Selenium Fractionation and Speciation in Paddy Soils and Accumulation in Rice Under Field Conditions in Jinhua Zhejinang Province, China

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Abstract. Soils, as well as paddy tissue samples, were collected in the Se-rich area of Jinhua County, Zhejiang Province, China. Sequential extraction procedure was used for selenium (Se) fractionation, including soluble Se, exchangeable Se, carbonate-bound Se, iron and manganese oxide-bound Se, humic acids-bound Se, organic matter-bound Se, and the residual Se fraction. The results showed that soluble Se, exchangeable Se, carbonate-bound Se, iron and manganese oxide-bound Se fractions accounted for less than 2% of the total Se, respectively. Organic matter-bound Se was the dominant fractions. The average concentrations (mg kg⁻¹) of Se in the paddy tissues were 0.069 in seed, 0.263 in root, 0.09 in stalk, and 0.17 in leaf. The organic matter-bound Se had a significant indirect effect on Se accumulation in paddy tissues. In conclusion, organic matter-bound Se was an important fraction and source of plant Se in agricultural soil.

1 Introduction

Selenium (Se) has been recognized as an essential trace element for humans and animals [1] because of its critical role in organic antioxidant defense systems [2] and cancer prevention [3]. Therefore, an adequate daily Se intake is required to maintain health. However, in many regions worldwide, such as in central China, Finland, and Sweden, the estimated Se intake rate from food consumption is generally lower than the 40 µg per day recommended by the World Health Organization [4-5].

This condition is attributable to the generally insufficient Se content in environment, resulting in inadequate Se in food [6]. Se supplementation by spraying with Se-enriched fertilizers to increase Se concentrations in agricultural products are some prevalent approaches [7], for example, the crop plant and vegetation Se biofortification strategy in the UK [8]. By contrast, it might be a more efficient and effective option to exploit Se-enriched food in soils naturally abundant in Se, which distribute in some certain regions of the world, such as Enshi and Ziyang, China [9-10], South Dakota, USA [11] and Punjab, India [12]. Among them, 10 to 30 mg/kg Se in soil from Ziyang County in Shaanxi Province, China has been reported [9], and soils containing more than 3.0 mg kg⁻¹ total Se can be defined as excessive for human nutrition [13].

However, Se uptake and accumulation by plants depend more on the abundance of Se in different fractions and species than the total Se concentration in soil [14-16]. Extensive studies have been conducted previously on the uptake and accumulation, as well as

translocation and distribution of selenate or selenite in higher plants utilizing exogenic Se[17-18]. While Se from anthropogenic sources oftentimes differs from that in natural soils. To date, studies have primarily focused on the sources and distribution of Se in the environment in Jinhua, and little is known about the fractions and species of Se in these soils, as well as plant Se accumulation, distribution, and translocation.

Therefore, the specific objectives of the present study were mainly to (1) determine Se fractions in naturally seleniferous soil in Jinhua; (2) investigate the effects of soil Se fractions on Se accumulation in paddy tissues; (3) to establish the relationship between plant Se accumulation and soil Se bioavailability quantified using sequential extraction procedures.

2 METHODOLOGY

2.1 Field sampling

Field survey and sampling were performed in Wucheng District, Jinhua County, Zhejiang Province (Fig. 1). 64 surface topsoil samples and 33 rice samples along with soil samples in the same sampling fields were collected. Additionally, 10 paddy tissues samples were also collected. Surface samples were stored in airtight fabric bags, and paddy tissues samples were stored in plastic bags during transport from the field to the laboratory.

2.2 Sample preparation

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After removal of plant and detrital materials, soil samples were ground to pass through a 100 mesh nylon sieve (0.15 mm) for chemical analyses.

Paddy tissues samples were washed with running tap water and then with deionized water to remove dust and soil. Washed paddy tissues samples were separated into the various plant parts (roots, stalks, leaves, and seeds), and then oven-dried at 95 °C for 30 min, and then at 60 °C to a constant weight. All plant samples were ground into fine powder using a steel mill and then kept in a dark room at room temperature.

Fig. 1. Locations of different samples

2.3 Chemical analysis

2.3.1 Extraction of Se fractionations in soil

Soil samples were analyzed for Se fractions using the five-step sequential extraction method as follows:

(1) Soluble Se: 10 ml 0.25 mol l⁻¹ KCl was added to 1.0 g soil. Then, the tube was shaken at 200 rpm at 25 °C for 1 h. The mixture was centrifuged for 10 min at 4000 rpm, and filtered through a 0.45 µm filter. The supernatant was collected for further speciation analysis, and the remaining precipitate was used for the next step of extraction. The same centrifugation and filtration steps were conducted after each of the following extraction procedure.

(2) Exchangeable Se and carbonate-bound Se: 10 ml 0.7 mol l⁻¹ KH₂PO₄ (pH 5.0) was added to the above tube and was shaken at 200 rpm and 25 °C for 4 h.

(3) Iron (Fe)/manganese (Mn) oxide-bound Se: 10 ml 2.5 mol l⁻¹ HCl was added to the remaining soil. The capped vials were then heated in a water bath at 90 °C for 50 min. The centrifuge vials were also shaken intermittently.

(4) Organic matter-bound Se and humic acid Se: 8 ml 5% $K_2S_2O_8$ and 2 ml concentrated HNO_3 were added to each remaining soil sample, and the vial was heated for 3

h in a water bath at 95 °C. The tube was intermittently shaken from time to time.

(5) Residual Se: the soil residuals were transferred into a Teflon crucible with 8 ml concentrated HNO_3 and 2 ml concentrated HClO_4 . The crucibles were covered and heated at 170 °C in a sand bath until the soil appeared white or gray. After acid digestion, the solution was transferred to a 25 ml volumetric flask with deionized water.

The centrifuge tube was weighed before and after each extraction to calculate the remaining extractant entrained in the soil residue. Se concentration in the remaining solution was subtracted from the measured Se concentration of each extraction.

2.3.2 Digestion of rice tissues

Concentrations of total Se in rice samples were determined using the Standard Method GB/T 5009.93-2003. Briefly, 1.0 g of each sample was weighed into a 100 ml glass tube. Concentrated HNO_3 (8 ml) and 2 ml concentrated HClO_4 were added, and the digestion tubes were kept overnight at room temperature (25 °C). Acid digestion was conducted at 170 °C until the digestion solution became clear. After acid digestion, sample solutions were cooled and transferred into a 25 ml volumetric flask with deionized water.

2.3.3 Determination of Se concentration

Se concentration in the solution was determined using atomic fluorescence spectrophotometer with hydride generation according to GB/T 5009.93-2003. solutions obtained by extraction steps 1 and 2 were treated as described in Section 2.3.1 before determination; solution obtained by extraction steps 4 and 5 and acid digestion were heated at 100 °C for 15 min in 6 mol l⁻¹ HCl solution.

3 RESULTS

3.1 Distribution of Se in soils

The total Se concentrations in soil samples ranged from 0.23 to 0.58 mg kg⁻¹ with an average of 0.39±0.08 mg kg⁻¹ (Table 1). The only one sample contained extremely high concentration of Se, which were located at cropland with the concentration of 1.58 mg kg⁻¹. Accordingly, we suggest that the mean of the 64 results represents a normal range for soils in most of the field area. The geometric mean of Se concentration in these soils is 0.39 mg kg⁻¹, is approximately 3 times greater than the average Se content (0.125 mg kg⁻¹) in Se-deficient areas and approximated that in Se-rich areas (0.4 mg kg⁻¹) in China, respectively [19]. However, the moderate coefficient of variation (45%) for Se in soil indicates that distribution of Se in soils is uneven.

Table 1. Summary of Se concentrations in the different samples.

Samples	Min (mg kg ⁻¹)	Max (mg kg ⁻¹)	A (mg kg ⁻¹)	N
Soil	0.23	1.58	0.39	64
Rice	0.023	0.32	0.069	34
Root	0.21	1.00	0.263	10
Stalk	0.055	0.12	0.09	10
Leaf	0.11	0.25	0.17	10

N: number of samples; A: average concentration

3.2 Se fractions in soil

The Se fractionation results were shown in Fig. 2. Concentrations of soluble, exchangeable, carbonate-bound and Fe-Mn oxides Se in soils were 0.005 mg kg⁻¹, which suggested these four fractions have reached saturated state.

Fig.2. Se fractions in the paddy soils

OM-Se is the main fraction of Se in the soil, ranged from 0.114 to 0.193 mg kg⁻¹ with an average of 0.155 mg kg⁻¹. And its corresponding ratio to total Se was 44.50±6.75%. OM-Se primarily originates from plant decomposition, with a small contribution from rainfall. Se is a pro-biological element that exists and bio-accumulates as organic compounds in plants or organisms [20].

The range of the RES-Se content in soils was 0.029-0.080 mg kg⁻¹, and its corresponding ratio to the total Se was 15.29±2.76%. Residual Se primarily occurs in silicate minerals and cannot be easily damaged by acid-base reactions.

3.3 Distribution of Se in rice

The concentrations of Se in rice samples ranged from 0.023 to 0.10 mg kg⁻¹ with an average of 0.069 ± 0.05 mg kg⁻¹. Only one sample with the concentration of 0.32 mg kg⁻¹, which were located at brickkiln. This suggests a coal combustion that might lead to the extreme Se enrichment. The average concentration exceeded the national standard of Se-rich rice. However, the spatial distribution of Se in rice was also uneven. The highest Se concentration samples located at the village of Da yuan tou, and the lower Se concentration samples located at the southern of the town of Langya.

3.4 Accumulation and distribution of Se in paddy tissues

Se concentrations of root, stalk and leaf were 0.263 ± 0.04 mg kg⁻¹, 0.09 ± 0.02 mg kg⁻¹ and 0.17 ± 0.04 mg kg⁻¹, respectively. And the Se concentration of rice was 0.069 ± 0.01 mg kg⁻¹. Overall, the total Se concentrations in the various paddy tissues were in the following order: root > leaf > stalk > rice. A significant correlation ($P < 0.05$) was found among the Se concentrations of the different paddy tissues.

Se concentration in paddy tissues was significantly correlated with the OM-Se concentration in soil, and the correlation coefficient reduced gradually in leaf and rice (Table 2). HM-Se had relatively greater positive effect on Se accumulation in paddy root than in stalk, leaf and rice. RES-Se had no direct effect on Se accumulation in rice, leaf, stalk and root.

Table 2. Correlations between Se contents in paddy tissues and Se concentrations in different fractions in the soils

Se fractions	Se contents in various paddy tissues			
	Root	Stalk	Leaf	Rice
HM-Se	0.454*	0.221	0.195	0.112
OM-Se	0.772*	0.665*	0.678*	0.588*
RES-Se	0.321	0.191	0.171	0.122

* Statistically significant ($p < 0.05$)

4 DISCUSSION

The bioconcentration factor ($BCF = [Se]_{\text{plant}}/[Se]_{\text{soil}}$) of Se in the present study was 0.181, investigation on soil from seleniferous area in Enshi showed the average ratio of whole grain Se concentrations to soil Se concentrations (BCF) was 0.23 [21]. Similarly, Dhillon [22] found that, in two types of weed plants, Se accumulation in natural seleniferous soil was almost 50% of that in the selenate-enriched soil. These results implied moderate soil Se bioavailability in the study soil, relatively lower than in soil treated with inorganic Se, which was easily conceivable given the soluble Se concentration in soil. Therefore, the limitation of Se bioavailability was one key obstacle to the exploitation of Se resource in the study region.

In addition to the variation in total Se concentration, the fractions of Se differed individually among the soil samples in this study (Fig. 2). Soluble, exchangeable, carbonate-bound and Fe-Mn oxide-bound Se accounted for less than 2%, respectively. Tan [19] found that the percentage of water-soluble Se varied from 1.07% to 6.69% in 20 main soil types in China. Experiments using parallel single extraction procedure also indicated that water soluble Se accounted for 1.4% to 14% of the total soil Se [23]. In contrast with other high selenium soil, concentrations of water-soluble and exchangeable Se in upland soil from Enshi and surface soil from polluted surface mine environment showed similar results with the present study [24]. However, an investigation in the states of Punjab and Haryana found that more than 50%

of the total Se was extracted by water and phosphate buffer solution in seleniferous soil. Whereas in soils of nonseleniferous regions, 90% and more of Se could be determined in the remaining fraction [25].

In the long-term process of bedrock weathering and atmospheric deposition, Se primarily existed in the form of inorganic colloids or was bound to organic matter [26]. Organic matter-bound Se accounted for the majority of soil Se, approximately 50% of the total Se. Hence, it might be inferred that the ultimate accumulation of Se in the organic matter-bound fraction would be the main process in the biogeochemical cycle of Se in the study region. Concentrations of Se in paddy tissues significantly correlated with OM-bound Se. Thus, Se releasing from the organic matter-bound fraction became dominant over other fractions during plant-growing season. This might attenuate Se deficits in soilsolution and enhance Se accumulation in rice. Soil organic matter-bound Se, affected by soil microbial activity, was recognized as potentially available to higher plants. Therefore, clarifying the biotransformation of Se might be crucial to understand soil capability of supplying Se and Se accumulation in plants.

HM-Se accounted for approximately 30% of the total Se. Humic acid is a polymer with a stable structure, thus, plants have difficulty absorbing the Se bound by humic acid. In comparison, the structure of fulvic acid is relatively simple, and the fulvic-acid-bound Se can be easily mineralized and decomposed into inorganic forms and low-molecular-weight organic compounds, such as Se amino acids, for easy absorption by plants. The corresponding ratio of RES-Se to the total Se was approximately 15%. No significant correlation ($p > 0.05$) was observed between the residual Se in the soils and the total Se in the paddy tissues. Residual Se primarily occurs in silicate minerals (e.g., feldspar, quartz, mica, etc.) and cannot be easily damaged by acide base reactions. Obviously, such forms of Se are unavailable to plants. However, under certain condition, residual Se could be converted into available Se to plants, so RES-Se was usually considered a library source of Se.

5 CONCLUSIONS

Concentrations of Se in agricultural soils of this study ranged from 0.23 to 0.58 mg kg⁻¹, and 0.023 to 0.010 mg kg⁻¹ in rice. Organic matter-bound Se accounted for the majority of total Se in soil. So the ultimate accumulation of Se in the organic matter-bound fraction remained as the main process in the biogeochemical cycle of Se. Concentrations of soluble, exchangeable, carbonate-bound and Fe-Mn oxide-Se have no significant change with a saturated state, which had no indirect effect on Se accumulation in rice. Therefore, Se released from the organic matter-bound fraction was dominant during short planting season, which might attenuate the deficit of Se in soil and enhance Se accumulation in rice.

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