The average value is clearly lower than the national average (0.29 ± 0.26 mg/kg; Li et al., 2009), the global average (0.44 mg/kg; Ullah et al., 2019) and the geometric mean of total Se in cultivated soil in China (0.188 mg/kg; Tan et al., 2002); however, it is close to the Se content of cultivated topsoil in KBD areas in Tibetan Plateau (0.13 ± 0.04 mg/kg; Zhang et al., 2011), suggesting that the Se content in the surface soil from 14 agricultural counties in central Tibet is not only at a low level in China, but also at a low level in Tibet. According to Tan et al. (2002), half of the surveyed counties belonged to Se-deficient (<0.125 mg/kg) areas, which was close to the proportion of Se deficiency areas in China (51%; Dinh et al., 2018). From the perspective of spatial distribution, the selenium content of soil on the North Bank of Yarlung Zangbo River is significantly lower than that on the south bank ($p < 0.01$; Supplementary Table 1), which was consistent with a previous study on the distribution of KBD in Yarlung Zangbo River bank. The selenium in cultivated soil, dietary Se intake in the south side of Yarlung Zangbo River bank (0.23 mg/kg,13.99 µg/day) were significantly higher than those in the north side (0.16 mg/kg, 4.02 µg/day), respectively (Guo et al., 2017).

Reasons for the deficiency of dietary Se intake of Tibetan adults living along Yalung Zangpo River

Highland barley (*Hordeum vulgare* L.) is the main food crop in Tibet, accounting for 43 and 38 % of the whole crops' planting area and yields in Tibetan Plateau (Wang J. et al., 2017; Feng et al., 2018). The present study found that highland barley contributed 34.2% of dietary Se intake of participants, which revealed that it is the primary source of the dietary Se for local Tibetan residents and was close to the contribution rate of grain to dietary selenium intake of Chinese residents (34–57%; Li et al., 2014). In the last century, 70% of the Se intake of rural Chinese residents came from their staple diet (Zhang et al., 2021); after 2000, cereals were still major Se source food in the daily diet with the development of the economy, such as 23% in the Suzhou area, a developed area in China (Gao et al., 2011). However, according to a WHO report, the typical high-Se food were organ meats and seafoods (0.4–1.5 mg/g; World Health Organization, 1987), thus in the UK, for instance, meat and poultry make a more important contribution than bread and cereals to dietary Se intake (Rayman and Callahan, 2006). Various ecological, environmental, geographical, and socio-cultural factors have catalyzed to evolve unique food systems of the local Tibetans (Kala, 2021). Highland barley is an irreplaceable staple food for Tibetan adults living along Yalung Zangbo River, not only for its high consumption frequency, but also for its unique nutritional contribution (Zhou et al., 2022). Furthermore, the monotonous diet in which there was little animal food might be an influence on the intake of selenium. The intake of fish, legumes, and fresh

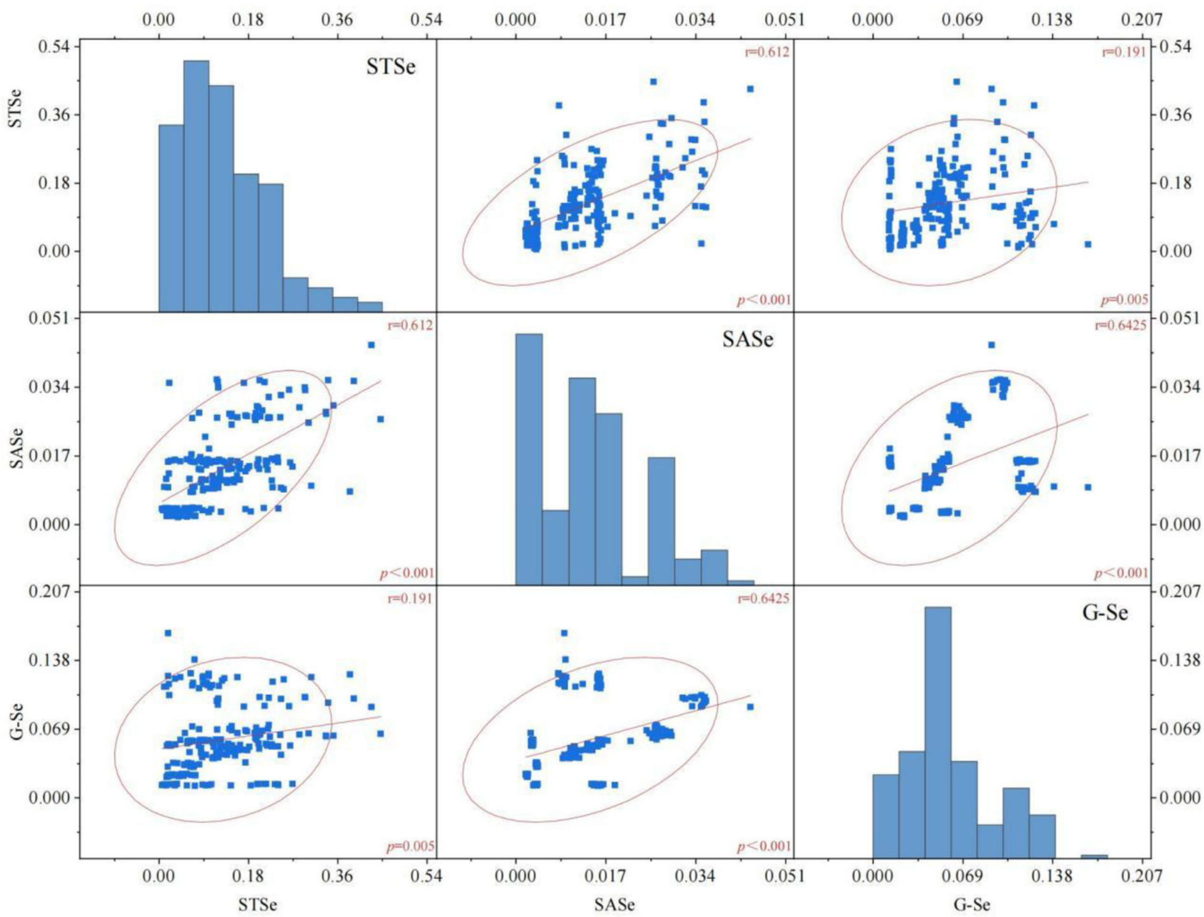

FIGURE 7
Matrix scatter plot for the analysis of the correlation among soil total selenium content (STSe), soil available selenium content (SASe), and barley grain selenium content (GSe).

TABLE 1 The vector value of environmental factors on the RDA ranking axis.

Statistics	STSe		SASe		GSe	
	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2
Eigenvalue	0.416	0.584	0.643	0.357	0.471	0.529
Explained variation (cumulative)	47.58	100	64.3	100	47.11	100
Pseudo-canonical correlation	0.6898	0	0.8019	0	0.6863	0
Cumulative percentage of species-environment relationships	100		100		100	

fruits positively associated with selenocysteine-bound selenium in body (Filippini et al., 2018). However, these food were not readily available in Tibet transportation and religious reasons. Balanced dietary pattern could play a key role in selenium nutrition of Tibetan population.

Interestingly, we also found that the portion of dietary Se provided by highland barley accounted for <10% of the dietary Se (*D-Se*). Soil total Se (STSe) and available soil Se(SASe) showed significantly positive relationship with grain selenium (GSe; Figure 7), which was consistent with previous studies (Tan et al., 2002; Temmerman et al., 2014; Wang Q. et al., 2017; Chang et al., 2019). Se enters the food chain through plants and the amount of Se in foods is directly affected by Se levels in the soil in which they are grown (Rayman, 2008). According

TABLE 2 Contribution rate and *p*-value of dominant environmental factors to STSe, SASe, GSe.

STSe			SASe			GSe		
Name	**Contribution %**	***P***	**Name**	**Contribution %**	***P***	**Name**	**Contribution %**	***P***
TOC	41	0.002	Clay	52.5	0.002	clay	14.3	0.002
Fe	21.3	0.002	Alt	19.2	0.002	TOC	10.8	0.004
clay	10	0.006	pH	10.2	0.002	Cu	15.5	0.002
MAP	6.6	0.002	Fe	7.8	0.002	silt	10.3	0.002
Zn	5.2	0.002	MAP	4.1	0.002	pH	8.4	0.006
sand	2.9	0.004	MAT	3.5	0.002	MAT	8.8	0.002
Mn	5.1	0.008	Mn	2.3	0.002	MAP	12	0.002
MAT	4.1	0.044	Sand	0.2	0.39	Zn	5.2	0.008
silt	1.8	0.054	Zn	<0.1	0.742	Alt	4.5	0.01
pH	1.4	0.108				Al	9.6	0.002
Al	0.3	0.714				Fe	0.3	0.498
Alt	0.2							
Cu	0.2							

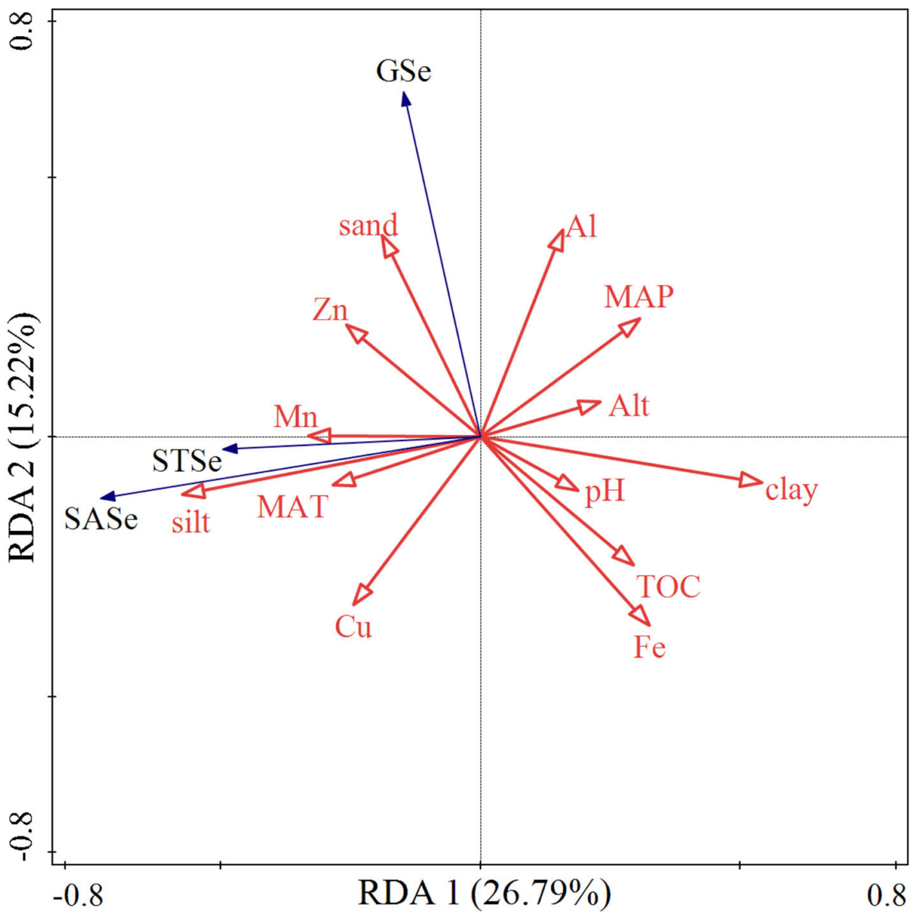

FIGURE 8
RDA sequence diagram of STSe, SASe, GSe, and environmental factors.

a previous study on exploring the quantitative relationships between Se concentration in various parts of highland barley plant and that in different species of corresponding soil, Se levels in highland barley were too low to meet the minimum requirements of human for daily intake of Se in Tibet and the restricting step for Se translocation was from soil to root (Wang J. et al., 2017). Previous studies have revealed the low selenium content of crops in KBD-affected areas is related to the relatively low content of available selenium in cultivated soil of those areas, not the soil total Se concentration. This is well-illustrated by data from the Keshan disease area of Hebei Province, China, that showed a high soil Se content but very low Se bioavailability owing to high organic matter content and lower pH than other soils in the region (Ge et al., 2000). According to a survey in KBD areas in Songpang county, located in the transition area between the eastern edge of the Tibetan Plateau and the northwestern plateau of Sichuan Province, the total soil selenium and the crops' Se concentration in some endemic areas was significantly higher than that of non-KBD areas. Meanwhile, the content and extraction rate of available selenium in KBD-affected areas were significantly lower than those in non-KBD areas. There is no significant correlation between any parts of plant Se and total soil Se ($p < 0.05$), but a distinct positive correlation between plant-available selenium and highland barley selenium ($r = 0.875$, $p = 0.001$; Wang et al., 2013; Wang J. et al., 2017). According to Fordyce (2013), it is important to understand that even soils that contain adequate or high total Se concentrations can result in Se-deficient crops if the element is not in a form amenable to plant uptake.

Moreover, the current study also revealed highland barley was the primary source of the dietary Se for local Tibetan residents. Therefore, it can be inferred that the insufficient dietary Se intake of Tibetan adult population living along Yalung Zangbo River is mainly caused by the low Se content in highland barley grain, which was result from the low Se content in cultivated soil and directly leaded to the low Se concentration in *tsampa* (a local Tibetan food flour obtained from roasted highland barley grains). Zhang et al. (2011) surveyed the arithmetic average value of highland barley grain in KBD areas in Tibetan plateau was 10.51 ± 5.18 μg/kg and the average value of *tsampa* was 16.82 ± 6.83 μg/kg, and the coefficient of Se in *tsampa* and highland Barley grains reaching a significant level ($r = 0.46$, $p < 0.05$). Wang et al. (2021) estimated the health loss from KBD in Qamdo district of Tibet using the years lived with disability (YLD) metric and investigated the influence of environmental selenium (Se) on it by multiple regression model. The multiple linear regression further revealed that Se contents in cultivated soil and highland barley were main influencing factors for the health loss of KBD, which could explain 90.5% of the variation in YLD rates.

Environmental determinants of Se concentration in food system of Tibetan adults living along Yalung Zangpo River

The present study, to the best of our knowledge, was the first to explore the environmental determinants of Se concentration in food system of Tibetan adults living along Yalung Zangpo River. In general, Se in the soil is ultimately derived from the parent material. Its content markedly depends on the origin and geological history of soil, and is controlled by mineralogy, weathering degree, and prevailing soil formation processes (Hartikainen, 2005), especially, low Se soils are typically derived from igneous rocks and found in regions with limited atmospheric deposition and high erosion rates (Christophersen et al., 2013). Previous studies in KBD endemic areas of the Tibetan Plateau revealed that the soil available selenium and ecological features are important factors that restrict the dietary selenium flux for local residents (Fordyce, 2005; Wang J. et al., 2017). Therefore, environmental factors that affect soil availability of Se will also affect soil total Se concentration (Wang et al., 2013).

In the present study, TOC had the strongest combined effect on STSe and SASe, with a contribution rate of 41.0 and 10.8%, respectively (Table 2). Total organic carbon (TOC) is the amount of carbon that represents the total amount of organic matter (OM) in the soil. Although the effect of OM on the availability of Se is double-edged (Dinh et al., 2018), we found TOC had significantly negative effect on STSe and GSe (Figure 8). Wang et al. (2012) also found that OM reduces the bioavailability of Se in 16 different soils with various physicochemical properties in China. Tolu et al. (2014) conducted a study on 26 soil samples and observed that OM content is negatively correlated with available Se content; when the OM content was <20%, the ambient Se solubility is mainly controlled by the adsorption process. A study conducted in Northeast China, a KD affected area, reported that although the concentrations of total soil Se in some of the regions are not Se deficient, the strong immobilizing effect of high OM content (6–10%) resulted in lower soil Se bioavailability as well as a low daily Se dietary intake of 7–11 μg by local residents (Wang and Gao, 2001). Moreover, we cannot ignore the impact of the long-term application with agricultural residues like manure in Tibet. Although the effect of manure application on the bioavailability of Se is still complicated, long-term application with agricultural residues like manure changes soil properties, improves soil nutrient and OM content, thus altering the bioavailability of Se (Schnitzler et al., 2007; Hamner and Kirchmann, 2015). An earlier study found that Se accumulation decreased from 7 to 10 times in canola leaves when animal manure was used to Se (VI)-treated soil (Ajwa et al., 1998). The same effect was also observed in a filed study about Se accumulation in wheat and oilseed rape grains, with a decreasing rate from 23 to 95% after the use of poultry manure and farmyard manure (Sharma et al., 2011). Soil OM content not only reduces soil Se content and its availability, but also affects the uptake of soil Se by crop, which in turn affects Se concentration in different tissues of crops. A pot experiment further confirmed that the Se content in wheat grain reduced from 1.35 to 0.16 mg/kg when the soil OM content is increased (Johnsson, 1991).

Consistent with previous findings, we also found that pH and clay had negative effects on STSe, SASe, and GSe (Figure 8). Studies on 18 main types of Chinese soil demonstrated that irrespective of Se (IV) or Se (VI) form, pH played a negative role in the adsorption process. Selenium adsorption decreased when pH increased (Li et al., 2016; Wang D. et al., 2017). In general, the availability of Se is higher in alkaline soils than in acidic soils (Lee et al., 2011). In neutral and acid soils, the tetravalent selenite state is the major form and is generally fairly insoluble, while in well-aerated, alkaline soil, the hexavalent selenate state is the dominant form, which is easy to dissolve in water and absorb by plant (Wang D. et al., 2017). Clay-Se interactions occur mainly *via* the adsorption process, and thus the available Se is negatively correlated with the content of soil clay (Li et al., 2015), mainly for clay minerals are positively charged and thus able to adsorb Se oxyanions (Loganathan et al., 2014). Xu et al. (2010) found the clay with particle sizes <0.025 mm increased the amount of Se adsorbed to soil particles, thus reducing bioavailable Se in the soil of Hainan province in China, whereas soil particles with sizes >1 mm have no fixed effect on Se.

We also found that the contents of Fe and Al were negatively correlated with the contents of STSe and SASe. Fe/Al/Mn oxides are regarded as the major factors for the adsorption process because of their extensive chelating ability and specific surface area (Muller et al., 2012). Li et al. (2015) found that Fe/Al oxides are important for Se(IV) adsorption on 18 types of Chinese soils and are positively correlated with the adsorption capacity. Feng et al. (2016) also concluded that amorphous Fe is the largest Fe-oxide, and could form stable inner-sphere complexation with Se(VI); its hydroxide was able to co-precipitate with Se, and Se bioavailability was thereby reduced in the 18 types of Chinese soils.

Climate also has an important influence on the bioavailability of Se by affecting the amount of Se in the soil or the absorption of Se by plants through direct mechanisms such as deposition or indirectly by the soil retention of Se such as sorption (Ham and Tamiya, 2006; Jones et al., 2017). In the present study, it was found that the average annual precipitation (MAP) had a negative effect on STSe, SASe, and GSe (Figure 8). High precipitation results in excessive losses of plant available Se by leaching was been revealed as early as 1968, Geering et al. (1968) have shown that precipitation influences the shift from oxic to more anoxic soil redox conditions, leading to an increase in less available Se forms, and thereby reducing Se accumulation in plants (Geering et al., 1968). However, other studies have reported that precipitation brings a large amount of Se from the atmosphere into the soil and increases the transportation of dissolved Se in the soil solution (Blazina et al., 2014; Winkel, 2016). Jones et al. (2017) identified climate-soil interactions as main controlling factors in the context of global change and predicted future (2080–2099) soil Se losses from 58% of modeled areas (mean loss = 8.4%), especially higher in croplands, with 66% of croplands predicted to lose 8.7% Se. However, a positive correlation between STSe, SASe, and MAT was found in the present study (Figure 8). Some studies have reported that low temperatures can reduce Se accumulation in plants, soil Se concentration decreased sharply during the growing season, but this trend was stopped toward the end of the growing season when plant growth slowed down as winter and cooler temperatures approached (Bisbjerg, 1972; Gissel-Nielsen, 1975).

Altitude range of Tibet Autonomous Region is from 610 to 4,795 m, and unique geographical conditions may lead to differences in the accumulation of nutrients and mineral elements in highland barley within Tibet Autonomous Region (Zhang et al., 2021). Therefore, altitude was considered as a terrain factor for this study, which had a negative effect on STSe and SASe. From the perspective of geographical environment, previous studies indicated that there was a highly significant impact from elevation and its hydrothermal conditions on the geographic differentiation of soil selenium. Peng and Wang (1995) studied the selenium species of cultivated soil in the Jiabawa Plateau in southern Tibet and found that the residual selenium content increased significantly as the altitude increased. Wang et al. (2013) conducted the correlation analysis of the trend between available selenium content of cultivated soil and corresponding altitude, found that the available selenium content of cultivated topsoil significantly decreases as the altitude increases ($r = -0.801$, $p = 0.010$).

Strengths and limitations

The present study, to the best of our knowledge, was the first to systematically evaluate the status of Se in Tibetan food system (soil-highland barley-dietary intake) and explore the environmental determinants of Se concentration in soil-highland barley system of Tibetan adults living in 14 agricultural counties along Yalung Zangpo River. Although the scope of the survey was relatively wide, and basically covered all major grain-producing counties in Tibet, it still had some shortcomings. Firstly, although the effect of individual environmental factors on Se flux is explored in the present study, the combination of factors affecting Se flux in Tibetan food system was not been fully discussed. For example, regarding the combined effect of Fe/Al/Mn oxides and pH, Dhillon and Dhillon (1999) claimed that Se is principally adsorbed in the amorphous iron surface in acidic soil, which is correlated with the formation of inner-sphere complexation between Fe-oxides and Se(IV). Regarding the combined effect of OM and pH, low-molecular-weight organic acids can dissolve and release Se that is immobilized onto the soil solid phase; thus, the bioavailability of Se is promoted under low pH and high OM condition (Sharma et al., 2015; Dinh et al., 2018). Regarding the combined effect of precipitation and altitude, Wang D. et al. (2017) found precipitation in KBD endemic areas in Tibet was high and concentrated at high elevations, the terrain changes considerably and the soil eluviations is significant, which exacerbates the loss of water-soluble and exchangeable Se in soil, and eventually leads to relatively low soil available Se and inefficient translocation of Se in the food system. Secondly, analysis about how soil-to-crop transfers of Se and intake of Se into food systems in Tibet was not conducted, and there was a lack of reliable biomarkers of Se status to identify where Se is in deficit and where it is adequate. Direct assessment of human Se deficiency requires blood sampling, which is a complex task for large-scale population studies, especially in outlying and poverty-stricken areas in Tibet with backward economy and transportation. Therefore, a typical diet survey with the utilization of the national food-composition tables giving the Se content of foods is much more effective, for it is not confounded by the variety and variation of local dietary pattern (Zhou et al., 2021).

Conclusion

There was a widespread deficiency of Se in "soil-highland barley-dietary intake" food chain of Tibetan adult population living in 14 agricultural counties along Yalung Zangpo River. It can be inferred that the insufficient dietary Se intake of Tibetan adult population is mainly caused by the low Se content in highland barley grain, which was result from the low Se content in cultivated soil and directly leaded to the low Se concentration in *tsampa* (a local Tibetan food flour obtained from roasted highland barley grains). Although highland barley was the first contributor of dietary Se in local adult residents (34.23%), the dietary Se intake provided by highland barley only about 10% of the EAR value (50 μg/day/adult) currently. There was still a lot of room for improvement in the role of highland barley in reaching the dietary selenium intake standard of local residents. In order to enable adult participants in the present study to achieve recommended Se-intake levels, agronomic fortification with selenized fertilizers applied to highland barley could be a great solution, which has the merit of using plants as effective biological barrier that protects the target population from the effects of accidental overdose (Aro et al., 1998), developing Se-enriched agricultural products to improve human Se nutrition and health (Newman et al., 2019). An successful agronomic Se biofortification example is in Finland. The Finnish government who have made it mandatory to add selenate to all multi-element fertilizers to overcome Se deficiency, which resulted in an increase in Se intake from 0.025 mg/d/10MJ before fortification in the 1970s to 0.08 mg/d/10MJ in 2013 (Alfthana et al., 2015). The entrance of Se into the terrestrial food chain is primarily dictated by the availability of Se in soil for plants. Therefore, an essential part of a resource efficient and sustainable agronomic fortification strategy includes proper use of Se fertilizers that takes the spatial soil variability, climatic and terrain conditions, and cropping systems into consideration.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Chinese Center for Disease Control and Prevention (CDC) of Tibet Autonomous Region. The patients/participants provided their written informed consent to participate in this study.

Author contributions

FZ and WC: supervision. ML and CZ: data collection and survey. RX and CZ: data analysis. CZ: manuscript writing. QW: soil sampling and determination. WC: conception. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.1007876/full#supplementary-material

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