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Effects of two different selenium fertilizers on accumulation of selenium and heavy metals in rice grains in field trials

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Abstract

Selenium (Se) is a nutritionally important micronutrient for humans. The use of Se fertilizers is often the most feasible approach to precisely increase Se contents in rice grains, but the effects of common commercial Se fertilizers on producing Se-rich rice remain to be investigated. In this study, we compared the effects of liquid and granular Se fertilizers on the accumulation of Se and heavy metals in rice grains of four different varieties in field. Results showed that Se accumulation in rice grains was affected by the form of fertilizers, applied concentrations of Se fertilizers, and rice varieties. Liquid fertilizer displayed significantly higher Se transfer efficiency than granular fertilizer, whereas the granular fertilizer had a slightly higher effect on organic Se accumulation. More than 95.5% Se in grains were organic Se, with selenomethionine (SeMet) being the dominant one. The proportion of organic selenocysteine (SeCys2) in grains was significantly higher in treatments with liquid fertilizer. Both forms of fertilizers significantly reduced the accumulation of cadmium, lead, and arsenic. Collectively, our study provides a reference for producing Se-rich rice in a more competitive manner in practice.

Keywords: heavy metals; rice (*Oryza sativa* L.); selenium fertilizer; selenium fortification; selenium speciation.

Practical Application: Comparing the effects of two forms of Se fertilizers provides a reference for Se fortification.

1 Introduction

Selenium (Se) is an essential trace element for humans that plays a critical role in relieving oxidative stress, improving the immune system, and reducing cancer risk (Rayman, 2012; Gashu et al., 2021; Gupta et al., 2021). Long-term Se deficiency leads to a series of human diseases, such as Keshan disease, Kashin-Beck disease and other endemic diseases (Loscalzo, 2014). On the contrary, excessive intakes of Se may cause hair and nail loss, garlic breathing, nervous system disorders, skin diseases and poor dental health (Johnson et al., 2010). To avoid diseases caused by Se deficiency, the World Health Organization (WHO) (World Health Organization, 1996) recommended no less than 34 μg/d for male and 26 μg/d for female with an age ≥19 for daily Se intake. However, to ensure adequate Se uptake, most developed countries, including USA, European Union, England, Canada and Australia, recommended no less than 55 μg/d for people with an age ≥19 for daily Se intake (Yuan et al., 2016). In most regions, especially in Asia and Africa, the main foods, such as grains, fruits and vegetables, in daily diet, have low Se contents, often resulting in an insufficient Se intake (Gao et al., 2011). More than one billion people worldwide were estimated to be suffering from Se deficiency (Lyons et al., 2003). A survey for daily dietary Se intakes on local residents of Suzhou, a developed region in Eastern China, showed an average Se intake of 43.9±3.8μg per day, lower than the value recommended by most developed countries and the Chinese Nutrition Society (2014) (60 μg/d) (Gao et al., 2011). Due to multiple benefits of Se, sufficient Se intake has been considered a feasible approach to improve human immunity and protect cardiovascular and myocardial health (Whanger, 2004). Comparatively, natural Se-rich dietary supplement is one of the safest and most effective methods to increase Se intake. Studies have shown that the most important source for daily Se intake is meats. However,

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cereals remain the major staple food for most people, especially in Asia and Africa. Therefore, Se-fortified cereals become an important way to solve the problem of insufficient Se intake (Alexander, 2007).

Rice is a staple food for more than half of the world's population, especially in many parts of Asia and Africa. It contains nearly 100 kinds of nutritional and bioactive compounds, including carbohydrates, proteins, lipids, amino acids, minerals, vitamins dietary fiber, and micronutrients (Zhao et al., 2020), and supplies up to 80% of daily energy, nutritional and bioactive compounds needed by the human body (Yue et al., 2015; Meng et al., 2005). However, a survey of global rice samples shows that 75% of rice cannot meet human daily Se intake (Williams et al., 2009). Therefore, development of Se-rich rice is of great significance to improve daily intake of Se. Although growing rice in Se enriched soil is the most economic strategy to obtain Se-rich grains, up to 40 countries were designated as low or Se deficient soil according to WHO. Application of Se fertilizers, as a result, has been considered an idealized and sustainable approach to produce Se-rich rice (Ros et al., 2016; Li et al., 2018). According to application methods, Se fertilizers can be generally divided into liquid and granular forms. Both fertilizers had been found to significantly increase the Se content in rice grains (Ros et al., 2016). Granular Se fertilizers generally go through three stages -- root assimilation, stem and leaf transport and grain accumulation -- to allow Se to reach grains. In contrast, liquid fertilizers applied by foliar spraying are thought to be directly transported from leaves to grains (Deng et al., 2017). Non-biologically active, toxic inorganic Se can be bio-converted in plants into organic forms of Se that are easily absorbed by and beneficial to human body (Longchamp et al., 2015). Organic forms of Se mainly include selenomethionine (SeMet), selenocystine ($SeCys_2$), and methylselenocysteine (SeMeCys), while inorganic forms of Se mainly refer to tetravalent and hexavalent selenium (Se^{4+}, Se^{6+}), including selenite and selenate. In the field, however, the effects of bioconversion efficiency of different Se fertilizers on different rice varieties have seldom been investigated and remain largely unknown so far.

Besides serving as a beneficial nutrient to the human body, Se has also attracted considerable attention for its ability to reduce the risk of heavy metals in rice grains. Among common heavy metals potentially contaminating grains, cadmium (Cd) ranks number one, and rice is considered as the dominant source of Cd exposure in the human diet (Meharg et al., 2013). Previous studies have shown that exogenous Se could significantly reduce Cd accumulation in rice grains (Lin et al., 2012; Hu et al., 2014; Wan et al., 2016; Huang et al., 2018). Furthermore, this Se-Cd antagonism has also been observed in several other plants (He et al., 2004; Wu et al., 2016). Recently, Huang et al. (2018) found that this fantastic effect of Se-Cd antagonism probably attributes to the fact that Se may mitigate Cd accumulation through altering soil pH, Cd bioavailability and translocation from roots to shoots. In addition, Se was also found to have significant antagonistic effects on rice uptake of other heavy metals, including arsenic (As), mercury (Hg) and antimony (Sb) (Wang et al., 2013). However, contrary to these previous studies that were mostly done in pot experiments, Yang et al. (2019) found that Se did not reduce Cd uptake and translocation in rice in field trials in either naturally Se-enriched region or highly Cd-contaminated land. Furthermore, Feng et al. (2013) found that Se helped to increase Cd uptake in rice under the condition of extremely high Cd levels. These inconsistent outcomes were speculated to be mainly determined by the soil conditions, the dosages and chemical forms of both elements (Feng et al., 2013; Wan et al., 2016; Yang et al., 2019). Notably, Boldrin et al. (2013) found that the application of Se is beneficial for plants to absorb iron (Fe) and zinc (Zn), which are beneficial to the human body (Gashu et al., 2021; Gupta et al., 2021). However, in the field without contamination of these metals, whether Se application affects their accumulation remains unclear.

In the present study, we applied two kinds of Se fertilizers and three concentrations for each to treat four different *Japonica* rice varieties in field trials. We comprehensively evaluated the effects of different forms of Se fertilizers on grains for Se accumulation and speciation in different rice varieties, as well as for the accumulation of seven common metals (Fe, Zn, Mn, Cu, As, Cd and Pb) in field trials. The results will provide a theoretical reference for developing Se-rich rice with low cost and high efficiency in practice, which will ultimately contribute to reducing the potential health risks derived from insufficient Se intake.

2 Materials and methods

2.1 Experiment materials and field trial design

Two forms of Se fertilizers, liquid fertilizer (product code: setek-bf-003) and granular fertilizer (product code: setek-bf-001), were purchased from Suzhou Setek Co., Ltd. Se contents were 5000 mg/L for liquid fertilizer and 1000 mg/kg for granular fertilizer. Three concentrations were used for each Se fertilizer: 7.5 kg/667 m^2, 15 kg/667 m^2 and 22.5 kg/667 m^2 for granular fertilizer, and 50 mL/667 m^2, 100 mL/667 m^2 and 150 mL/667 m^2 for liquid fertilizer.

Four *Japonica* rice varieties were tested: S1785, NJ5055, NJ46 and ZD. Among them, ZD was developed by our laboratory and the other three were varieties authorized by the government of Jiangsu Province, China, and have been widely planted in Jiangsu province. All these varieties were planted in the experimental farm of Yangzhou University, Yangzhou, China. Each variety was treated with the two Se fertilizers at three different concentrations. Each treatment unit or plot in the field contained 30 m^2. All varieties were sowed on 15 May, and transplanted on 15 June, 2019. Granular and liquid Se fertilizers were applied at the booting stage and at 3 days after heading, respectively. Mock control (CK) contained no Se fertilizer application. The experiment was conducted in a completely randomized block design with three replicates for each treatment.

2.2 Sample collection and measurement of selenium contents

The soil samples from five different positions in the field were collected before rice planting. In each position, no less than 0.5 kg soil at 15 cm below the ground was collected and air dried. At around 50% water content, the soil samples were crushed and sieved through 200 mesh for subsequent Se measurement. To determine the Se content in rice grain, the rice grain samples were harvested at around 50 days after heading. In each experimental plot, no less than 1.0 kg rice grains were harvested from at least 40 plants. All grain samples were dried to 15% of water content, and then processed into brown and milled rice, respectively. Around 10 g of brown rice and milled rice from each experiment plot were then ground into powder for Se measurement subsequently.

To measure Se content, 0.5 g of each dried sample was placed in a prepared digestion tube, then added 5 mL of HNO_3, 3 mL of dd H_2O and 20 μL of H_2O_2. The following extraction procedure was used: 800 W, heat up to 180 °C for 10 minutes until the solution became colorless, and digest for 10 minutes at 180 °C. After cooling, filter the digestion solution, and place in a 50 mL volumetric flask. After mixing, measure 10 mL of the solution, place it in a centrifuge tube, and directly determine the total Se content by ICP-MS.

To estimate the Se transfer efficiency from fertilizer to brown rice, we harvested all rice plants from each experimental plot and measured the grain yield in each plot. The average value of three replications was defined as the grain yield of the corresponding treatment (GWT). One thousand grains were processed into brown rice; this process was repeated twice and the values were used to determine the proportion of brown rice yield (pBRY%). The weight of the brown rice in each plot was calculated by GWT × pBRY%. The Se transfer efficiency from fertilizer to brown rice was determined based on the Se content measured in brown rice and the Se input in each treatment.

2.3 Determination of Se speciation in grains

Se speciation was measured using milled rice powder of each variety treated with the highest concentration of a Se fertilizer. The sample was prepared as described above. Weigh 0.5 g sample into digestion tank, and add 15 mg protease XIV and 10 mL ddH_2O for microwave extraction. The following extraction procedure was used: 200 W, 10 min temperature rise to 37 °C, 30 min extraction at 37 °C. The extract was separated by high performance liquid chromatography (HPLC; LC-20AT; Shimadzu, Kyoto, Japan). After centrifugation (6000 rpm, 5 min), the extract was filtered through a 0.45 μm water-based membrane for Se speciation analysis. The blank test was conducted in the same way. Quantitation was performed based on peak area measurements of the chromatographic signals by monitoring the ^{78}Se isotope. The Se standard samples included SeMet (Tokyo Chemical Industry, Co., Japan), $SeCys_2$, and SeMeCys (Sigma-Aldrich, St. Louis, MO), selenate and selenite (National Reference Material Centre, China).

2.4 Determination of other metal elements

To measure Fe, Zn, Mn, Cu, As, Pb and Cd, 0.5 g of dried grains were weighed and acid-digested with 4 mL of concentrated HNO_3 plus 20 mL of concentrated $HClO_4$ (Sigma-Aldrich, Saint Lousi, MO, USA) at 120 °C for 1 h, then at 220 °C until $HClO_4$ fumes were observed. Total contents of Fe, Zn, Mn, Cu, As, Pb and Cd were then determine by ICP-MS.

2.5 Data analysis

Statistical analysis of variance (ANOVA) was conducted with SPSS 13.0 for Windows (SPSS Inc., Chicago, IL) to determine the significant differences ($P<0.05$ and $P<0.01$) between control and treatments. Microsoft Excel software was used to process data.

3 Results

3.1 Selenium contents in soils

The average Se contents in the field soils ranged from 71.41 to 77.56 μg/kg (Figure 1A), and no significant difference was found among different samples. The average Se contents in the experimental field were 72.92 μg/kg, which is clearly lower than 100 μg/kg, a recommended value to determine Se-deficiency soil (Blazina et al., 2014).

We also detected the Se contents in rice grains of four different varieties planted in field without Se fertilizer treatment. The results showed that the Se contents in these varieties ranged from 25 to 33 μg/kg in brown rice and from 19 to 27 μg/kg

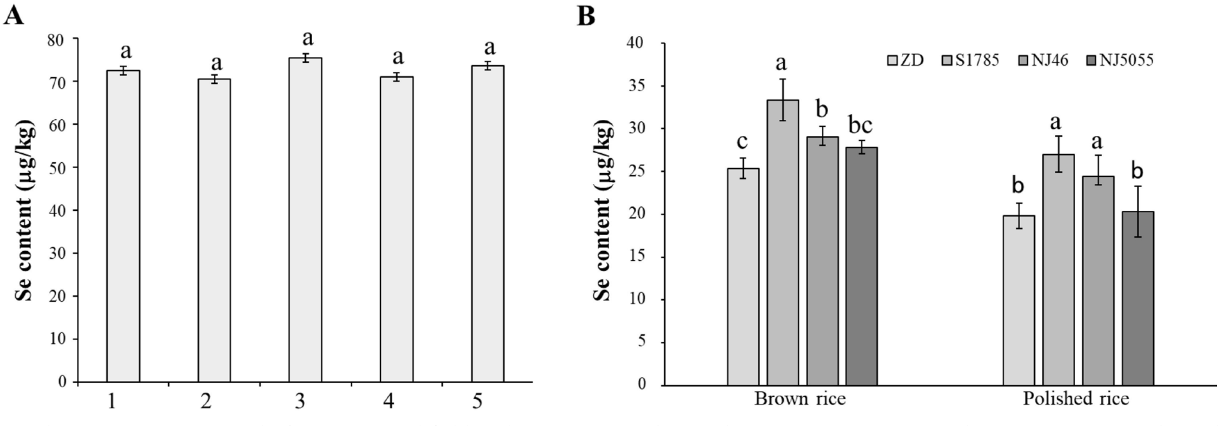

Figure 1. Selenium contents in soil of experimental field and rice grains without selenium treatment. (A) Selenium contents in the soils of the experimental field. Numbers 1 to 5 refer to the soil samples collected from 5 different positions in the experimental field; (B) Selenium contents in brown rice and polished rice of four varieties without selenium fertilizer. Different letters mean statistically significant differences at $P\leq0.05$ level.

in polished rice (Figure 1B). The S1785 variety contained the highest Se content (33 μg/kg) in both brown rice and polished rice, but it was still lower than the standard value (40 μg/kg) of Se-rich rice recommended by Chinese government. In addition, a significant difference was found among varieties on grain Se contents. These results show that different rice varieties clearly differ in their ability to accumulate Se in grains.

3.2 Selenium contents in grains of different varieties after Se fertilizers treatment

Two kinds of Se fertilizers were applied with three different concentrations on four varieties. By using three-factor ANNOVA (Table 1), we observed significant interaction effects among varieties, fertilizers and applied concentrations on Se contents in rice grains, which indicated that the Se accumulation in rice grains was affected by multiple factors. Through multi-comparison, we found that compared with control, both Se fertilizers significantly increased Se content in both brown rice and polished rice of different rice varieties (Table 2). Even in the treatment of low concentration fertilizer, the Se contents of polished rice of all varieties clearly exceeded the standard value of Se-rich rice. The Se in polished rice shared the most part of Se in brown rice, accounting for 48.33% to 83.67% in all treatments, indicating that the majority of Se was accumulated in polished rice.

With regard to fertilizer concentrations, we found that grain Se contents were positively correlated with the applied concentrations, while the positive effects declined evidently for almost all varieties and the negative effects showed slightly difference among fertilizers and varieties (Figure 2). Take brown rice of the ZD variety for example, we observed increases of 182.83 μg/kg for liquid fertilizer and 222.49 μg/kg for granular fertilizer treatment in Se contents comparing low Se treatment (T1) with control (T0); whereas we observed the effects were reduced to 116.33 μg/kg (liquid fertilizer) and 93.66 μg/kg (granular fertilizer) comparing medium Se treatment (T2) with T1 treatment, and to 115μg/kg (liquid fertilizer) and 66 μg/kg (granular fertilizer) comparing high Se treatment (T3) and T2, respectively. Comparatively, application of the low concentration of both Se fertilizers was enough for all varieties to produce Se-rich rice grain although increased concentrations of Se fertilizers could further enhance Se accumulation. Notably, among treatments with high concentrations, the Se contents in polished rice of two varieties (S1785 and NJ5055) and in most brown rice had exceeded the upper limit (400 μg/kg) of Se-rich rice. This result demonstrates that the concentration of Se fertilizer being applied need to be fully considered in order to produce Se-rich rice.

Together our results indicate that granular fertilizer application resulted in most Se accumulation in rice grains with an average value of 367 μg/kg in brown rice and 269 μg/kg in polished rice than liquid fertilizer application (313 μg/kg in brown rice and 221 μg/kg in polished rice). For rice varieties, S1785 displayed highest ability to accumulate grain Se, followed by NJ46. However, significant interaction effects were also detected among fertilizers, varieties and applied concentrations (Table 2). These results indicate that in practice, appropriate rice varieties, fertilizers and application concentrations should be adopted to reach the cost-saving and benefit-maximizing aim in producing Se-rich rice.

3.3 Transfer efficiency of selenium from fertilizer to rice grains

Although granular fertilizer tends to produce more Se in rice grains, it requires much more Se input than liquid fertilizer. Because brown rice is the edible part of rice for humans, we compared the transfer efficiency of Se from Se fertilizer to brown rice. We found that liquid fertilizer presented apparently higher transfer efficiency, ranging from 19.43% to 33.04%, than granular fertilizer which resulted in all less than 1.3% (Table 2). With granular fertilizer treatments, no significant difference was found on Se transfer efficiency among different varieties or applied concentrations; whereas significant differences were found with liquid fertilizer treatments. The S1785 and NJ5055 varieties showed higher Se transfer efficiencies than the other two varieties. In terms of applied fertilizer concentrations, we found that Se transfer efficiency displayed an apparently decreasing trend with the increase of applied concentration. Together, these data indicate that liquid fertilizer clearly offers higher Se transfer efficiency than granular fertilizer.

3.4 Comparison of selenium speciation in rice grains treated by selenium fertilizers

The biological activity of Se in the human body mainly depends on its speciation which is generally classified into two

Table 1. Three-factor ANNOVA of selenium contents in rice grains among different treatments.

Factors	Pr (>F)	Brown rice	Pr (>F)	Polished rice
Varieties (V)	< 2e-16	***	< 2e-16	***
Fertilizers (F)	< 2e-16	***	< 2e-16	***
concentrations (C)	< 2e-16	***	< 2e-16	***
Groups (G)	0.776	NS	0.492	NS
V× F	< 2e-16	***	6.24E-14	***
V× C	< 2e-16	***	< 2e-16	***
F × C	4.45E-13	***	< 2e-16	***
V×F×C	< 2e-16	***	1.29E-14	***

Note: "NS" denotes no significant difference at the statistical level of 0.05; ***denotes significant differences at statistical level of 0.001.

Table 2. Multi-comparison of Se contents (μg/kg) in rice grains, proportion of Se in polished rice and Se transfer efficiency among different treatments.

Varieties	Selenium fertilizers	Treatments	Se contents in brown rice (μg/kg)	Se contents in polished rice (μg/kg)	Proportion of Se in polished rice (%)	Se transfer efficiency (%)
ZD	Liquid	T1	207.66^{lm}	135.77^{lm}	65.33^{h}	24.62^{e}
		T2	324.00^{gh}	187.13^{i}	57.67^{i}	23.10^{f}
		T3	439.00^{1c}	240.33^{hi}	54.67^{i}	20.75^{h}
	Granular	T1	247.33^{jk}	138.49^{lm}	56.33^{i}	1.05^{i}
		T2	341.00^{fg}	257.11^{fg}	75.33^{cdef}	0.87^{i}
		T3	407.34^{e}	332.33^{c}	81.67^{abc}	0.82^{i}
S1785	Liquid	T1	263.00^{ij}	127.33^{mn}	48.33^{j}	27.84^{cd}
		T2	346.33^{ef}	270.00^{f}	78.00^{bcd}	31.4^{b}
		T3	452.00^{bc}	363.66^{b}	80.67^{bc}	28.01^{d}
	Granular	T1	229.33^{kl}	157.52^{j}	68.67^{fgh}	1.17^{i}
		T2	430.67^{d}	321.67^{c}	75.00^{cdef}	1.22^{i}
		T3	536.33^{a}	415.33^{a}	77.67^{cd}	1.07^{i}
NJ46	Liquid	T1	172.02^{n}	120.00^{n}	69.67^{efgh}	32.12^{b}
		T2	280.35^{i}	234.67^{i}	83.67^{ab}	22.33^{g}
		T3	457.67^{bc}	314.00^{d}	68.67^{fgh}	19.43^{h}
	Granular	T1	196.67^{lm}	153.67^{k}	78.33^{bcd}	1.045^{i}
		T2	346.34^{ef}	234.34^{i}	67.67^{gh}	0.96^{i}
		T3	464.33^{b}	352.00^{b}	76.00^{cde}	0.85^{i}
NJ5055	Liquid	T1	162.67^{n}	142.17^{klm}	87.67^{a}	33.30^{a}
		T2	312.67^{h}	243.00^{gh}	78.00^{bcd}	28.66^{c}
		T3	350.00^{ef}	283.33^{e}	80.67^{bc}	25.50^{e}
	Granular	T1	240.81^{jk}	174.34^{j}	72.67^{efg}	1.36^{i}
		T2	434.00^{cd}	298.33^{d}	68.67^{fgh}	1.16^{i}
		T3	530.34^{a}	404.00^{a}	76.00^{cde}	0.95^{i}

Note: Different letters mean statistically significant differences at the level of $P \leq 0.05$.

types: organic and inorganic (Pyrzynska, 2009; Rayman et al., 2008). Organic Se mainly includes SeCys$_2$, MeSeCys and SeMet, which are beneficial for health; while inorganic Se mainly consists of Se^{4+} and Se^{6+}, which are believed to be harmful to the human body. Therefore, we compared the Se speciation in rice grains treated with the two different Se fertilizers (Table 3). We found that all varieties contained organic Se exceeding 97% of total Se in grains with no significant differences among varieties, except for S1785 which showed 95.78% in for liquid fertilizer, indicating that most Se accumulated in rice grains were organic Se. Comparatively, the granular fertilizer displayed a slightly better effect on organic Se accumulation in rice grains than liquid fertilizer. For the components of organic Se, we observed that most organic Se speciation were SeMet, followed by SeCys$_2$; MeSeCys only accounted for a very small proportion. Interestingly, we found that liquid fertilizer tended to produce more SeCys$_2$ but less SeMet compared with granular fertilizer, demonstrating that different Se fertilizers or applied methods have effects on accumulation of different organic Se. For rice varieties, we found that NJ5055 accumulated more SeCys$_2$ but less SeMet than the other varieties in both Se fertilizer treatments. Taken together, we conclude that most Se accumulated in rice grains are organic Se, and that liquid fertilizer application favors SeCys$_2$ accumulation in grains.

3.5 Contents of valuable trace elements and heavy metals in rice grains treated by selenium fertilizers

Several previous studies have reported that applying Se is able to reduce the risk of heavy metals in rice grain. Therefore, we measured seven common metals (Fe, Zn, Mn, Cu, As, Cd and Pb) for all treatments. Compared with control, we found that the contents of Fe, Zn and Mn in rice grains were apparently increased and positively correlated with the applied concentrations of both Se fertilizers (Table 4). On average, Fe was increased by 1.65, 2.2 and 3.14 times for low, medium and high concentrations of liquid fertilizer, and by 2.16, 3.12 and 3.99 times for granular fertilizer from low to high concentrations compared to the control. Zn was enhanced by 1.73, 2.2 and 3 times for liquid fertilizer and to 1.4, 2.7 and 3.87 times for granular fertilizer compared to control. Considering that both Se fertilizers enhanced Fe, Zn and Mn contents, we therefore could not attribute the increased accumulation of these three elements to the possible synergistic effects between Se and these three elements. Contrary to Fe, Zn and Mn, the contents of Cd were reduced more than 50% in low Se treatments for all varieties, and were on average reduced by 79% and 90.25% in treatments with medium and high concentrations of liquid fertilizer, and 78.5% and 85.5% in

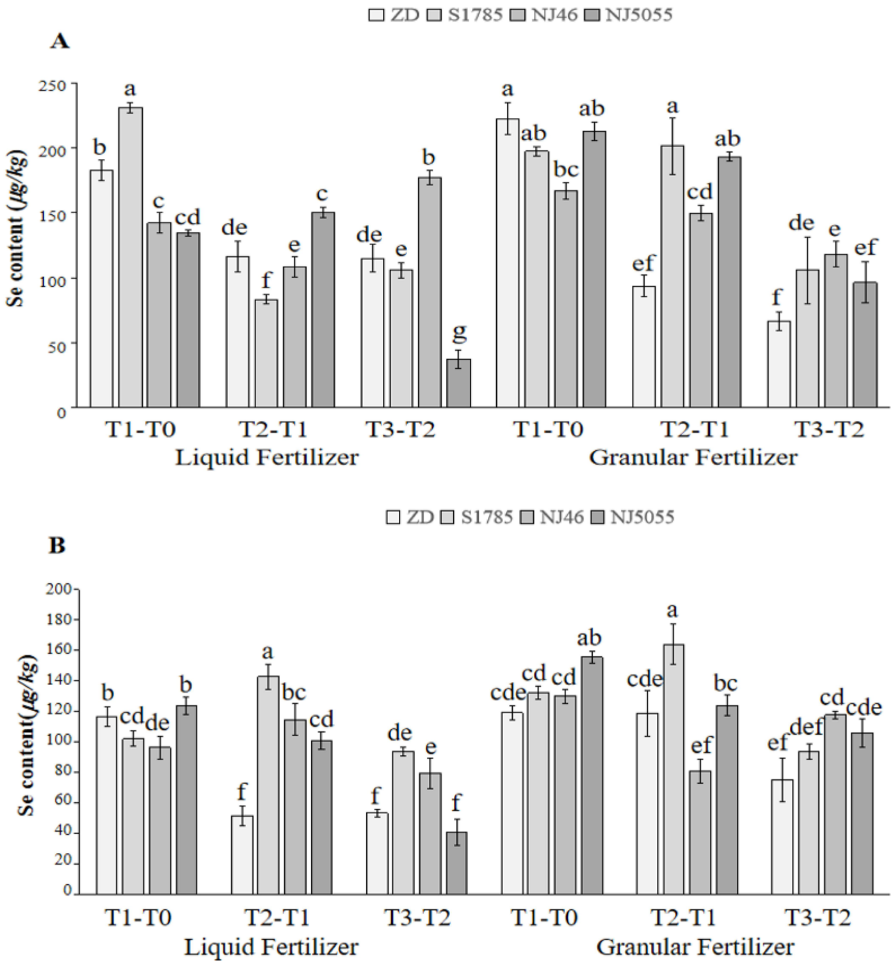

Figure 2. Comparison of relatively increased selenium contents in rice grains between the treatments of applied concentrations. Comparison of increased selenium contents in brown rice and polished rice treated with granular fertilizer (A) or liquid fertilizer (B). T0 refers to no treatment of Se fertilizer. T1, T2 and T3 refer to the treatment of low, medium and high concentration of Se fertilizer, respectively. T1-T0 indicates the difference in Se contents between T1 treatment and control, and similarly for the others. Different small letters mean statistically significant differences at *P*≤0.05 level.

Table 3. Multi-comparison of the contents of organic and inorganic selenium and their components in polished rice among different treatments.

Selenium fertilizers	Varieties	Total Organic Se (%)	$SeCys_2$	SeMeCys	SeMet	Total Inorganic Se (%)	Se^{4+}	Se^{6+}	unknown
Liquid	ZD	97.13^{bc}	27.26^{a}	0.10^{c}	69.77^{f}	1.95^{a}	0.05^{b}	1.90^{b}	0.70^{c}
	S1785	95.78^{c}	12.32^{b}	0.09^{c}	83.30^{d}	1.44^{b}	0.39^{a}	1.04^{c}	2.10^{a}
	NJ46	97.52^{abc}	11.07^{bc}	0.66^{a}	85.80^{c}	0.53^{c}	0.02^{b}	0.21^{e}	1.20^{b}
	NJ5055	98.12^{ab}	26.21^{a}	0.05^{c}	71.85^{e}	1.65^{ab}	0.04^{b}	2.21^{a}	0.04^{c}
Granular	ZD	98.18^{ab}	7.64^{d}	0.02^{c}	90.52^{a}	0.38^{cd}	0.05^{b}	0.33^{d}	1.40^{b}
	S1785	98.59^{ab}	6.14^{e}	0.38^{b}	92.07^{a}	0.31^{d}	0.18^{a}	0.13^{e}	0.60^{c}
	NJ46	98.43^{ab}	6.75^{de}	0.42^{b}	91.27^{a}	0.23^{d}	0.04^{b}	0.19^{e}	1.50^{b}
	NJ5055	99.28^{a}	10.59^{c}	0.04^{c}	88.60^{b}	0.23^{d}	0.03^{a}	0.50^{d}	0.02^{c}

Note: Different letters mean statistically significant differences at the level of *P*≤0.05.

treatments with medium and high concentrations of granular fertilizer, respectively. Similarly with Cd, the contents of As and Pb were all significantly reduced with application of Se fertilizers, and also presented an apparent negative correlation with the applied concentrations (Table 4). The accumulation of Cu in grains was also suppressed by applying both Se fertilizers, although to a lesser extent than As, Cd and Pb, and seemly associated with varieties. Taken together, we conclude that in the field, application of Se fertilizers is able to reduce the risk of heavy metals accumulated in rice grains.

Table 4. Multi-comparison of contents (µg/kg) of trace elements and heavy metals in rice grains among different treatments.

Varieties	Selenium fertilizers	Treatments	Fe	Zn	Cu	Mn	As	Pb	Cd
ZD	-	CK	133.37^{f}	115.26^{g}	84.85^{a}	72.38^{g}	354.50^{a}	318.30^{a}	44.58^{a}
	Liquid	T1	173.48^{e}	163.34^{f}	64.04^{b}	89.64^{f}	268.10^{b}	223.87^{b}	18.33^{b}
		T2	247.38^{d}	212.12^{e}	59.26^{b}	106.10^{e}	262.18^{b}	125.23^{e}	9.19^{c}
		T3	321.43^{c}	284.98^{c}	40.66^{c}	123.64^{c}	135.2 ^{d}	242.17^{c}	5.26^{c}
	Granular	T1	259.54^{d}	239.67^{d}	82.55^{a}	114.92^{d}	346.46^{a}	159.28^{d}	17.27^{b}
		T2	420.62^{b}	312.30^{b}	60.07^{b}	135.30^{b}	237.52^{c}	109.63^{f}	14.10^{b}
		T3	544.90^{a}	374.24^{a}	34.86^{c}	156.67^{a}	112.44^{e}	189.94^{d}	8.79^{c}
S1785	-	CK	125.89^{d}	76.84^{g}	75.28^{a}	35.44^{e}	243.17^{a}	247.99^{a}	52.08^{a}
	Liquid	T1	269.79^{c}	158.45^{f}	57.224^{b}	72.56^{d}	193.12^{b}	191.33^{c}	16.92^{b}
		T2	350.29^{b}	220.39^{d}	52.782^{b}	68.47^{d}	188.63^{b}	123.66^{c}	7.32^{c}
		T3	489.34^{a}	312.33^{b}	30.393^{d}	99.73^{c}	106.80^{d}	84.11^{e}	3.45^{d}
	Granular	T1	273.01^{c}	180.85^{e}	49.07^{cd}	65.26^{d}	173.92^{b}	222.11^{b}	18.78^{b}
		T2	348.33^{b}	280.22^{c}	56.42^{b}	111.91^{b}	184.81^{b}	117.21^{d}	10.45^{c}
		T3	478.88^{a}	387.13^{a}	24.23^{d}	141.67^{a}	136.42^{c}	61.11^{f}	8.99^{c}
NJ46	-	CK	287.86^{f}	143.37^{f}	73.20^{a}	103.66^{f}	176.65^{a}	136.16^{b}	42.43^{a}
	Liquid	T1	475.42^{e}	161.00^{e}	57.64^{b}	117.02^{e}	94.49^{d}	114.69^{c}	26.61^{b}
		T2	538.63^{d}	223.53^{d}	58.38^{b}	122.78^{e}	149.41^{b}	171.68^{a}	8.39^{d}
		T3	682.67^{b}	290.49^{b}	33.83^{c}	152.14^{c}	78.27^{d}	26.53^{f}	3.26^{d}
	Granular	T1	588.04^{c}	187.76^{c}	54.71^{b}	126.14^{d}	142.44^{b}	116.14^{c}	17.12^{c}
		T2	679.20^{b}	256.41^{c}	58.53^{b}	154.22^{b}	121.22^{c}	94.22^{d}	14.28^{c}
		T3	800.11^{a}	316.42^{a}	20.89^{d}	180.33^{a}	82.88^{e}	56.43^{e}	6.48^{d}
NJ5055	-	CK	121.80^{f}	81.314^{f}	54.32^{a}	65.30^{e}	198.77^{a}	253 .5^{a}	46.24^{a}
	Liquid	T1	216.67^{e}	196.66^{e}	60.96^{ab}	77.42^{d}	188.62^{a}	182.44^{b}	18.62^{b}
		T2	308.52^{d}	232.00^{d}	64.53^{a}	112.37^{c}	87.67^{d}	118.99^{d}	12.52^{c}
		T3	496.34^{c}	298.69^{c}	42.38^{b}	122.67^{b}	94.94 ^{d}	172.11^{b}	5.21^{d}
	Granular	T1	320.70^{d}	194.31^{e}	34.38^{b}	104.32^{c}	163.13^{b}	155.42^{c}	17.46^{b}
		T2	545.97^{b}	315.96^{b}	39.64^{b}	124.01^{b}	137.41^{c}	102.34^{e}	10.13^{c}
		T3	664.68^{a}	418.10^{a}	30.28^{b}	143.67^{a}	135.13^{c}	56.70^{f}	5.10^{d}

Note: Different letters mean statistically significant differences at the level of $P \le 0.05$.

4 Discussion

Due to the fact that many arable framing lands are located in soil Se-deficiency regions, the use of Se fertilizers is the most feasible approach to appropriately increase Se concentrations in grains (Ros et al., 2016; Deng et al., 2017). In practice, however, how to more economically and efficiently produce Se-rich rice remains to be investigated. In this study, we found that although increasing the applied concentration of Se fertilizers could significantly enhance the Se accumulation in grains in all varieties, it would also increase the cost and, even for some varieties, resulted in Se over-accumulation in grains. Also, different varieties and fertilizers were found to significantly affect the grain Se accumulation. All these together indicate that optimizing fertilizers and choosing appropriate varieties can commercially produce Se-rich rice in a more competitive manner. Notably, although applied granular fertilizer could result in slightly more Se accumulation in rice grains compared with liquid fertilizer, its Se-transfer efficiency was apparently lower than that of liquid fertilizer (Table 2). Broadley et al. (2010) previously has reported that the total recovery (grain and straw) of applied granular Se fertilizer was only 20%-35%. Transferring Se from granular Se fertilizers amended in soil to grains needs to go through a series of biological processes, which is apparently more complex than the transfer from leaf to grains when spraying liquid Se fertilizer (Zhang et al., 2019). This accounts for higher inputs of granular Se fertilizer needed. In addition, different soil conditions, including soil pH and organic matter, have also been found to significantly affect the transfer efficiency of Se from soil to grains (Yang et al., 2019). Foliar application of liquid Se fertilizer will not be disturbed by soil conditions that generally vary significantly among different regions, demonstrating the widely adaptable potential for liquid fertilizers in producing Se-rich rice.

Besides the total Se content in grains, the most important factor is the chemical forms of Se (Thiry et al., 2012; Rayman, 2012). However, there has been seldom reports for the contents and its components of organic Se in rice grains so far. Here, we found that the proportion of organic Se in grains of four varieties treated by both fertilizers all exceeded 97%, except for S1785 (95.78%) in one treatment. This indicates that most Se accumulated in rice grains are beneficial to the human body. Comparatively, granular Se fertilizer displayed slightly higher proportions of total organic Se than liquid Se fertilizer, but the proportions of different organic Se-compounds in liquid Se fertilizer treatments showed a wider distribution (Table2). Consistently with the previous reports in most commonly consumed foods, SeMet is the principal species of Se in rice grains, followed by $SeCys_2$ and MeSeCys. Unlike SeMet, the potential beneficial effects of $SeCys_2$, MeSeCys and other organic Se species remained unclear so far. These organic Se are considered to be of high importance, even in low quantities, regarding the health effect of Se (Ogra et al., 2005; Thiry et al., 2012). Therefore, the increases in the contents of other organic Se-compounds could be of great significance. As a consequence, liquid Se fertilizer should be a better choice in producing Se-rich rice due to its higher potential of generating relatively high contents of $SeCys_2$.

Several previous studies have shown that application of Se has various effects on the accumulation of heavy metals (Wan et al., 2016; Huang et al., 2018). However, whether the application of Se affects the accumulation of micronutrients and poisonous heavy metals in the field without excess amounts of these elements remained unknown. Like Se, Fe and Zn have also been considered important micronutrients to human health, and increasing their contents are desired by consumers and rice producers (Gashu et a., 2021; Gupta et al., 2021). Here, we found that application of both forms of Se fertilizers significantly increased Fe and Zn contents in rice grains for all varieties tested. Although we could not completely exclude the possibility that it might be due to possible residual contents of these micronutrients in the two fertilizers, it at least implies that application of Se fertilizers would not affect Fe and Zn uptake and accumulation in rice grains. Notably, we found that the application of both fertilizers in the field was able to significantly reduce Cd, As and Pb accumulation. In polluted soil, these heavy metals (Pb, Cd, As, etc.) are easily enriched in the crops and animals through food chain, and eventually threaten human health. Due to react with heavy metals, proteins, ribose, vitamins, hormones and other substances in the human body may lose their original biochemical functions, resulting in disease and even death, and can also weaken or even lose the enzyme activity by acting on the active parts of the enzyme in the body, disrupting the normal metabolism of the body (Jiang et al., 2021). Therefore, although these three heavy metals in control did not reach their standard levels of heavy metals-contaminated rice, further reducing their contents is still highly desirable in practice. As a consequence, our study demonstrates that applying Se fertilizers clearly carries positive effects on reducing the contents of poisonous metals in rice grains.

5 Conclusion

We found that commercially produced Se-rich rice could reach the cost-saving and benefit-maximizing aim by optimizing the type of fertilizers, the applied concentration and the rice varieties. Liquid Se fertilizer displayed significantly higher Se transfer efficiency than granular fertilizer, while granular Se fertilizer presented a slightly higher effect on organic Se accumulation. More than 95.5% Se in rice grains were organic Se; the proportion of organic $SeCys_2$, followed by SeMet, was significantly higher in treatment with liquid fertilizer. Both fertilizers were found to significantly reduce the accumulation of Cd, As and Pb. Collectively, our study provides a reference to practically produce Se-rich rice in an economical and efficient manner.

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