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Selenium Distribution and Translocation in Rice (*Oryza sativa* L.) under Different Naturally Seleniferous Soils

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Abstract: Selenium (Se) accumulation in plant foods may be providing dietary Se to minimize the health problems related to Se deficiency. In this study, rice plants were cultivated in different naturally seleniferous soils (0.5–1.5 mg Se kg^{-1}). Se concentration in rice plant tissues was analysed, and the distribution and translocation of Se in rice were also studied. The effect of exogenous Se on yield and Se concentration in rice grain was also investigated by spraying Na_2SeO_3 (15 mg L^{-1}, 15 g ha^{-1}). Results show that Se concentration in root, straw and grain of rice was increased with increased concentrations of Se in seleniferous soils. The root accumulated higher Se than straw and grain under the same naturally seleniferous soil. Spraying Se significantly increased Se concentration in grain, hull, brown rice and polished rice compared with spraying water. Se concentration in the grain fractions was in the following order: Bran > brown rice > whole grain > polished rice > hull. About 13.7% Se in wholegrain was discarded by milling process if about 6.9% of it was polished as bran. Se-enriched rice could be produced in naturally seleniferous soils with Se concentration from 0.5 to 1.0 mg kg^{-1}, and this polished rice would provide enough Se (60–80 µg day^{-1}) to satisfy the human requirement. Therefore, naturally seleniferous soils may be an effective way to produce Se-enriched rice without spraying Se fertilizer, which will be more economically feasible and environmentally friendly for without exogenous Se added to the soils or plants. However, the polished rice and brown rice, produced by spraying Na_2SeO_3 (15 g ha^{-1}) or grown in soil with total Se upto 1.5 mg kg^{-1} was not suitable for daily human consumption, unless diluted with Se-deficient rice to meet the standard ($\leq$0.3 mg Se kg^{-1}). This study imparted a better understanding of the utilization of seleniferous soils and Se-enriched rice for human health and food safety.

Keywords: rice *Oryza sativa* L.; selenium; distribution; accumulation; polished rice

1. Introduction

Selenium (Se) is considered as an essential micronutrient for animals and humans [1–3], and has an important role in improving the immune system and reducing the risk of cancer [2–4]. However, Se deficiency is an important cause of some human diseases, including Keshan disease and Kaschin-Beck disease [5]. The recommended level of Se recommended by the World Health Organization (WHO) for adults is 40 µg day^{-1} [3,6,7]. However, in many regions in China, the daily Se intake of people is lower than the suggested level [3], and its levels in some Se-deficient areas are less than 10 µg day^{-1} [8].

People could acquire Se from their diet to reduce the occurrence of diseases related to Se deficiency, as previously reported [3,9]. Rice (*Oryza sativa* L.) is a staple food in many countries, particularly in China [3,10]. Nevertheless, Se concentration in rice is too low to meet the people's daily demand in many countries, including many areas in China [10,11]. Se concentration in rice varies according to Se status of the paddy soil. The rice grown in soil with Se concentration within the range of 0.5 to 47.7 mg kg^{-1} could produce Se in wholegrain ranged from 0.084 to 9.67 mg kg^{-1} [3]. Some field experiments have indicated that Se concentration in rice grain can be significantly increased by spraying Se fertilizers [8,12,13]. However, this method is associated with high costs, and exogenous Se spraying may cause soil and water pollution [10,14,15]. Therefore, the production of high-Se food under natural Se-rich soil may be an effective Se biofortification scheme.

The accumulation of Se in the edible parts of the plant depends on Se concentration in soil and the Se distribution in plant organs [3,9]. Evidence indicated that rice grain accumulated more Se in high-Se soils than that in the low-Se condition [3]. However, when plants were grown in high Se concentration, the growth was significantly inhibited, even led to death [1,2]. The Se concentration between deficiency and toxicity is narrow [2], which has a major implication for human health. The upper limitation of Se intake is 400 μg d^{-1} for adults recommended by the WHO [16]. The upper standard Se concentration in cereal for food is 0.3 mg kg^{-1} in China [3]. For the purpose of this study, a limit of 0.3 mg kg^{-1} is also applied to rice grain, including the whole grain, brown rice and polished rice. Therefore, to ensure the safety of Se in food, understanding Se absorption by the roots, accumulation and distribution within the rice, is important to predict the Se concentration in grain under seleniferous soils.

The concentration of total Se in soil ranges from less than 0.1 mg kg^{-1} to more than 100 mg kg^{-1} [17]. In general, 0.1 to 0.6 mg kg^{-1} total Se in soil is considered deficient [18]. The average Se content in the soil of Shitai district, Chizhou city, Anhui province, China, is 0.67 mg kg^{-1}, which is categorized as medium-high Se content in China [19]. Se concentration in rice varies depending on the Se concentration in which rice is grown [3,14]. Currently, only few pieces of research have focused on Se accumulation and distribution in rice plant and the grain fractions [3], especially in natural seleniferous soils. Therefore, understanding the amount of Se stored in the different components of the grain and detecting the absolute loss of Se during the dehusking process of rice are of great importance.

To understand the accumulation and distribution of Se in rice, a local rice variety "Wandao 205" was selected and planted in three different levels of naturally seleniferous soil of Shitai district, Chizhou city, Anhui province, China. And the effect of exogenous Se on yield and Se concentration in rice grain was also investigated by exogenous Se spraying. This study aimed to confirm the suitable Se concentration range of the soil for achieving Se-enriched rice and to elucidate the correlations between Se concentration in rice and the seleniferous soils.

2. Material and Methods

2.1. Experimental Site and Growth Conditions

Two pot experiments were carried out in this study, including experiment of seleniferous soil (Exp. 1) and the experiment of exogenous Se application (Exp. 2). The experiments were conducted under greenhouse conditions in major rice-growing areas of Anhui province, China [20]. Rice (*O. sativa*) cultivar "Wandao 205" was used in this study.

Experiment. 1: Three paddy soils with different Se concentrations (0.50, 0.97, and 1.47 mg Se kg^{-1}) were selected to investigate the effect of naturally seleniferous soil on yield and Se content of rice in this study. Before the present study, selenium content in paddy soils was investigated by collecting 22 surface soil (0–20 cm) samples in Shitai county, Anhui province in 2011. The total Se content in soil was between 0.31 and 1.99 mg kg^{-1}, with an average of 0.60 mg kg^{-1} in all soil samples. Soil Se concentration was measured following the methods of Sun et al. [3]. Two pots were used for one treatment. A randomized block design with three replicates was used in the experiment. Therefore, 6 pots were included in each treatment.

Experiment. 2: Exogenous application of Se was conducted by spraying on rice plants grown in paddy soil with low Se concentration (0.31 mg Se kg^{-1}). The Se applied was Na_2SeO_3 with the concentrations of 0 and 15 mg L^{-1} (at a rate of 15 g Na_2SeO_3 ha^{-1}, i.e., 3 mL for one hill). The Na_2SeO_3 was sprayed at the heading stage of rice (50 d before maturity). For 0 mg Na_2SeO_3 L^{-1} treatment, the rice plants were sprayed with distilled water. Two pots were used for each treatment, and three replicates were used.

Rice seedlings (three-leaf stage) were transplanted to experimental pot (35 cm of diameter, 28 cm of height) containing 15 kg of different naturally seleniferous soils on 1 August 2012. Two rice seedlings per hill were transplanted with space of 20 cm × 15 cm. Four hills were contained in one experimental pot. Two pots were used for one treatment. Three replicates were used. Therefore, 6 pots (total 24 hills) were contained for each treatment. The basal fertilizer (60 kg N ha^{-1}, 30 kg P_2O_5 ha^{-1}, 45 kg K_2O ha^{-1}) was applied before transplanting, and the first top-dressing (15 kg N ha^{-1} and 15 kg K_2O ha^{-1}) and second top-dressing (15 kg N ha^{-1} and 15 kg K_2O ha^{-1}) was applied at 20 d and 40 d after transplanting, respectively. The temperature was 25–33 °C for daytime and 15–23 °C for night-time, with a relative humidity of 60%–85% in the greenhouse during the experiment. The physicochemical properties of the experimental soils were provided in Table 1.

Table 1. Main physicochemical properties of the experimental soils.

Experiments	**Treatments**	**pH 2.5:1**	**Organic Matter ($g\ kg^{-1}$)**	**Available N ($mg\ kg^{-1}$)**	**Available P ($mg\ kg^{-1}$)**	**Available K ($mg\ kg^{-1}$)**
Exp. 1	Low-Se	4.8	19.4	117.1	44.3	249.0
	Middle-Se	4.9	22.6	120.4	81.2	285.5
	High-Se	4.7	33.3	153.9	51.1	301.7
Exp. 2	Spraying water Spraying Se	4.9	22.2	130.9	50.4	251.2

Note: Spraying Se: Na_2SeO_3 was applied with the concentrations of 15 mg L^{-1} at the heading stage of rice.

2.2. Grain Yield and Yield Components

At harvest, rice plants were separated into roots, straws and grains. The straws and roots were cleaned and dried at 60 °C to a constant weight. The grains were air dried at room temperature, and weighed. The number of panicles, spikelets per panicle, filling rate, and 1000 seed weight, were also scored [21].

2.3. Total Se Analysis

The grain parts were dehusked by a roller sheller (SY2001-NSART100, SANGYONG, Korea), and were divided into hull and brown rice. A portion of the brown rice was milled into polished rice and rice bran using a rice polisher (Kett Electric Laboratory, Tokyo, Japan) [22]. The grain and its four fractions (i.e., hull, brown rice, bran, and polished rice) were dried at 60 °C to a constant weight [22]. All plant samples, including the root and straw, were milled into powder with a DFY-1000C mixer mill (Shanghai Bilon Instrument, Shanghai, China).

Se concentration of the samples was determined by the method of Gao et al. [23] and Zhou et al. [24] The dried powders of all samples (0.5 g) were digested by 10 mL HNO_3:$HClO_4$ (9:1) in a polypropylene sample tube at room temperature overnight. Then, the digestion solution was heated at 60 °C and 100 °C for 2 h and 1 h, respectively. The addition of 10 mL of HNO_3:$HClO_4$ (9:1) in the tube was maintained at 170 °C to generate white fume and till the white fume was completely out. The tubes were allowed to cool, and then added 5 mL of HCl (1:1) and maintained at least for 3 h until it became colorless. After cooling, the digests were filtered and added with deionized water to 50 mL. The digest solutions were determined for Se concentration using an inductively coupled plasma mass spectrometry (ICP-MS) [X Series ICP-MS (Thermo Electron Corporation, Waltham, MA, USA)] [25,26]. During the digestion procedure and measurement, a reagent blank and standard reference material [GBW10010(GSB-1) (polished rice) [27], purchased from Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences, Langfang, Hebei, China] were used to validate the accuracy and precision. The average recovery of Se was between 95% and 98% [26]. All materials were determined in three replicates. All the above reagents used in the experiment were super pure grade.

2.4. Statistical Analysis

Data were analyzed using ANOVA and Duncan's multiple range test at $P < 5\%$ with SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Figures were drawn using Microsoft Excel 2013.

3. Results

3.1. Grain Yield and Its Components

Se concentration in soil significantly affected the grain yield of rice (Figure 1A). Grain yield in high-Se treatment was significantly lower than that in middle-Se treatment. However, foliar application of Se did not significantly affect the grain yield compared with spraying water (Figure 1B). As shown in Figure 2, the panicle number of panicles decreased as the Se concentration in soil increased. However, no significant difference among the treatments was detected. By contrast, compared with that of the low-Se treatment, the spikelets per panicle, filling rate and 1000-seed weight were all slightly increased in the middle-Se treatment, but decreased in the high-Se treatment (Table 2).

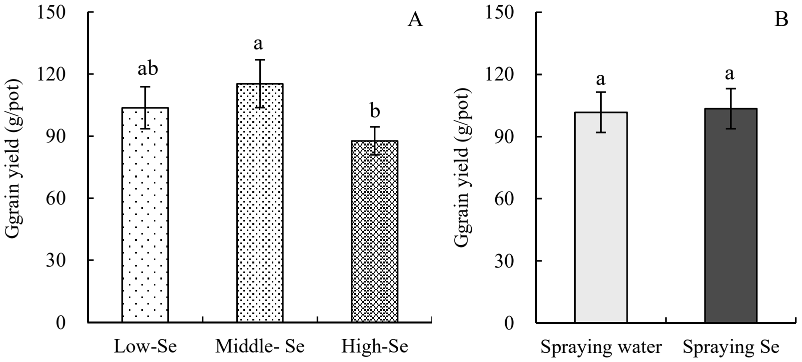

Figure 1. Grain yield of rice under different naturally seleniferous soils (**A**) and foliar of Se application (**B**). Columns withed different lowercase letters indicate a significant difference ($P < 5\%$).

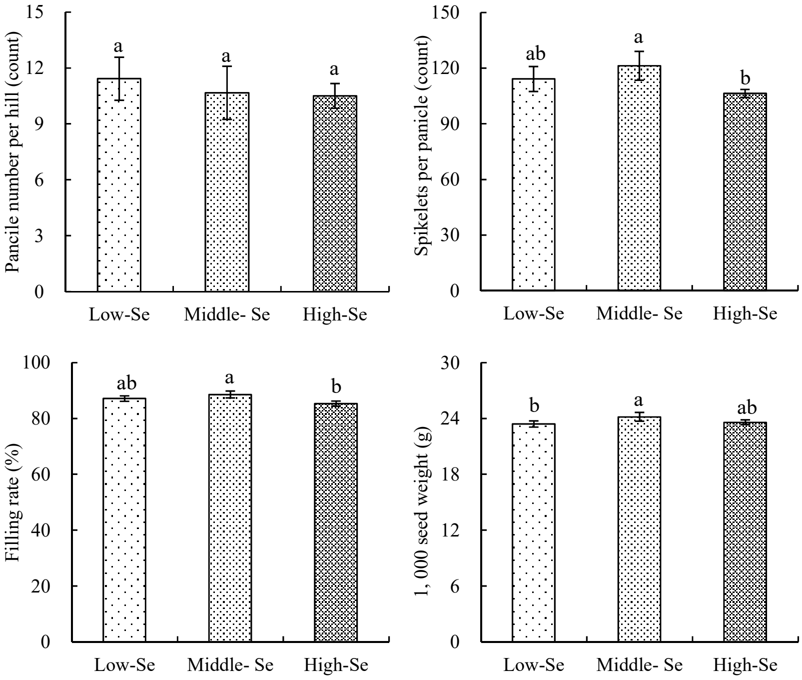

Figure 2. Analysis of seed production parameters in different soil Se concentrations of treated plants. Columns with different lowercase letters indicate a significant difference ($P < 5\%$).

3.2. Se Concentration and Coefficient of Accumulation in Rice Plants

The increasing levels of Se in soil significantly increased ($P < 5\%$) the concentration of Se in the root, straw, and grain (Table 2). However, Se concentration in root was significantly higher than that in straw and grain under the same treatment. In general, Se concentration in the rice plant fractions was in the following order: Root > straw > grain. For example, under high-Se treatment, Se concentration in root reached up to 2.72 mg kg^{-1}, which was 4.9 and 8.2 times of that in straw and grain, respectively. Both the coefficient of Se accumulation in straw and grain were highest in low-Se treatment, which were significantly higher than that of middle-Se and high-Se treatments (Table 2).

Table 2. Se concentration and coefficient of accumulation in different organs of rice plants under different treatments.

Treatment	Se Concentration (mg kg^{-1})			Coefficient of Accumulation	
	Root	**Straw**	**Grain**	**Straw**	**Grain**
Low-Se	1.16 c	0.30 c	0.16 c	0.59 a	0.32 a
Middle-Se	1.68 b	0.38 b	0.21 b	0.38 b	0.21 b
High-Se	2.72 a	0.56 a	0.33 a	0.37 b	0.22 b

Coefficient of accumulation was presented as ratio of plants Se/soil Se concentration. Within rows, values for the different Se level followed by different lowercase letters indicate a significant difference ($P < 5\%$).

3.3. Relationship between Se Concentration in Soil and the Rice Plants

As shown in Figure 3, Se concentrations in the root, straw, and grain were significantly positively correlated with Se concentration in naturally seleniferous soil, and the R^2 values was 0.9691, 0.9287 and 0.9544, respectively. The amount of absorbed Se in the root, straw, and grain was closely associated with Se concentration in the seleniferous soil.

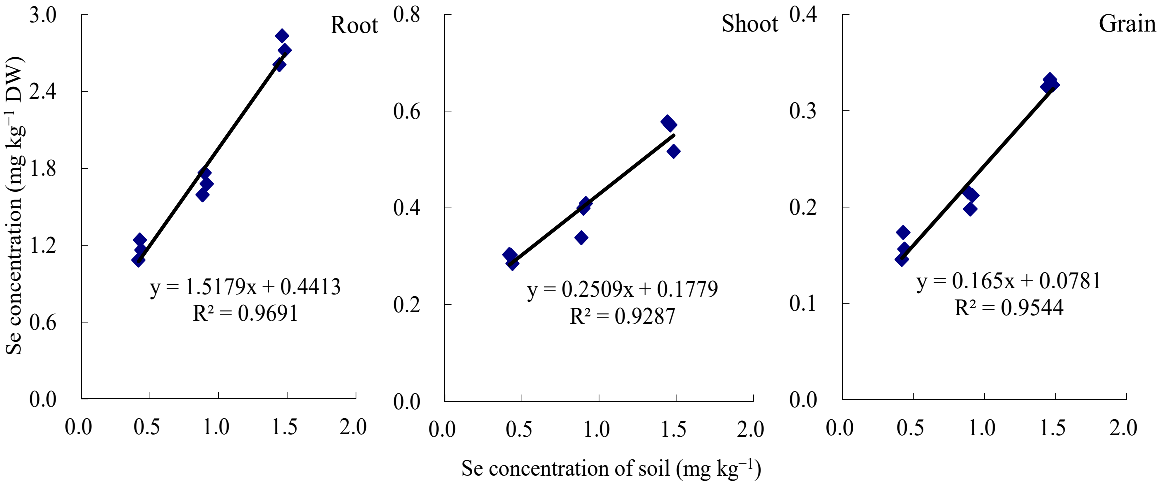

Figure 3. Relationship between Se concentration in naturally seleniferous soil and Se concentration in different parts of rice. Data were the means of three replicates.

3.4. Se concentration in Different Fractions of Rice Grain

As shown in Figure 4, Se concentration in the grain, hull, brown rice, bran, and polished rice was significantly increased with increasing Se in seleniferous soil ($P < 5\%$). Se concentration of polished rice (0.32 mg kg^{-1}) in the high-Se treatment was 2.1 and 1.6-fold higher than those in low-Se and middle-Se soils, respectively. Likewise, Se concentration of brown rice (0.35 mg kg^{-1}) in high-Se treatment was 1.1 and 0.5-fold higher than those in low-Se and middle-Se treatments, respectively. Under the same treatment, Se concentration in the grain fractions was in the following order: Bran

> brown rice > whole grain > polished rice > hull. As shown in Figure 5, spraying Se significantly increased Se concentration in the grain, hull, brown rice and polished rice compared with spraying water treatment. The Se concentration in brown rice and polished rice was 0.55 and 0.51 mg kg^{-1} under spraying Se treatment, respectively, which was significantly higher than that in spraying water treatment (0.11 mg kg^{-1} in brown rice and 0.10 mg kg^{-1} in polished rice). Similar to seleniferous soil treatments, for both spraying water and spraying Se treatment, Se concentration in the grain fractions was in the following order: Brown rice > whole grain > polished rice > hull.

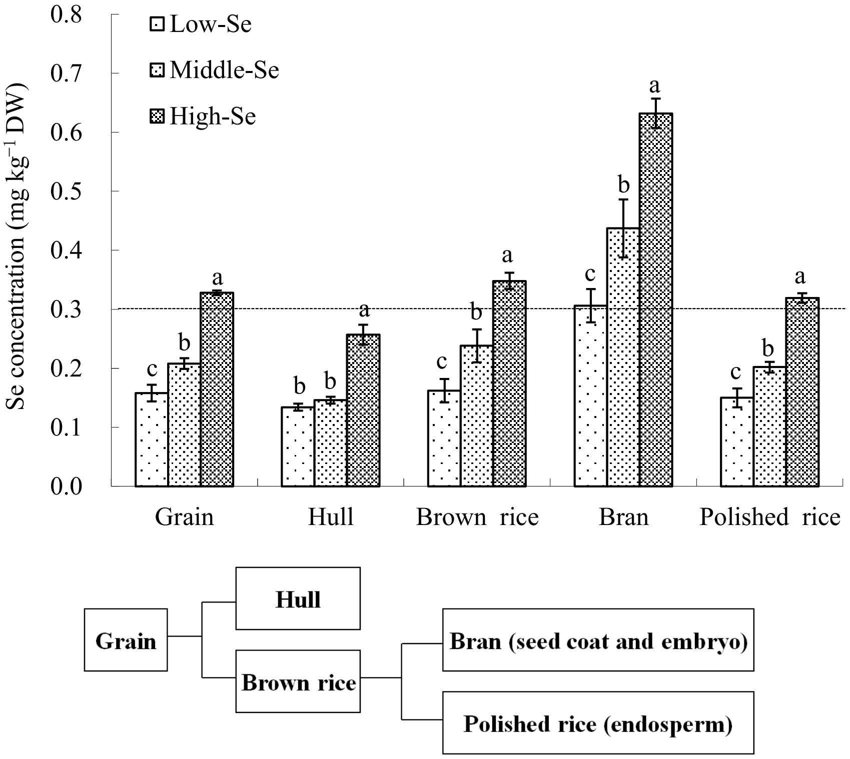

Figure 4. Selenium concentration in different grain fractions of rice under different naturally seleniferous soil. Data were the means of three replicates. Columns with different lowercase letters indicate a significant difference ($P < 5\%$).

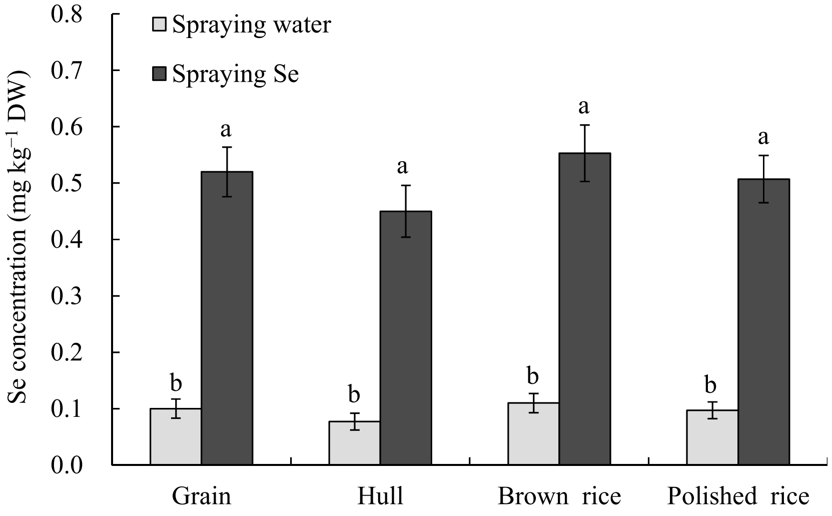

Figure 5. Selenium concentration in different grain fractions of rice under foliar application of Se. Columns with different lowercase letters indicate a significant difference ($P < 5\%$).

As shown in Figure 6, the weight percentages for hull, bran, and polished rice were 18.4%, 6.9%, and 74.6% of the whole grain, respectively. Both the weight percentages for bran and polished rice were not significantly affected by Se concentration in the naturally seleniferous soils. However, the weight percentage for hull was higher in high-Se than that in low-Se treatment. The percentage distributions of Se in the three rice grain fractions were shown in Figure 6. Se accumulation in grain was preferentially distributed to polished rice (more than 70%), followed by hull or bran. The weight percentage for hull was 1.7-fold higher than that for bran, while the Se percentage in the hull and bran was nearly the same.

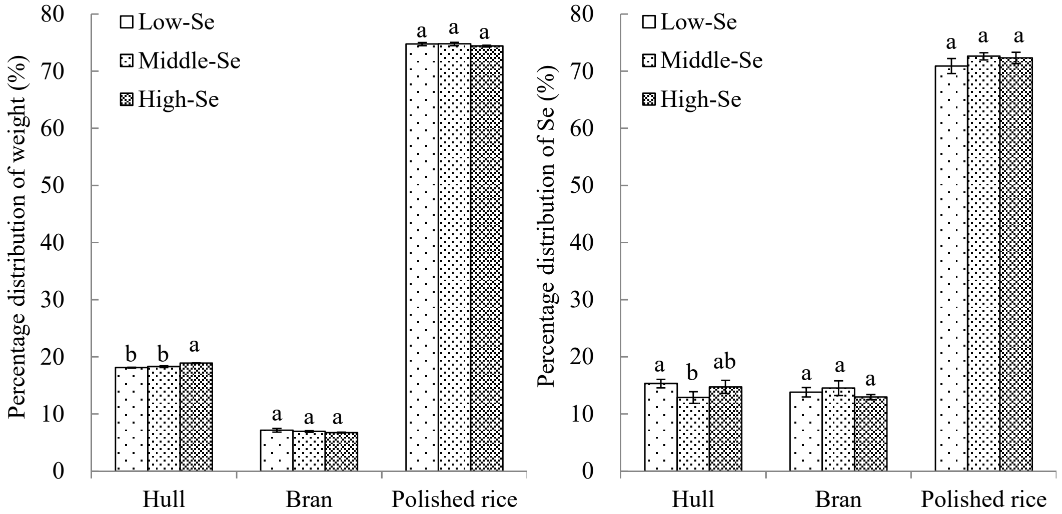

Figure 6. Percentage distribution of weight and Se in different fractions of rice grain. Columns with different lowercase letters indicate a significant difference ($P < 5\%$).

4. Discussion

Selenium is an important element for human and animal nutrition. However, Se is not considered essential for plants [1,28]. Based on reports, when plants were grown in high Se concentration, the growth would exhibit symptoms of injury, even led to death [1,2,29,30]. In the present study, we studied the effect of Se on the plant growth and Se concentration of rice under difference naturally seleniferous soils. In this study, the spikelets per panicle and filling rate of rice under high-Se were significantly lower than that in middle-Se treatment, and the grain yield (GY) was significantly higher in middle-Se treatment than that in high-Se treatment (Figure 1). The grain yield was mainly affected by four components, including panicles per plant, spikelets per panicle, filling rate and total seed weight [21,31]. Although the difference was not significant, GY of high-Se was 12% lower than that of low-Se treatment. Accordingly, the spikelets per panicle was of high-Se was 7% and 12% lower than that of low-Se and middle-Se treatment, respectively. Therefore, high-Se in soil affected the GY of rice mainly by reducing the spikelets per panicle of rice. Our results are different with those found by Hu et al. [32], which showed no significant biomass difference in root, straw and grain of rice under 0.2, 0.7 and 1.2 mg Se kg^{-1} soil concentration by applying exogenous Se. The effect of spraying Se on rice grain yield was also investigated in the present study. The grain yield did not significantly affect by spraying Se, and the spikelets per panicle, filling rate and 1000-seed weight were not significantly affected by spraying Na_2SeO_3 with the concentration of 0 and 15 mg L^{-1}. The difference results may be due to the differences in selenium concentrations and the morphologies of Se in the soil [31]. Therefore, more studies should be conducted, in order to investigate the effect of exogenous Se added in soils and naturally seleniferous soils on plant growth and development.

In the present study, there were large differences in the concentrations of organic matter, and available nitrogen, phosphorus and potassium among different kinds of experimental soils. It was reported that soil fertility, particularly organic matter, is the basis for high rice yield in paddy soil [33]. Moreover, the application of nitrogen and phosphorus fertilizer pays an important role in a high yield of rice [34,35]. High-Se obtained the lowest rice yield, spikelets per panicle and filling rate in all treatments, although the organic matter, available nitrogen and available potassium in high-Se soil were higher than that in low-Se and middle-Se soil. Therefore, our results suggested that selenium content in soil significantly affected rice yield, and the negative effects of high selenium on yield did not alleviate by higher soil organic matter, nitrogen and potassium in high-Se soil. Taken together, it suggests that the main physical and chemical properties in soils generally improved for plant growth (Table 1), however, it implies that 1.5 mg Se kg^{-1} in the naturally seleniferous soil is probably too high to maintain rice GY, and it also implies that Se level plays leading role compared with the other parameters. However, it is unclear whether the inhibition of Se on plant growth can be alleviated by significantly increasing nitrogen and organic matter in soils, which may require further research.

Rice accumulated different Se content in different plant tissues. Some studies have been conducted to compare the uptake among the tissues of plants [15,36]. However, the mechanism of Se absorption and accumulation in rice plants under different Se levels in the soil are still unclear [15]. As shown in Table 2, Se concentration in rice plant tissues was in the following order: Root > straw > grain. Se concentration in the root, straw, and grain was increased significantly ($P < 5\%$) with increasing concentrations of Se in the natural seleniferous soil. Similar results obtained in rice plants were reported by Sun et al. [3]. A previous study has also illustrated that Se concentration in rice plant tissues was positively associated with the available Se concentration of Se-deficient paddy soils [37]. Moreover, Se concentrations in different grain fractions were quite different. The rice grain is composed of four fractions, including hull, bran, and polished rice (endosperm) [3,22]. Under the same treatment, Se concentration in the grain fractions was following order: Bran > brown rice > whole grain > polished rice > hull (Figure 4). And a similar result was detected in both the spraying Se and the spraying water treatment (Figure 5). Our result was slightly different from that obtained by Sun et al. [3]. Nevertheless, Sun et al. [3] also agreed with the order: Bran > whole grain > polished rice. Other research has also indicated that Se concentration in bran was higher than that in polished rice (endosperm) [2,3,11].

In this study, Se-enriched rice grain (0.16–0.33 mg Se kg^{-1}) can be produced in naturally seleniferous soils (0.5–1.5 mg Se kg^{-1}). Se-enriched food is an efficient resource for human dietary Se intake [11,38]. However, under high-Se treatment, Se concentration in polished rice and brown rice was 0.32 and 0.35 mg kg^{-1} (Figure 4), respectively, which was higher than the maximum standard of Se concentration (0.3 mg kg^{-1}) in cereal [3]. The tolerable upper Se intake level recommended by the WHO for adults is 400 μg day^{-1} [16]. The polished rice and brown rice, which were grown in paddy soil with total Se up to 1.5 mg kg^{-1}, were not suitable for daily consumption of humans, unless diluted with Se-deficient rice to meet the standard ($\leq$0.3 mg Se kg^{-1}) [3]. Lots of studies showed that Se-enriched rice was achieved by spraying Se fertilizer [38]. The experiment of exogenous Se application also found that Se concentration in brown rice and polished rice reached 0.55 and 0.51 mg kg^{-1} by spraying Se (Figure 5). However, Se concentration in polished rice and brown rice considerably exceeded 0.3 mg kg^{-1}. In order to achieve Se-enriched rice within the standard of Se concentration (0.3 mg kg^{-1}) by spraying Se, more studies should be carried to investigate optimal spray concentration of Se. Nonetheless, our results suggested that Se-enriched rice could be produced in natural conditions in the study area, which will be more feasible and environmentally friendly as exogenous Se pollution is avoided.

Understanding the percentage distributions of Se in the different fractions of the grain is important for the efficient use of rice. The weight percentages for the hull, bran, and polished rice were 18.4%, 6.9%, and 74.6% of the total whole grain, respectively, while the Se percentages were 14.3%, 13.7%, and 71.9%, respectively (Figure 6). Rice bran is very popular for nutrition because it's rich in lipid, protein, vitamin, and dietary fiber [3,39,40]. We found that Se concentration in bran was 2.0-fold and 1.8-fold of polished rice and brown rice under the same treatment, respectively (Figure 4). The present study suggested that 13.7% Se in the wholegrain will be discarded by the milling process if 6.9% of it was polished as bran, which will reduce the Se value of the rice grain. Therefore, the bran is very important for Se intake, due to its characteristic enrichment of Se, and can be used for Se-enriched food resources.

5. Conclusions

In summary, the rice grown on the naturally seleniferous soil with suitable Se concentration (1.0 mg kg^{-1}) showed a high grain yield; however, 1.5 mg Se kg^{-1} in natural soil is inappropriate for getting optimum rice production in this study. Under the same naturally seleniferous soils, root accumulated higher Se than straw and grain. Se concentration in the grain fractions was in the following order: Bran > brown rice > whole grain > polished rice > hull. Se-enriched rice could be produced in naturally seleniferous soils with Se concentration from 0.5 to 1.0 mg kg^{-1}, and this polished rice would provide enough Se (60–80 μg d^{-1}) to satisfy the human requirement. However, both the brown rice and polished rice, produced by spraying Na_2SeO_3 (15 g ha^{-1}) or grown in paddy soil with total Se up to 1.5 mg kg^{-1}, was not suitable for daily human consumption, unless diluted with Se-deficient rice to meet the standard ($\leq$0.3 mg Se kg^{-1}).

Author Contributions: J.S., Y.Y. and C.J. performed the experiments; C.J. and C.Z. designed the research; J.S. and C.J. collected and analyzed data, and wrote the paper; C.J. and C.Z. revised the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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