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Selenium Speciation in Soil and Rice: Influence of Water Management and Se Fertilization

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Rice (*Oryza sativa*) is the staple food for half of the world's population, but the selenium (Se) concentrations in rice grain are low in many rice-growing regions. This study investigated the effects of water management on the Se speciation dynamics in the soil solution and Se uptake and speciation in rice in a pot experiment. A control containing no Se or 0.5 mg kg^{-1} of soil of selenite or selenate was added to the soil, and plants were grown under aerobic or flooded conditions. Flooding soil increased soluble Se concentration when no Se or selenite was added to the soil, but decreased it markedly when selenate was added. Selenate was the main species in the +selenate treatment, whereas selenite and selenomethionine selenium oxide were detected in the flooded soil solutions of the control and +selenite treatments. Grain Se concentration was 49% higher in the flooded than in the aerobic treatments without Se addition. In contrast, when selenate or selenite was added, the aerobically grown rice contained 25- and 2-fold, respectively, more Se in grain than the anaerobically grown rice. Analysis of Se in rice grain using enzymatic hydrolysis followed by HPLC-ICP-MS and in situ X-ray absorption near-edge structure (XANES) showed selenomethionine to be the predominant Se species. The study showed that selenate addition to aerobic soil was the most effective way to increase Se concentration in rice grain.

KEYWORDS: Selenium; selenium speciation; *Oryza sativa*; soil pore water; HPLC-ICP-MS; XANES

INTRODUCTION

Selenium (Se) is an essential micronutrient for humans. Suboptimal intake of Se is associated with a range of health effects such as oxidative stress, impaired immune function, reduced fertility, and increased risk of some cancers (*1*). Globally, between 0.5 and 1 billion people are estimated to have an insufficient intake of Se (*2*). A recent global survey of rice (*Oryza sativa*), the staple food for more than half of the world's population (*3*), showed that 75% of the samples had a Se concentration insufficient for human requirements (*4*). Because Se enters the food chain through plants, biofortification of food crops through Se fertilization or genetic improvement is considered to be an effective way of raising Se intake in a target population (*1*, *5*, *6*). This approach has the merit of using plants as effective buffers to prevent accidental overdose of Se. Moreover, the assimilation of inorganic Se into organic forms by plants also enhances its nutritional efficacy (*7*). Selenium biofortification of crops through fertilization has been successfully practiced in Finland since the mid-1980s (*8*).

The accumulation of Se by plants is determined by the ability of a plant to take up Se, which varies widely among plants species (*1*, *9*, *10*), and the bioavailability of Se in the soil. Plant-available Se in soil is strongly influenced by Se speciation, with the redox potential and pH playing a central role (*11*, *12*). Selenate tends to be the predominant species in aerobic and neutral to alkaline environments; selenite becomes the dominant species in the environment with an intermediate redox potential, especially with an acidic pH, whereas elemental Se and selenide may be produced in anaerobic environments (*13*). The various chemical forms of Se differ widely in their water solubility and sorption to soil. Selenate and selenite are highly water-soluble. However, selenate behaves mainly as a nonsorbing solute, whereas selenite can be adsorbed strongly by the soil solid phase (e.g., iron oxides/hydroxides), resulting in a lower solubility in the soil solution (*14*−*16*). Thus, selenate is generally more available for plant uptake than selenite (*11*, *17*−*19*). A number of studies have investigated the effects of redox potential, pH, and the addition of organic matter on the transformation of Se species and Se mobility (*20*−*22*); however, these studies have focused on Se-contaminated soils or sediments with the aim of reducing Se mobility in the environment and Se toxicity to the organisms. Moreover, the forms of Se were quantified by subtraction of different chemically reactive pools, which may not be specific to individual Se species. Much less is known about Se speciation, transformation, and bioavailability in low Se soils, which produce low Se crops in many regions of the world.

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Rice plants normally grow in flooded paddy soils, where the behavior of Se is likely to be very different from that in aerobic soils. Under flooded soil conditions, elemental Se and selenide are the stable forms (*13*), which are less bioavailable for plant uptake. On the other hand, the reductive dissolution of iron oxides/hydroxides occurring in anaerobic soils may release the sorbed metals/metalloids into the soil solution phase (*23*, *24*). Mikkelsen et al. (*20*) showed that flooding and the addition of organic manure decreased Se uptake and toxicity to rice when selenate was added to a Se-contaminated soil. There are no studies on the dynamics of Se speciation in soil and the bioavailability to plants as influenced by water management during rice cultivation in the context of Se biofortification.

In addition to total Se concentration, the speciation of Se in food is important because the bioavailability and the health effects may differ depending on the Se species present. Organic forms of Se are more bioavailable to humans than inorganic Se species (*7*). Selenium methylselenocysteine (SeMeSeCys) and its γ-glutamyl derivative are low molecular weight nonprotein amino acids found in Se accumulators and in a number of edible plants of the *Allium* and *Brassica* families (*7*, *25*). There is evidence from animal studies that these Se species have potent antitumor effects (*7*). In rice grain, the protein-bound selenomethionine (SeMet) is the predominant Se species, typically accounting for >80% of total Se (*26*, *27*). However, a recent study using synchrotron-based X-ray absorption near-edge structure (XANES) showed a high proportion of SeMeSeCys (~50%) in a high-Se rice grain (*4*). It is not known if this difference is due to different analytical methods used or variation among rice samples. If Se fertilizers are to be used to biofortify crops, it is important to know whether inorganic Se is efficiently assimilated into organic forms.

The objective of the present study was to investigate the effects of water management on the dynamics and speciation of Se in the soil solution phase and subsequently the bioavailability of Se to rice plants. Furthermore, the speciation of Se in rice grain was determined using two different methods.

MATERIALS AND METHODS

Pot Experiment. The soil used is a silty clay loam (Aquic Paleudalf, USDA classification) collected from the plow layer (0−20 cm) of an arable field on the Rothamsted farm, southeastern England. The soil contained 2.08% organic C, 0.19% total N, and 0.48 mg kg^{-1} total Se and had a pH of 5.3. The soil was air-dried, sieved to < 5 mm, and homogenized. Basal fertilizers (150 mg of N kg^{-1} of soil as NH_4NO_3, 25 mg of S kg^{-1} of soil as $MgSO_4$, 30 mg of P kg^{-1} of soil, and 75.5 mg of K kg^{-1} of soil as K_2HPO_4) were added to the soil and mixed thoroughly. A factorial experimental design was used with two water management regimes (flooded or aerobic) and three Se treatments: control (no addition of Se) and +0.5 mg of Se kg^{-1} of soil in the form of selenate (Na_2SeO_4) or selenite (Na_2SeO_3). Each treatment was replicated in four 1 L plastic pots each containing 1.2 kg of soil. The pots were randomly arranged on a bench inside a glasshouse (day/night temperatures of 28/25 °C, light period of 16 h per day with natural sunlight supplemented with sodium vapor lamps to maintain a light intensity of >350 μmol m^{-2} s^{-1}).

A soil pore-water sampling device (Rhizon MOM 10 cm length, 2.5 mm o.d., Rhizosphere Research Products, Wageningen, The Netherlands) was buried diagonally in the middle of the soil of each pot for collecting soil solution. Deionized water was then added to maintain soil moisture of around 70% of the soil's water-holding capacity for the aerobic treatment and to full saturation with 2−3 cm of standing water above the soil surface for the flooded treatment. The two water management regimes were maintained throughout the experiment by daily additions of deionized water. A common japonica rice cultivar (*O. sativa* L. cv. Oochikara) was used. Five pregerminated rice seeds were planted in each pot. Further doses of fertilizers (175 mg of N, 30 mg of P, and 75.5 mg of K kg^{-1} of soil) were added into each pot at the tillering, stem extension, and flowering stages. Three plants per pot were removed at the growth stage of stem elongation (30 days after planting). The remaining plants were harvested at grain maturity (110 days after planting). Stems were cut at 2 cm above the soil surface, rinsed with deionized water, and dried at 60 °C for 48 h. The samples were separated into straw, husk, and unpolished rice grain. Soil solution samples were collected on days 9, 30, 57, and 97.

Analysis of Se Speciation and Concentration in Soil Solution. One milliliter of 0.1 M Na_2EDTA was added to 9 mL of soil solution immediately after collection to prevent iron oxide/hydroxide precipitation. The solution was filtered through a sterilized 0.2 μm filter and analyzed for Se speciation using HPLC-ICP-MS (Agilent LC1100 series and Agilent ICP-MS 7500ce, Agilent Technologies, Santa Clara, CA). Selenium species in soil solution were separated by an anion-exchange column (Hamilton PRP-X100) fitted with a guard cartridge. The injection volume was 50 μL per sample. The mobile phase contained 50 mM NH_4NO_3, 0.01 mM Na_2EDTA, and 2% methanol (pH adjusted to 9.4 with ammonia), which was pumped through the column isocratically at 1 mL min^{-1}. The outlet of the separation column was connected to a concentric nebulizer and a water-jacketed cyclonic spray chamber of the ICP-MS. An internal standard (germanium, Ge) was mixed continuously with the postcolumn solution through a peristaltic pump. Signals at m/z 72 (Ge), 78, and 80 (Se) were collected with a dwell time of 0.3 s. The Se signals were normalized using the Ge signal to correct any signal drift during the analysis. Polyatomic interference was removed by the Agilent Octopole Reaction System operating in the hydrogen gas mode (flow rate = 4 mL min^{-1}). Other ICP-MS instrumental conditions were as follows: RF forward power, 1550 W; sample depth, 8 mm from the load coil; carrier gas flow rate, 0.9 L min^{-1}; and spray chamber temperature, 2 °C. Peaks were identified by comparison with the retention times of standard compounds (see the Supporting Information (SI), Figure S1a). The Se standards [Na_2SeO_3, Na_2SeO_4, selenomethionine (SeMet), selenocystine ($SeCys_2$), selenium methylselenocysteine (SeMeSeCys)] were obtained from Sigma (St. Louis, MO) and prepared in ultrapure (> 18 MΩ) water. Selenomethionine selenium oxide (SeOMet) was prepared by reacting SeMet with 3% (v/v) H_2O_2 under sonication for 1 h (*28*). The identified Se species in the samples were quantified by external calibration curves with peak areas. Analysis of Se species was carried out immediately following sample collection and completed within 12 h. The total soluble concentration of Se in soil solutions was determined by ICP-MS operating in the H_2 gas reaction mode. The concentrations of Fe and Mn in soil solution were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES; Fisons ARL Accuris, Ecublens, Switzerland). The pH values of the soil solutions (untreated with EDTA) were measured using a pH electrode, and dissolved organic C (DOC) was measured by UV persulfate oxidation (Shimadzu TOC-V Instrument). The redox potentials (Eh) of soils were determined using a combined platinum and silver/silver chloride electrode system (HI 3230B, Hanna Instruments, Woonsocket, RI), with the electrode being inserted at approximately 1 cm below the soil surface.

Analysis of Se Speciation and Concentration in Rice Samples. Selenium speciation in the rice grain was determined by HPLC-ICP-MS following extraction with protease and lipase (*29*). Four milliliters of 10 mg mL^{-1} protease XIV solution, 4 mL of 5 mg mL^{-1} lipase solution, and 2 mL of water were added to 0.4 g of ground rice powder in a 10 mL centrifugal tube. To test the recoveries of standard Se compounds, rice powder samples from the control treatment were spiked with the appropriate amounts of SeMet, SeMeSeCys, or $SeCys_2$, followed by the additions of the protease and lipase solutions. The mixture was incubated at 37 °C and gently shaken (125 rpm) for 24 h. The extract was centrifuged at 3400g for 45 min at room temperature. The supernatant was filtered through a 0.45 μm syringe filter. The filtrate was immediately analyzed for Se speciation using HPLC-ICP-MS as described previously (*28*). An anion-exchange column (Dionex AS14) fitted with a guard column (Dionex AG14) was used, and the mobile phase was 6 mM Na_2CO_3 and 2% methanol (pH 9.5) at a flow rate of 1 mL min^{-1}. Selenium species in the extracts were identified by standard addition and by matching the retention times of standard compounds (SI, Figure S1b). The recoveries of SeMet and SeMeSeCys were 90 and 82%, respectively, and that of $SeCys_2$ was only 10%. Because $SeCys_2$ was not detected in the samples from the present study, its low recovery in the spiking experiment was considered not to present a problem. In addition, two other HPLC methods were tested on selected samples; the first method was the same as that used for soil solution Se speciation described above, and the second method used the same

anion-exchange column (Hamilton PRP-X100) but with a mobile phase of 5 mM citric acid and 1% methanol (pH 5.0) (SI, Figure S1c). These additional two methods confirmed the results obtained with the first method using the Dionex AS14 column.

For analysis of total Se, ground samples of rice straw, grain, and husk were digested with 5 mL of high-purity $HNO_3/HClO_4$ (87:13 v/v). The Se concentration in the digest solution was determined by ICP-MS (Agilent 7500ce) operating in the reaction cell mode with H_2 gas. A certified reference material (NIST 1568a rice flour) and blanks were included for quality assurance. The recovery for NIST 1568a was 93.3 ± 1.4%.

Selenium Speciation in Rice Grain Using Synchrotron XANES. XANES spectra of three selected rice grain powder samples were collected at BL20-B at the Photon Factory, Tsukuba, Japan. BL20-B is equipped with a water-cooled Si (111) monochromator, which was calibrated using an elemental Se foil (*K*-edge 12658 eV). The samples were mounted in a cryostat sample holder to hinder beam-induced artifacts and analyzed at about 18 K. XANES spectra were collected in fluorescence mode with a 36 pixel array detector (Canberra-Eurisys). Selenium standards were analyzed as powders (diluted in boron nitride) and included sodium selenite, sodium selenate, $SeCys_2$, SeMet, and SeMeSeCys. The XANES data of the samples (average of 6−13 scans) were normalized and analyzed by linear combination fitting (LCF) using Athena software (*30*).

Statistical Analysis. Two-way analysis of variance was performed to test the significance of water management, Se treatments, and the interactions between these two factors.

RESULTS

Dynamics of Se in Soil Solution. Flooding of the soil decreased the soil redox potential markedly and increased the pH of soil solution compared with the aerobic treatment (SI, Figure S2). Soil Eh ranged from 487 to 613 mV in the aerobic treatment and from 128 to 267 mV in the flooded treatment, representing about a 300 mV difference between the two water management regimes (SI, Figure S2a). Note that Eh was measured near the soil surface, which was likely to be higher than the values at a deeper position of the soil in the flooded treatment. Soil solution pH was around 6.5 in the flooded treatment at all sampling times; the pH values in the aerobic treatment were 0.7−2 units lower in the first three samplings but approached those of the flooded treatment in the last sampling (SI, Figure S2b).

Analysis of the total soluble concentration of Se in soil solutions revealed highly significant differences between the two water management regimes and between the Se treatments, as well as strong interactions between these two factors (**Figure 1**). In the control treatment (no addition of Se), total Se concentration in soil solution was 3−11 times higher in the flooded treatment than under the aerobic conditions throughout rice growth (**Figure 1a**). In the treatment with selenite addition, soil solution Se was also higher (by 4−6 times) under the flooded than under the aerobic conditions (**Figure 1b**). In contrast, in the selenate treatment, Se concentration in soil solution was significantly lower in the flooded treatment than in the aerobic treatment in the first two samplings, although this pattern was reversed in the last two samplings when Se concentration had decreased to levels comparable to those in the control and +selenite treatments (**Figure 1c**). The addition of selenate increased soil solution Se initially much more than the addition of selenite. Soil solution Se decreased with sampling time, especially in the +selenate treatment,when Se concentration decreased by 3- and 75-fold in the aerobic and flooded treatments, respectively, from day 9 (first sampling) to day 30 (second sampling). Approximately 50 and 3% of the added selenate and selenite, respectively, were found in the soil solution under the aerobic conditions in the first sampling.

Analysis of Se speciation in soil solutions using HPLC-ICP-MS also showed a marked difference between the flooded and aerobic treatments (**Figure 2**). Three Se species, selenite, selenate, and SeOMet, were detectable in the soil solutions (SI, Figure S3).

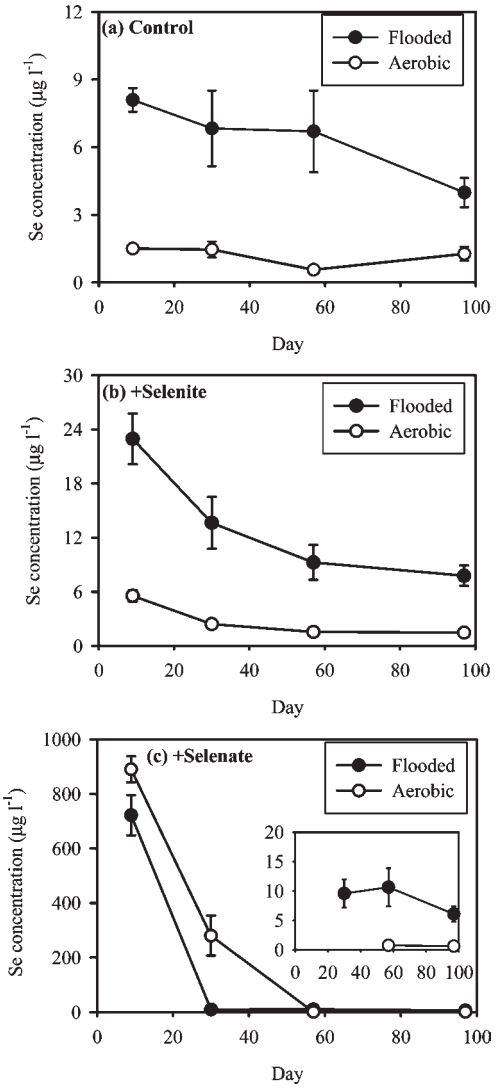

Figure 1. Dynamics of total soluble Se concentrations in soil solution as influenced by water management and Se addition in the pot experiment: control (**a**), +selenite treatment (**b**); +selenate treatment (**c**). Data are mean ± SE (n = 4).

SeOMet was detected only in the flooded treatment in the first sampling. Selenite was detected only in the flooded control and +selenite treatments and only in the first one or two samplings. Large concentrations of selenate were found in the +selenate treatments; its concentration was significantly higher under the aerobic than under the flooded conditions, but decreased rapidly with time in both water management regimes. Small concentrations of selenate were also found in the +selenite treatments, more under the aerobic than under the flooded conditions. None of the three Se species was detected in the last two samplings. Comparison of the total Se concentration determined by ICP-MS and the sum of the three Se species determined by HPLC-ICP-MS (**Figures 1** and **2**) reveals that a nearly full recovery (85−108%) by the latter method was achieved in the aerobic or flooded + selenate treatments in the first sampling and the aerobic + selenate in the second sampling. In all other samples, the species sum accounted for 0−68% of the total soluble Se in the soil solutions, suggesting the existence of other undetected Se species.

There was also a marked mobilization of Fe and Mn into the soil solution in the flooded treatments, whereas their concentrations remained very low in the aerobic treatments (SI, Figure S4). The concentrations of Fe and Mn in soil solutions increased

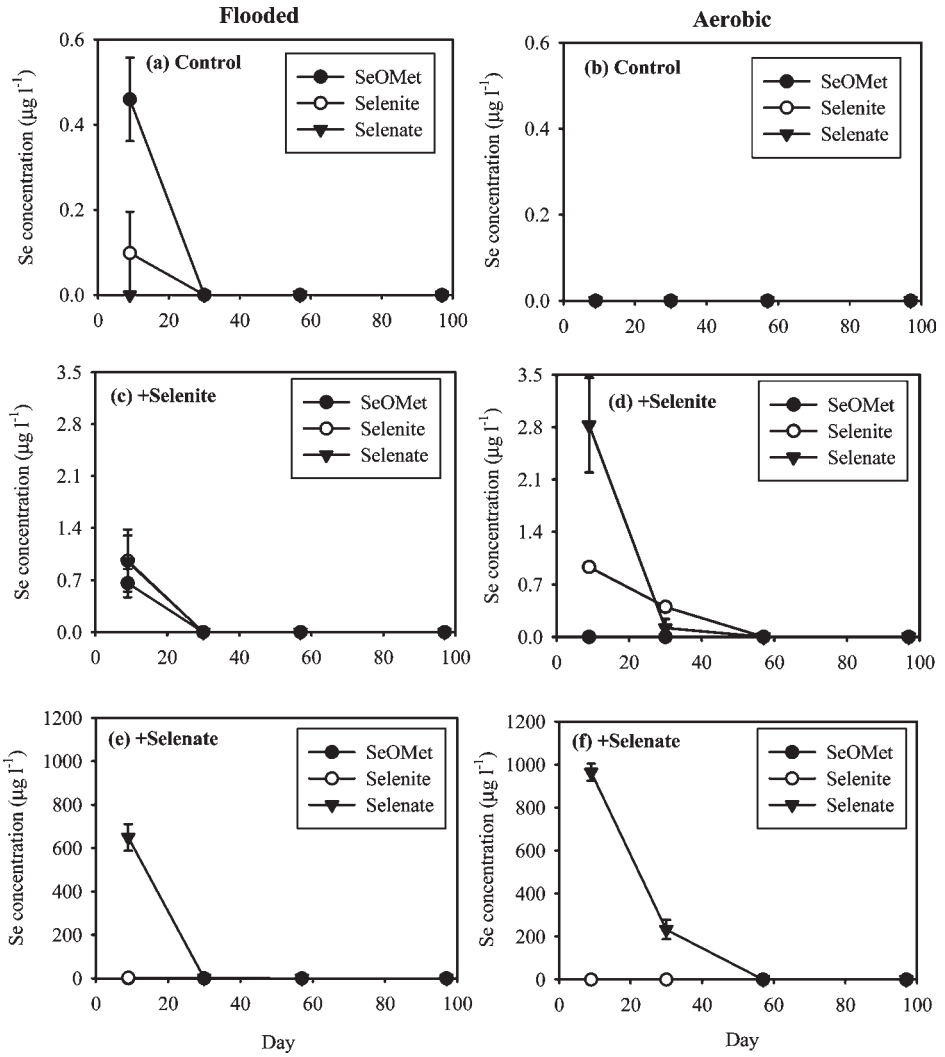

Figure 2. Dynamics of Se speciation in soil solution as influenced by water management and Se addition in the pot experiment: flooded (**a**, **c**, **e**); aerobic (**b**, **d**, **f**); control (**a**, **b**); +selenite treatment (**c**, **d**); +selenate treatment (**e**, **f**). Data are mean ± SE (n = 4).

dramatically with flooding within 30 days after planting and thereafter showed small fluctuations.

Plant Growth and Se Concentration in Plants. Rice grew better in the aerobic than in the flooded treatments, with significantly higher grain and straw yields in the former (SI, Figure S5 and Table S1). There was no significant effect of Se addition on grain or straw biomass.

The concentrations of Se in rice straw, grain, and husk were significantly ($P < 0.0001$) influenced by water management regime, Se additions, and the interactions between these two factors (**Figure 3** and SI, Table S1). The concentrations of Se were similar between grain and straw and larger than those of husk, but the influence of treatments was consistent among the three types of plant tissues. Therefore, only the grain Se data are considered in more detail below. Flooding increased the Se concentration of rice grain in the control treatment by 49%, but decreased it by 51 and 96% in the +selenite and +selenate treatments, respectively (**Figure 3**), indicating a strong interaction between the water management regime and the Se treatments. Grain Se concentration was about 2- and 25-fold higher in the aerobic treatment than in the flooded treatment when selenite or selenate, respectively, was added to the soil. Compared with the control treatment, an addition of selenite or selenate increased grain Se concentration by 2.3- and 1.9-fold, respectively, under the flooded conditions. The increase was much greater under the aerobic conditions, by 7- and 70-fold, respectively.

Selenium Speciation in Grain. Two methods were used to determine Se speciation in rice grain: enzymatic hydrolysis followed by HPLC-ICP-MS analysis for all samples and XANES for three selected samples.

In the HPLC-ICP-MS analysis, SeMet was the predominant Se species detected in the enzymatic hydrolytes of the rice grain samples from the pot experiment. There were some unidentified peaks (SI, Figure S6), but these were quantitatively unimportant. The concentration of SeMet determined by this method accounted for 47−75% (mean = 65%) of the total Se concentration determined by acid digestion and ICP-MS measurement (**Table 1**). This percentage was not significantly influenced by water management or Se treatments; therefore, samples with a high total Se concentration were also high in SeMet concentration. For comparison, we also collected four rice grain samples from paddy fields in Enshi, Hubei province, China, where the soils are naturally enriched with Se (*31*). The total Se concentration in rice ranged from 0.2 to 1.5 mg kg^{-1}. SeMet was by far the most important Se species in the enzymatic hydrolytes of the Enshi grain samples, accounting for 94−98%; the remainder (2−6%) was selenate (**Table 1**). The recovery of the Se species by HPLC-ICP-MS was 61−93% of the total grain Se in the Enshi samples.

Of the three rice grain samples analyzed by XANES, two samples (the aerobic + selenate treatment in the pot experiment with a total Se concentration of 5.1 mg kg^{-1} and the Enshi sample 3

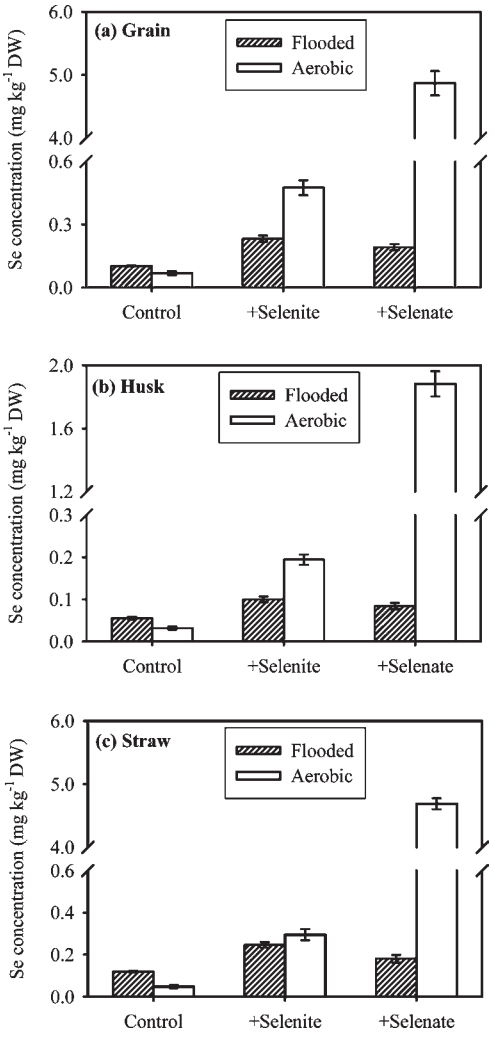

Figure 3. Effects of water management and Se addition on the total Se concentration in rice grain (**a**), husk (**b**), and straw (**c**) at maturity. Data are mean ± SE (n = 4).

Table 1. Selenium Speciation in Rice Grain (Determined by Enzymatic Hydrolysis and HPLC-ICP-MS) As Influenced by Water Management and Se Addition in the Pot Experiment and in Four Rice Grain Samples Collected from Enshi, China[a]

treatment	SeMet determined by HPLC-ICP-MS (mg of Se kg^{-1})	SeMet as a % of the total grain Se
aerobic + control	0.04 ± 0.00	68.2 ± 9.0
flooded + control	0.07 ± 0.00	69.5 ± 3.8
aerobic + selenite	0.22 ± 0.03	47.1 ± 4.8
flooded + selenite	0.15 ± 0.01	66.3 ± 4.8
aerobic + selenate	2.90 ± 0.15	61.7 ± 4.9
flooded + selenate	0.15 ± 0.02	75.3 ± 3.6
Enshi sample 1	0.63	61.7
Enshi sample 2	0.18	63.3
Enshi sample 3	1.05	72.7
Enshi sample 4	0.20	93.3

[a] Data are mean ±SE (n = 4) for the samples from the pot experiment.

with a total Se concentration of 1.5 mg kg^{-1}) could be fitted satisfactorily using the spectra of the Se standards (**Figure 4**). For the aerobic + selenate sample, LCF results indicated that the Se in the sample was dominated by SeMet (88 ± 5%) with much smaller contributions from SeMeSeCys and selenite (9 ± 5 and 2 ± 1%, respectively). Similarly, the best fit of the XANES spectra

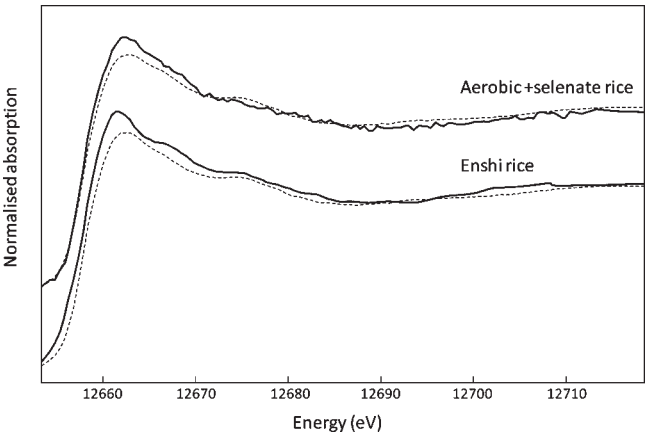

Figure 4. XANES analysis of Se speciation in rice grain from the aerobic + selenate treatment of the pot experiment and from Enshi, China. The solid and dotted lines represent the spectra of the samples and the results of the linear combination fitting analysis, respectively.

of Enshi grain sample 3 indicated the prevalence of SeMet (88 ± 10%), followed by SeMeSeCys (11 ± 10%). The third rice grain sample (the flooded + selenate treatment in the pot experiment) had a Se concentration (0.2 mg kg^{-1}) too low to be detected by XANES at the beamline used.

DISCUSSION

Because plant roots take up nutrients mainly from the soil solution (*32*), the dynamics of Se speciation in soil pore water may to a large extent control the bioavailability of Se to plants. The Se speciation method used in the present study was able to determine a number of inorganic and organic Se species in soil solutions, including selenate, selenite, SeMet, SeOMet, and $SeCys_2$. However, in many of the soil solution samples analyzed, the sum of the Se species quantified was lower than the total concentration of soluble Se (**Figures 1** and **2**); the only exception was the +selenate treatment in which selenate accounted for most of the soluble Se in the first two samplings. Similarly, Stroud et al. (*33*) found that the Se species quantified by HPLC-ICP-MS accounted for only 17−48% of the total extractable Se by 0.016 M KH_2PO_4 in a range of U.K. soils. They showed that a treatment with hydrogen peroxide of the soil extracts released SeOMet and an unidentified Se species, as well as converting selenite to selenate. Therefore, it is likely that the unaccounted portion of soluble Se in soil solutions was organic, possibly associated with dissolved organic matter.

The results of the present study show that the speciation and concentration of Se were influenced greatly by water management regime and the forms of Se addition (**Figures 1** and **2**). In the +selenate treatment, large concentrations of Se, mostly as selenate, were initially present in the soil pore water. The selenate concentration decreased rapidly, more so in the flooded than in the aerobic soil. This decrease can be attributed to plant uptake, microbial assimilation, or transformation to other less soluble Se species. Under the prevailing redox and pH conditions in the aerobic soil (SI, Figure S2), selenate is the thermodynamically stable species (*13*); therefore, plant and microbial uptakes were likely to be the main explanations for the decrease in the selenate concentration over time. Under the flooded conditions, selenate is expected to be reduced to selenite, elemental Se, or even selenide (*20*−*22*). However, no production of selenite was found in the soil pore water in the flooded treatment (**Figure 2e**,**f**). It is possible that selenite was strongly adsorbed by the soil solid phase, for example, iron oxyhydroxides (*34*), thus leaving little selenite in the

solution phase, or that selenate was converted to other insoluble forms (e.g., elemental Se or metal−selenide precipitates). In the +selenate treatment, the data of Se uptake by rice plants are consistent with the solution dynamic data, with the aerobic treatment producing approximately 25-fold higher Se concentrations in grain and straw than the flooded conditions (**Figure 3**). Under these two water management regimes, 30 and 1%, respectively, of the added selenate was taken up by rice plants, indicating a much higher bioavailability of the selenate fertilizer under the aerobic conditions.

In contrast to the +selenate treatment, flooding the soil increased the concentration of Se in soil pore water compared with the aerobic treatment when either no Se or selenite was added (**Figure 1a**,**b**). There are two possible explanations why flooding increased Se solubility in soil. First, flooding the soil resulted in a reductive dissolution of iron oxyhydroxides, which are important sorbents of Se species such as selenite (*11*, *12*, *34*). This was confirmed by approximately 2 orders of magnitude higher Fe concentrations in the soil pore water in the flooded soil than the aerobic soil (SI, Figure S4). Second, more organic matter was dissolved into the soil pore water under the flooded than under the aerobic conditions (SI, Table S2), possibly bringing some organic Se species into the solution phase. In support of this, small concentrations of SeOMet were detected in the pore water extracted from the flooded soil, but not from the aerobic soil (**Figure 2**). Our results are different from those of Masscheleyn et al. (*21*), who found that Se solubility decreased with decreasing redox potential in the sediments of the Kesterson Reservior in California. These sediment samples had high concentrations of total Se ($\sim$9 mg kg^{-1}) with iron selenide controlling the solubility of Se. In contrast, the soil used in our study was an arable soil for growing upland crops and had a low Se concentration; therefore, the solid phase Se speciation was unlikely to be dominated by iron selenide, at least not during the initial period of soil flooding or in the aerobic treatment.

When no Se was added to the soil, flooding increased plant Se uptake (**Figure 3**), consistent with increased soluble Se in the soil pore water (**Figures 1a**, **2a**,**b**). In the +selenite treatment, however, increased Se concentration in the soil pore water (**Figure 1b**) resulting from soil flooding did not lead to a higher Se uptake by rice; in fact, the opposite occurred (**Figure 3**). This can be explained by the oxidation of some added selenite to selenate under the aerobic conditions (**Figure 2c**,**d**). It is known that selenate is more bioavailable to plants than selenite, because it is much more weakly adsorbed by the soil solid phase (*14*−*16*) and is also more readily translocated from plant roots to the above-ground tissues (*28*,*35*,*36*). Comparison between the +selenite and +selenate treatments showed that the latter had a much higher solubility in soil pore water and a higher bioavailability to rice under the aerobic conditions (**Figure 3**). However, there was little difference in their bioavailability to rice under the flooded conditions.

Speciation of Se in grain is important because the nutritional value may differ between different Se species (*7*, *25*). The wide range of Se concentration in rice grain obtained in this experiment presented an opportunity to test whether Se assimilation into organic forms was affected when Se concentration was boosted by fertilization. Our results show that SeMet was by far the predominant species in the enzymatic hydrolytes of rice flour from both the pot experiment and from the field samples collected in Enshi, China (**Table 1**). This is consistent with previous findings of SeMet being the predominant Se species in cereal grain including rice (*7*, *25*, *27*, *37*−*39*). Furthermore, the assimilation of inorganic Se to SeMet in rice was unaffected by water management or Se fertilization, indicating that the Se assimilation capacity was not exceeded even with the highest Se concentration obtained in the pot experiment ($\sim$5 mg kg^{-1}). This finding was in agreement with the study of Fang et al. (*27*), who found that 87% of the total Se in rice grain from a crop treated with foliar spray of Se was SeMet. Fang et al. (*27*) also identified a number of minor Se species in rice grain following enzymatic hydrolysis, including inorganic Se, $SeCys_2$, and SeOMet, but no SeMeSeCys. A recent study by Cubadda et al. (*39*) reported the presence of SeMeSeCys in wheat grain, but only at $<0.5\%$ of the sum of all Se species determined. The in situ Se speciation analysis using XANES on a high-Se sample from the pot experiment and an Enshi rice sample showed the dominance of SeMet, with SeMeSeCys and selenite being the minor components (**Figure 4**). In contrast, the XANES study of Williams et al. (*4*) reported SeMeSeCys as a major Se species in the bran and endosperm of a rice sample collected from Enshi, China, accounting for 47−55% of the total Se. However, reanalysis of the XANES data of Williams et al. (*4*) using a set of spectra of Se standards, all collected at the same time and the same beamline, showed that SeMeSeCys accounted for only 10−15% with the rest being SeMet (E. Lombi, unpublished data), which is similar to the results of the present study. Although the results from XANES and HPLC-ICP-MS were consistent in showing the predominance of SeMet in rice grain, they differed in the detection of SeMeSeCys. Because the enzymatic hydrolysis followed by HPLC-ICP-MS analysis recovered only about two-thirds of the total Se in the rice grain in our study, it is possible that other forms of Se may be present in the unreleased fraction. On the other hand, whereas XANES offers the advantage of in situ analysis, the outcome of the linear combination fitting of the XANES spectra is affected by, often a priori, the choice of the standards that are used in the fitting procedure (*40*). Further studies are needed to resolve this discrepancy.

In conclusion, the present study showed that the soil indigenous Se was more bioavailable to rice under flooded than under aerobic conditions. However, the most effective way to increase Se concentrations in rice was the addition of selenate to soil that was maintained under the aerobic conditions. In comparison, additions of selenate or selenite to flooded soil were clearly much less effective in boosting grain Se concentration. SeMet was found to be the predominant Se species in rice grain over a wide range of total Se concentration, indicating that rice was efficient at assimilating inorganic Se into organic forms.

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Supporting Information Available: Two tables and six figures. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Rayman, M. P. Food-chain selenium and human health: emphasis on intake. *Br. J. Nutr.* **2008**, *100*, 254−268.
(2) Combs, G. F. Selenium in global food systems. *Br. J. Nutr.* **2001**, *85*, 517−547.
(3) HarvestPlus, http://www.harvestplus.org/content/rice.
(4) Williams, P. N.; Lombi, E.; Sun, G. X.; Scheckel, K.; Zhu, Y. G.; Feng, X. B.; Zhu, J. M.; Carey, A. M.; Adomako, E.; Lawgali, Y.; Deacon, C.; Meharg, A. A. Selenium characterization in the global rice supply chain. *Environ. Sci. Technol.* **2009**, *43*, 6024−6030.
(5) Broadley, M. R.; White, P. J.; Bryson, R. J.; Meacham, M. C.; Bowen, H. C.; Johnson, S. E.; Hawkesford, M. J.; McGrath, S. P.;

Zhao, F. J.; Breward, N.; Harriman, M.; Tucker, M. Biofortification of UK food crops with selenium. *Proc. Nutr. Soc.* **2006**, *65*, 169−181.
(6) Hawkesford, M. J.; Zhao, F. J. Strategies for increasing the selenium content of wheat. *J. Cereal Sci.* **2007**, *46*, 282−292.
(7) Rayman, M. P.; Infante, H. G.; Sargent, M. Food-chain selenium and human health: spotlight on speciation. *Br. J. Nutr.* **2008**, *100*, 238−253.
(8) Hartikainen, H. Biogeochemistry of selenium and its impact on food chain quality and human health. *J. Trace Elem. Med. Biol.* **2005**, *18*, 309−318.
(9) Terry, N.; Zayed, A. M.; de Souza, M. P.; Tarun, A. S. Selenium in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2000**, *51*, 401−432.
(10) Zhu, Y. G.; Pilon-Smits, E. A. H.; Zhao, F. J.; Williams, P. N.; Meharg, A. A. Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation. *Trends Plant Sci.* **2009**, *14*, 436−442.
(11) Gissel-Nielsen, G.; Gupta, U. C.; Lamand, M.; Westermarck, T. Selenium in soils and plants and its importance in livestock and human nutrition. *Adv. Agron.* **1984**, *37*, 397−460.
(12) Mikkelsen, R. L.; Page, A. L.; Bingham, F. T. Factors affecting selenium accumulation by agricultural crops. In *Selenium in Agriculture and the Environment*; SSSA Special Publication 23; Jacobs, L. W., Ed.; Soil Science of America and American Society of Agronomy: Madison, WI, 1989; pp 65−94.
(13) Elrashidi, M. A.; Adriano, D. C.; Workman, S. M.; Lindsay, W. L. Chemical equilibria of selenium in soils: a theoretical development. *Soil Sci.* **1987**, *144*, 141−152.
(14) Neal, R. H.; Sposito, G. Selenate adsorption on alluvial soils. *Soil Sci. Soc. Am. J.* **1989**, *53*, 70−74.
(15) Neal, R. H.; Sposito, G.; Holtzclaw, K. M.; Traina, S. J. Selenite adsorption on alluvial soils. 1. Soil composition and pH effects. *Soil Sci. Soc. Am. J.* **1987**, *51*, 1161−1165.
(16) Barrow, N. J.; Whelan, B. R. Testing a mechanistic model. 7. The effects of pH and of electrolyte on the reaction of selenite and selenate with a soil. *J. Soil Sci.* **1989**, *40*, 17−28.
(17) Singh, B. R. Selenium content of wheat as affected by selenate and selenite contained in a Cl-based or SO_4-based NPK fertilizer. *Fert. Res.* **1991**, *30*, 1−7.
(18) Cartes, P.; Gianfreda, L.; Mora, M. L. Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. *Plant Soil* **2005**, *276*, 359−367.
(19) Sharma, S.; Bansal, A.; Dhillon, S. K.; Dhillon, K. S. Comparative effects of selenate and selenite on growth and biochemical composition of rapeseed (*Brassica napus* L.). *Plant Soil* **2010**, *329*, 339−348.
(20) Mikkelsen, R. L.; Mikkelsen, D. S.; Abshahi, A. Effects of soil flooding on selenium transformations and accumulation by rice. *Soil Sci. Soc. Am. J.* **1989**, *53*, 122−127.
(21) Masscheleyn, P. H.; Delaune, R. D.; Patrick, W. H. Transformations of selenium as affected by sediment oxidation reduction potential and pH. *Environ. Sci. Technol.* **1990**, *24*, 91−96.
(22) Jayaweera, G. R.; Biggar, J. W. Role of redox potential in chemical transformations of selenium in soils. *Soil Sci. Soc. Am. J.* **1996**, *60*, 1056−1063.
(23) Fendorf, S.; Herbel, M. J.; Tufano, K. J.; Kocar, B. D. Biogeochemical processes controlling the cycling of arsenic in soils and sediments. In *Biophysico-chemical Processes of Heavy Metals and Metalloids in Soil Environments*; Violante, A., Huang, P. M., Gadd, G. M., Eds.; Wiley: Hoboken, NJ, 2008; pp 313−338.
(24) Grybos, M.; Davranche, M.; Gruau, G.; Petitjean, P. Is trace metal release in wetland soils controlled by organic matter mobility or Fe-oxyhydroxides reduction? *J. Colloid Interface Sci.* **2007**, *314*, 490−501.
(25) Whanger, P. D. Selenocompounds in plants and animals and their biological significance. *J. Am. Coll. Nutr.* **2002**, *21*, 223−232.
(26) Mar, J. L. G.; Reyes, L. H.; Rahman, G. A. M.; Kingston, H. M. S. Simultaneous extraction of arsenic and selenium species from rice products by microwave-assisted enzymatic extraction and analysis by ion chromatography-inductively coupled plasma-mass spectrometry. *J. Agric. Food Chem.* **2009**, *57*, 3005−3013.
(27) Fang, Y.; Zhang, Y. F.; Catron, B.; Chan, Q. L.; Hu, Q. H.; Caruso, J. A. Identification of selenium compounds using HPLC-ICPMS and nano-ESI-MS in selenium-enriched rice via foliar application. *J. Anal. At. Spectrom.* **2009**, *24*, 1657−1664.
(28) Li, H. F.; McGrath, S. P.; Zhao, F. J. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. *New Phytol.* **2008**, *178*, 92−102.
(29) Huerta, V. D.; Reyes, L. H.; Marchante-Gayon, J. M.; Sanchez, M. L. F.; Sanz-Medel, A. Total determination and quantitative speciation analysis of selenium in yeast and wheat flour by isotope dilution analysis ICP-MS. *J. Anal. At. Spectrom.* **2003**, *18*, 1243−1247.
(30) Ravel, B.; Newville, M. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. *J. Synchrotron Radiat.* **2005**, *12*, 537−541.
(31) Fordyce, F. M.; Zhang, G. D.; Green, K.; Liu, X. P. Soil, grain and water chemistry in relation to human selenium- responsive diseases in Enshi District, China. *Appl. Geochem.* **2000**, *15*, 117−132.
(32) Barber, S. A. *Soil Nutrient Bioavailability, a Mechanistic Approach*; Wiley: New York, 1984.
(33) Stroud, J. L.; McGrath, S. P.; Zhao, F. J. Selenium speciation in soil extracts using LC-ICP-MS. *Int. J. Environ. Anal. Chem.* **2010**, in press (DOI: 10.1080/03067310903111661).
(34) Balistrieri, L. S.; Chao, T. T. Adsorption of selenium by amorphous iron oxyhydroxide and manganese dioxide. *Geochim. Cosmochim. Acta* **1990**, *54*, 739−751.
(35) Zayed, A.; Lytle, C. M.; Terry, N. Accumulation and volatilization of different chemical species of selenium by plants. *Planta* **1998**, *206*, 284−292.
(36) Ximénez-Embún, P.; Alonso, I.; Madrid-Albarran, Y.; Camara, C. Establishment of selenium uptake and species distribution in lupine, Indian mustard, and sunflower plants. *J. Agric. Food Chem.* **2004**, *52*, 832−838.
(37) Beilstein, M. A.; Whanger, P. D.; Yang, G. Q. Chemical forms of selenium in corn and rice grown in a high selenium area of China. *Biomed. Environ. Sci.* **1991**, *4*, 392−398.
(38) Stadlober, M.; Sager, M.; Irgolic, K. J. Effects of selenate supplemented fertilisation on the selenium level of cereals − identification and quantification of selenium compounds by HPLC-ICP-MS. *Food Chem.* **2001**, *73*, 357−366.
(39) Cubadda, F.; Aureli, F.; Ciardullo, S.; D'Amato, M.; Raggi, A.; Acharya, R.; Reddy, R. A. V.; Prakash, N. T. Changes in selenium speciation associated with increasing tissue concentrations of selenium in wheat grain. *J. Agric. Food Chem.* **2010**, *58*, 2295−2301.
(40) Lombi, E.; Susini, J. Synchrotron-based techniques for plant and soil science: opportunities, challenges and future perspectives. *Plant Soil* **2009**, *320*, 1−35.

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