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Availability of fertilizer selenium in rice soils

Influence of submergence and subsequent drainage on the partitioning and lability of added selenium fertilizers in a Sri Lankan rice soil

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Summary

The influence of submergence (anaerobic conditions) and subsequent drainage (aerobic conditions) in rice soils on the partitioning and lability of Se fertilizers was studied. We used an isotope dilution method to examine the time-dependent changes in the partitioning and lability of Se in a Sri Lankan rice soil following fertilization with selenite (Se(IV)) and selenate (Se(VI)) (1 mg kg$^{-1}$) and incubation under anaerobic (30 d) and subsequent aerobic conditions (7 d) mimicking rice paddy water management practices. The K$_d$ values for Se(IV) were significantly (P $\leq$ 0.001) higher than those for Se(VI) in all treatments and sampling times. The K$_d$ values for Se(IV) and Se(VI) decreased significantly (P $\leq$ 0.001) with time during the anaerobic and subsequent aerobic phases. Applied Se(IV) fertilizer was rapidly removed (0 d = ~ 40% and 14 d ~100 %) into non-labile pools during the anaerobic phase, with no significant increase in the labile pool following short term aeration. The results suggest the rapid decrease in Se(IV) lability may be due to the strong non-reversible (at least for 7 days) sorption of Se (IV). In contrast, applied Se(VI) fertilizer was ~ 90 % labile at 0 d and decreased during the anaerobic phase to ~ 30% after 30d. There was no significant change in the lability of Se(VI) following the short term aerobic phase following anaerobic conditions. These results indicate that Se(IV) would not be an effective pre-plant fertilizer for rice production. Selenate is likely to be a more effective Se fertilizer, but losses to non-labile forms during the submerged phase of rice production may means that efficiency of pre-plant Se(VI) fertilization is also compromised.
Introduction

Rice is a staple food in the Sri Lankan daily diet with an average annual per capita consumption of ~100 kg (IRRI, 2007). Selenium concentrations in rice grains in many parts of Sri Lanka range from 25 to 58 µg kg\(^{-1}\) (Fordyce et al. 2000). According to a survey using rice grown from various countries it is said that concentration of Se < 50 µg kg\(^{-1}\) of rice grain is indicative of Se-deficient areas (Williams et al. 2009).

Grain Se concentrations < 40 µg Se kg\(^{-1}\) can be found in rice in many parts of the world (Williams et al. 2009). In soils where the total Se content or bioavailability of Se is low, the associated food chains may also be Se deficient (Combs, 2001; Lyons et al., 2003). Low background Se concentrations (0.01-0.2 mg kg\(^{-1}\)) in soils could be due to parent material mineralogy while low Se availability in soils may be due to use of sulfate fertilizers (sulfate competes with selenate for root uptake) and strong adsorption of labile Se or conversion to insoluble forms such as elemental Se (Se(0)) (Dhillon & Dhillon 2000; Elrashidi et al. 1989; Fordyce et al. 2010; Fordyce et al. 2000). There is increasing evidence in the literature to suggest that diets supplemented with Se have the potential to reduce incidences of certain types of cancers, such as prostate and breast cancer (Lyons et al. 2004). The use of biofortification strategies such as soil fertilization with inorganic Se species has been shown to be a successful management strategy to increase the uptake and accumulation of Se in food crops (Broadley et al. 2006; Curtin et al. 2006; Lyons et al. 2005; Stadlober et al. 2001; Yang et al. 2007).

Selenate (Se(VI)) is the dominant Se species found in aerobic solutions in the typical pH range found in soils (e.g. pH 4 to 8) (Séby et al., 2001), and is considered highly soluble with low adsorption and precipitation capacities (Fordyce 2007). Selenite (Se(IV)) is a weak acid (pKa\(_1\) = 2.70 and pKa\(_2\) = 8.54) that can exist as HSeO\(_3\)\(^{-1}\) or SeO\(_3\)\(^{-2}\) in soil.
solutions (Séby et al. 2001). In reduced soil environments, Se(IV) is the major Se species found present in solutions and its mobility is mainly governed by sorption/desorption processes on various solid surfaces such as metal oxy-hydroxides (Balistrieri and Chao 1987). Selenite may be further reduced to Se(0) or selenide (Se(-II)) in soils (Neal 1995). In addition, Se may substitute for sulfur in sulfide minerals forming seleniferous sulfides (e.g. ferroselite) (Alejandro & Laurent 2009).

The speciation, partitioning and lability of Se in rice paddy soils is influenced by the soil oxidation-reduction (redox) potential, pH, microbial activity, mineralogy and organic matter content, and the presence of other competitive anions (e.g. phosphate and sulphate) (Dhillon & Dhillon 2000; Goh & Lim 2004). The behavior of Se has been extensively investigated in anaerobic soil systems (Sposito et al. 1991; Tokunaga et al. 1991; Tokunaga et al. 1996; Zhang & Frankenberger 2003). These studies found the availability of soil Se to be reduced under anaerobic conditions, so that pre-plant fertilization of rice soils with Se(VI) may not improve Se uptake by rice during growth under flooded conditions. Rice paddies are generally drained prior to harvest for 1-2 weeks, and this practice may have a significant influence on the partitioning and lability of fertilizer Se added to soils. In flooded soils, reduction of added soluble Se(IV) or Se(VI) occurs within weeks with >80 % of the added Se being fixed into non-labile pools (Premarathna et al., 2010). Oxidation of reduced Se species has been shown to be slow in soils with < 20 % of Se(0) being oxidised to Se(VI) in 60 d (Collins et al. 2006; 1998; Premarathna et al. 2010). Tokunaga et al. (1991) found it required over 55 days to oxidize half of the newly formed (biologically) colloidal Se(0) to Se(IV) in a contaminated sediment. In a previous study, we found the oxidation of Se(0) added to soils to be slow (< 20 % in 60 d) even in aerobic rice soils (Premarathna et al. 2010). It is therefore uncertain if reduced forms of Se (either added or formed in situ in soil) formed following submergence in soils could be
sufficiently oxidized during pre-harvest drainage of rice paddies to increase the labile pool of Se in soil and therefore improve Se uptake into rice grain.

Sequential fractionation methods are often used to determine the associations of Se in soils with different phases and to suggest possible lability and availability (Nakamaru et al. 2005). However, these sequential fractionation procedures are operationally defined, and may lead to changes in Se speciation and associations during extraction (Martens & Suarez 1996; Wright et al. 2003). In addition, these sequential fractionation methods do not provide information on the strength of binding of Se with soil surfaces which may change with soil redox conditions (Ashworth et al., 2008). Isotope dilution methods using enriched stable or radioactive Se isotopes have been used with success to examine the partitioning and lability of both Se(IV) and Se(VI) species simultaneously in aerobic and anaerobic soils (Collins et al., 2006; Premarathna et al., 2010).

In this study, we used an isotope dilution method to examine the time-dependent changes in the partitioning and lability of Se(IV) and Se(VI) in a Sri Lankan rice soil following fertilization with Se(IV) and Se(VI) and incubation under anaerobic (submerged) and subsequent aerobic (drained) conditions closely mimicking the rice paddy water management practices. This study was undertaken using a closed reaction system to examine in a controlled environment the influence of redox processes on solution speciation, partitioning and lability of added Se(IV) and Se(VI). The information will be used to develop fertilizer formulations or management practices for rice growing on similar soils in Sri Lanka or other rice growing countries to increase Se uptake into rice grain.

**Materials and Methods**
**Soil Physical and Chemical Characteristics**

An agricultural rice soil (poorly drained, Thionic Histosol) was collected for this study from Benthota (low country wet zone) a rice research station in Sri Lanka. The soil was collected from the top 20 cm, air-dried, crushed, sieved to < 2 mm and homogenized. The physical and chemical characteristics for the soil can be found in Table 1.

**Closed Vessel Reaction Cell System**

A series of laboratory microcosms (closed reaction vessels) were made in-house using a modification of the design by (Patrick et al. 1973) (Figure 1). The sampling tube was installed vertically into each reaction vessel near the centre of the soil suspensions. The end of the tubing outside the reaction vessel was fitted with a three-way valve so that when sampling was not in progress the valve could be closed to prevent the penetration of oxygen into the reaction vessel. Soil suspension samples were taken from the reaction vessels using vacuum-evacuated vials. All the reaction vessels were temperature controlled in a water bath maintained at 25°C. The reaction vessels were initially aerobic and then forced to become anaerobic through the addition of nitrogen gas (N$_2$) to the vessels at a flow rate of 0.37 ml min$^{-1}$. At the end of the anaerobic (reduced) phase, N$_2$ was turned off and the vessel opened to allow aeration and the onset of aerobic conditions.

Ten grams of soil was placed into each reaction vessel (n = 3) with 400 ml of ultra pure deionised water (Milli-Q, Millipore). The Se fertilizers were added to the reaction vessels as solutions at a rate of 1 mg Se kg$^{-1}$ as either sodium selenite (Na$_2$SeO$_3$.5H$_2$O, ≥99%, Fluka) or sodium selenate (Na$_2$SeO$_4$.10H$_2$O, >99.9%, Aldrich) in ultra pure deionized water (Milli-Q, Millipore). In addition, there were control reaction vessels with no Se fertilizer added to soil.
Soil Suspension Sampling and Analysis

Redox potential and pH of the soil suspensions were measured using ORP redox electrodes (Sensorex, CA, USA) and portable pH meters (HI98103, Hanna Instruments, USA) that were installed into each reaction vessel. Redox and pH measurements were recorded from each reaction vessel daily from time 0 to 37 d. The recorded values were corrected using the collected calibration data of Eh and pH meters before installation and after completion of the experiment.

Soil suspension samples were collected at time 0, 14 d (when plants are normally transplanted in the field following soil submergence), 30 d (when Eh values were found to stabilise under anaerobic conditions), and 37 d (when Eh values were found to stabilise under aerobic conditions) (Figure 2). At each sample collection period, three samples of ~15 ml were collected after first flushing the sampling line with ~ 2 ml of soil suspension. The soil suspension samples were transferred to an anaerobic chamber filled with N2 (modular atmosphere controlled system; DW Scientific) for further handling and analysis. At each sampling time additional soil suspension sample was sampled into a centrifuge tube from each reaction cell to measure the soil solution ratio. Dry mass of soil was measured by evaporation of water in the centrifuge tubes at 40 ⁰C to a constant weight.

Solution chemistry was determined after filtration of ~ 15 ml of soil suspension through 1.2 µm (Whatman No. 42) and 0.22 µm (Sartorius) filters. The filtered solutions were stored in the N2-filled glove box until analysis. All filters and vials were stored in the N2-filled glove box for one week before sampling. Five millilitres of filtrate was acidified with 50 µL 6M HCl and used for determination of Se(IV) by hydride generation-ICPMS, total Se by ICP-MS and Se(VI) concentrations by difference (Se(VI) = total Se concentrations minus Se(IV) concentrations) (Premarathna et al., 2010). The limits of
quantification (LOQ) (5×blank SD) were 0.36 µg L⁻¹ for Se(IV) by HG-ICP-MS and 0.2 µg L⁻¹ for total Se by ICP-MS. Four ml of the non-acidified filtrate was used to determine dissolved nitrate (NO₃⁻), and sulphate (SO₄²⁻) concentrations by ion chromatography (Dionex ICS-2500 system). Concentrations of dissolved organic carbon (DOC) in solutions were determined by the difference between total carbon and inorganic carbon (Matejovic 1997b). Dissolved sulphide (S(-II)) and Fe(II) concentrations in filtered solutions were determined in the anaerobic N₂ glove box using colorimetric methods published by Greenberg (1992) and Sparks (1996). The handling of aerobic samples taken from reaction vessels occurred outside the N₂-filled glove box.

Selenium Tracer Stability

An initial study was undertaken to assess the stability of the ⁷⁵Se(IV) and ⁷⁵Se(VI) tracers during Kₐ value and labile Se (E value) determinations. In triplicate, ~1 g soil was weighed into 50 ml centrifuge tubes and 10 ml ultrapure deionised water (Milli-Q, Millipore) added. The soil suspensions were shaken for 48 h and spiked with ⁷⁵Se(IV) or ⁷⁵Se(VI) and shaken for a further 24 h period. The soil suspensions were then centrifuged at 2325 g for 20 min, filtered through a 0.22 µm filter and ⁷⁵Se species activities determined using high performance liquid chromatography (HPLC) and γ-spectroscopy detection (Premarathna et al., 2010). There was found to be no significant (P ≤0.05) redox conversion of the ⁷⁵Se(IV) or ⁷⁵Se(VI) tracers in soil suspensions during the 24 h isotope equilibration period.

Selenium Kₐ Values

Fifteen ml of soil suspension from each reaction vessel and time period were spiked with 100 µL of a solution containing 50 kBq of ⁷⁵Se(IV) or 30 kBq of ⁷⁵Se(VI). The spiked
solutions were shaken for 24 h on an end-over-end shaker, centrifuged at 2325 g for 20 min and filtered to < 0.22 µm (Sartorius). The $^{75}$Se activities in filtered solutions were measured by $\gamma$-spectroscopy (1480 Wizard, Wallac).

The $K_d$ values for Se(IV) or Se(VI) from each reaction vessel and time period were calculated using the following equation (Premarathna et al., 2010):

$$K_d \text{ value (L kg}^{-1}) = \frac{R - r}{r} \times \frac{v}{m}$$

where, $R$ is the total activity of $^{75}$Se(IV) or $^{75}$Se(VI) added to soil suspensions in Bq; $r$ is the activity of $^{75}$Se(IV) or $^{75}$Se(VI) remaining in solution after 24 h equilibration in Bq; $v$ is the volume in litres and $m$ is mass of soil in kilograms.

*Labile Selenium (E values)*

The labile Se (IV) and Se(VI) concentrations determined by isotopic dilution using $^{75}$Se(IV) ($E_{Se(IV)}$ value) or $^{75}$Se(VI) ($E_{Se(VI)}$ value) tracer in soil suspensions from each reaction vessel and time period were determined using the following equation:

$$E \text{ value (mg kg}^{-1}) = \frac{S}{r} \times R \times \frac{v}{m}$$

where, $S$ is the total concentration of Se(IV) or Se(VI) in soil solutions after equilibration (mg L$^{-1}$) determined by HG-ICP-MS or ICP-MS (Se(VI) by difference); $r$ is the activity of $^{75}$Se(IV) or $^{75}$Se(VI) remaining in soil solutions after 24 h equilibration in Bq; $R$ is the total
activity of $^{75}$Se(IV) or $^{75}$Se(VI) added to soil suspensions in Bq; $v$ is volume in litres and $m$ is mass in kilograms.

The lability of Se(IV) or Se(VI) in each treatment and time period expressed as a percentage (%) of the total fertilizer Se added into each reaction vessel was calculated using the following equation:

$$E_{\text{added value (%)}} = \frac{E_{\text{Total Fertilized}} - E_{\text{Control}}}{\text{Total Se}_{\text{Fertilized}} - \text{Total Se}_{\text{Control}}} * 100$$

where, $E_{\text{added}}$ is the labile Se(IV) or Se(VI) as a percentage of the added fertilizer Se; $E_{\text{Control}}$ is labile Se(IV) or Se(VI) concentrations in control soil suspensions in mg kg$^{-1}$; $E_{\text{Total Fertilized}}$ is the total labile Se(IV) or Se(VI) concentrations in fertilized soil suspensions in mg kg$^{-1}$; Total Se$_{\text{Fertilized}}$ is the total Se(IV) or Se(VI) concentration in soil suspensions in mg kg$^{-1}$; and Total Se$_{\text{Control}}$ is the total Se(IV) or Se(VI) concentrations in control soil suspensions in mg kg$^{-1}$.

**Kinetics of Reaction of Added Se(IV) and $^{75}$Se(IV)**

In preliminary experiments, Se(IV) added to soil was observed to be rapidly (> 90% in < 24 hr) moved into non-labile pools in this soil. A further experiment was therefore undertaken to examine the short-term reaction kinetics of added Se(IV) and $^{75}$Se(IV) in this soil and its potential influence on $E$ value determinations for Se(IV). The Sri Lankan soil was compared to a sandy soil (pH 5.9, organic carbon content = 0.5%) from Mount Compass, South Australia, Australia with a lower capacity for Se(IV) sorption.

In 500 ml beakers ($n = 4$), soil and ultra-pure deionised water was added at a 1:20 (m/v) ratio and stirred for 24 h using a stirring bar to allow equilibration of surface and
solution Se. The soil suspensions were spiked with a solution containing Se(IV) at a rate of 1 mg Se kg⁻¹ (Na₂SeO₃.5H₂O, ≥99%, Fluka) and ⁷⁵Se(IV) (activity 5 MBq mL⁻¹). The absence of ⁷⁵Se(VI) from the ⁷⁵Se(IV) spike solution was confirmed using HPLC and γ counting (Premarathna et al., 2010).

Subsamples of the soil suspensions were taken after 5 min, 6 h, 24 h and 30 h, centrifuged at 2325 g for 20 min and filtered to < 0.22 µm (Sartorius). Total Se(IV) concentrations in the solutions were determined using HG-ICP-MS and ⁷⁵Se(IV) activities by HPLC and γ-spectroscopy (no conversion of the ⁷⁵Se(IV) tracer to ⁷⁵Se(VI) was observed). The E values for Se(IV) were determined and specific activity of solution Se(IV) was calculated for each time period using the following equation:

\[
\text{Specific activity Se(IV) (Bq µg}^{-1}) = \frac{\text{Se(IV)} \text{activity (Bq L}^{-1})}{\text{Se(IV)} \text{(µg L}^{-1})}
\]

Statistical Analysis

Analysis of variance (ANOVA) was performed on Kₐ and E values for the randomized complete block design using Genstat statistical software (Tenth Edition).

Results and Discussion

Soil Suspension Chemistry

A similar trend in E_H and pH values was found between the control, Se(IV) and Se(VI) fertilizer treatments (Figure 2). Similar to previous studies on submerged soils, there was found to be an inverse relationship between E_H (e.g. decreasing to more negative values) and pH (e.g. increasing towards neutral pH values) values in soil suspensions (Harada & Takahashi 2008; Ponnamperuma 1972). Initially a rapid decrease in redox
potential was observed in reaction vessels following N₂ addition and the onset of anaerobic conditions (Figure 2). This was followed by a slower decrease in E_H values to ~ -150 mV after 30 d. After the addition of air to the reaction vessels and the onset of aerobic conditions (oxidation), redox potential increased rapidly to E_H values found at time 0 of ~ +450 mV.

The pH of soils suspensions was found to decrease slightly in the control and Se(VI) treatments (from pH 5.9 to ~ 5.5) following N₂ addition and then increase in all soil treatments to pH ~6.5 after 30 d (Figure 2). Once the soil treatments were exposed to air, a rapid drop in pH was observed to pH ~5.0 followed by an increase in pH to ~6.0 after 2 d (Figure 2). Similar pH changes in soils have been observed by other researchers following submergence and drainage (Ponnamperuma 1972 ). The initial decrease in soil solution pH may be due to accumulation of carbon dioxide (CO₂) and production of organic acids from the partial decomposition of organic matter (Ponnamperuma, 1972). The DOC concentrations in solutions were found to increase from 54.7 ± 0.9 mg L⁻¹ at time 0 to 90.0 ± 2.3 mg L⁻¹ at 30 d and then decreased following the addition of air to soil treatments (23.2±0.56 mg L⁻¹ at time 37 d). The observed increase during the anaerobic phase may be due to incomplete microbial degradation of soil organic C or remobilization of co-precipitated solid phases during reductive dissolution (Angeles et al., 2006). The observed decreases in DOC during the aerobic phase may be due to DOC becoming associated or co-precipitated with mineral phases (e.g. Fe oxyhydroxides). Loss of CO₂ from the vessels and consumption of H⁺ ions during reduction reactions (e.g. reduction of Fe(III) oxyhydroxides) may be the reasons for the increase in pH in the anaerobic phase. The oxidation of sulfides phases to sulphate (SO₄²⁻ ) and Fe(II) into Fe(OH)₃ may have resulted in the pH decrease found in soil solutions following aeration of the reaction vessels (Mathew et al. 2001).
Dissolved nitrate (NO$_3^-$) and SO$_4^{2-}$ (Figure 3) concentrations decreased and dissolved Fe(II) and S(-II) concentrations increased (Figure 4) following N$_2$ addition. The addition of air to the reaction vessels resulted in increased NO$_3^-$ (data not shown) and SO$_4^{2-}$ concentrations and decreased Fe(II) and S(-II) concentrations in soil suspensions (Figure 4). These results are similar to other reported findings in submerged and aerated soils (Harada and Takahashi 2008; Reynolds et al. 1999).

**Selenium Partitioning**

In the majority of cases the partitioning of Se(IV) or Se(VI) as determined by partitioning of $^{75}$Se was found to be similar in all soil treatments (Se fertilizer and control) at all sampling times (Figure 5). These findings suggest that the low rate of Se fertilizer used had little influence on the partitioning of Se (IV) or Se (VI) in the soil, as determined by $^{75}$Se partitioning.

Selenite K$_d$ values were found to significantly decrease with time ($P \leq 0.001$) during the anaerobic phase (Figure 5). At day 0, Se (IV) K$_d$ values were 550-600 L kg$^{-1}$ in all three treatments and decreased to $\sim$ 200 L kg$^{-1}$ after 30 d (Figure 5). The Se(VI) K$_d$ values were found to significantly decrease ($P \leq 0.05$) during the anaerobic phase from $\sim$ 50 L kg$^{-1}$ at day 0 to $\sim$ 1 L kg$^{-1}$ after 30 d (Figure 5). The partitioning of Se(VI) and Se(IV) did not significantly ($P \leq 0.05$) change following the onset of aerobic conditions (Figure 5). This suggests the establishment of aerobic conditions had little influence in the short-term on the partitioning of Se species in this soil. At the initial pH of the soil suspensions ($\sim$ 5.5) (Figure 2), applied Se(IV) was likely adsorbed rapidly onto Fe and Al oxides and OM abundant in this soil (Mandal et al. 2009; Saha et al. 2004b). According to the study of Saha et al. (2004), Se (IV) adsorption was found to be a very fast two step reaction; the
first complete in 0.083 - 0.5 hr and then a relatively slow second reaction (hrs). The observed decrease in $K_d$ values for both Se species during the reduced phase may have been due to dissolution of oxide/hydroxides active in Se sorption, which is supported by the increases in Fe(II) concentrations in solution in the reduced phase (Figure 4A). Premarathna et al., (2010) found a similar trend of decreasing $K_d$ values, for both Se(VI) and Se(IV) in this soil after flooding.

The $K_d$ values for Se(IV) were found to be significantly ($P \leq 0.001$) higher than those for Se(VI) in all treatments and sampling times (Figure 5). This finding is consistent with $K_d$ values previously reported for Se(IV) and Se(VI) in soils (Collins et al. 2006; Nakamaru et al. 2005). Selenite forms monodentate and bidentate surface complexes with soil clay minerals, OM and Fe and Al oxides and these complexes appear to be stable so that desorption is limited (and slow) (Essington 2004; Mandal et al. 2009). Barrow & Whelan (1989) also showed that Se(IV) sorbs more strongly to soil than Se(VI) and furthermore Se(IV) has a marked continuing reaction that follows the kinetics of diffusive penetration into crystal lattices – hence not only does Se(IV) adsorb more strongly to soil surfaces, but Se(IV) penetrates into soil surfaces so that the Se is likely to be rapidly removed from the labile pool when applied as Se(IV). This is discussed further below.

**Selenium Lability**

The labile pool of fertilizer Se, expressed as a percentage of added Se (% E value), was found to be lower in soils receiving Se(IV) compared to soils receiving Se(VI) (Figure 6). The lability of Se(IV) and Se(VI) was found to significantly ($P \leq 0.001$) decrease with time, especially for fertilizer Se(IV). Indeed, solution concentrations of Se(IV) were below the reporting limit ($< 0.36 \mu g \, L^{-1}$) and prohibited calculation of Se(IV) $E$ values
after the initial addition (Figure 6). Even at time zero, lability of Se(IV) was much less than 100%, which is unusual as there was a very short time period (30 min) between addition of Se(IV) fertilizer and subsequent addition of $^{75}$Se(IV) to determine Se(IV) lability. This suggests a very fast fixation reaction of added Se(IV) in this soil (note not adsorption as added $^{75}$Se(IV) equilibrates with reversibly sorbed Se(IV)). Strong adsorption of Se(IV) is often associated with rapid fixation of Se(IV) into non-labile (non-exchangeable) pools (Barrow and Whelan 1979) – this was examined further in the kinetics experiment. There was no labile Se(IV) or Se(VI) identified in Se(VI) or Se(IV) fertilizer treatments, respectively, during the anaerobic or subsequent aerobic phases (Figure 6).

The lability of added Se(VI) at 0 d was found to be ~ 90 % (Figure 6) and slowly decreased to ~ 30% after 30 d submergence. There was found to be no significant change in the lability of Se(VI) following the subsequent 7 d aerobic phase. The decrease in the labile Se (VI) pool under anaerobic conditions may have been due to the reduction of added Se(VI) to Se(IV), Se (0) or Se(-II). By 14 d, concentrations of Fe in soil solution had increased, likely due to reductive dissolution of Fe solid phases (Kirk 2004). The higher concentrations of Fe(II) in soil solutions could lead to precipitation of Fe(II) and Fe(III) oxides as green rust which could reduce Se(IV) or Se(VI) species to non-labile Se(0) or Se(-II) (Myneni et al. 1997). In addition, the lability of Se(VI) may have decreased due to reduction to Se(IV) and the strong non-reversible sorption of Se (IV) to this soil. The lack of any increase in the labile pool of Se(VI) with the onset of aerobic conditions suggests the oxidation of reduced Se species (e.g. Se(IV), Se(0) and Se(-II)) to Se(VI) was slow.

As mentioned in the previous section, the solid phase partitioning of $^{75}$Se species was found to decrease with time during the anaerobic and subsequent aerobic phases (Figure 5), indicating the soil surfaces were being altered, likely through reductive
dissolution of Fe oxides. At the same time, the lability of cold Se fertilizers added at time 0 was decreasing with time (Figure 6), indicating that exchangeable Se(IV) and Se(VI) on soil surfaces was being transformed over the incubation period to non-exchangeable forms. This transfer of Se(IV) from the labile pool to non-labile forms was likely the result either of diffusive penetration of Se(IV) into mineral lattices (Barrow and Whelan 1989), or due to reduction to insoluble forms (Mikkelsen et al. 1989). The transfer of Se(VI) from the labile pool to the non-labile pool over time could have been due to reduction to Se(IV), and from thence the same reactions as outlined above would be operative. The decrease in Se lability during the anaerobic phase is consistent with a recent rice growth study undertaken Li et al. (2010). Similar to our findings, the pore water concentrations of Se were higher in soils fertilized with Se(VI) compared to Se(IV), and rice grain had much lower Se concentrations when grown under submerged compared to aerobic conditions (Li et al. 2010). Premarathna et al. (2010) also reported similar results showing a decrease in lability of Se with time in flooded rice soils amended with either Se(IV) or Se(VI).

To evaluate the rapidity of the reaction of Se(IV) with this soil, we compared the short term kinetics of reaction of $^{75}$Se(IV) and Se(IV) in this soil with a soil low in reactive Al or Fe oxides (Mount Compass soil). The specific activity of Se(IV) in soil solutions was constant with equilibration time in the Mt Compass soil (Supporting information, Figure 1) and the labile (Se(IV)) remained at ~100% of added Se(IV) throughout the equilibration (as expected). In contrast, the specific activity of Se(IV) in soil solutions from the Sri Lankan soil were measurable after 5 mins but not measurable thereafter, as (Se(IV)) concentrations in solution fell to below the detection limit (Supporting information, Figure 1). Even after only 5 minutes, cold Se(IV) concentrations were only 1.0 µg L$^{-1}$, just above the detection limit. The $^{75}$Se(IV) activity was found to also decrease in soil solution with equilibration time but remained above the quantification limit (105 Bq
L\textsuperscript{-1}) throughout the equilibration period. Due to Se(IV) concentrations being below the detection limit no E values could be calculated and the validity of the Se(IV) E value after only 5 mins reaction time is also questionable given cold Se concentrations being so close to the detection limit. This indicates an extremely rapid and strong sorption of Se(IV) in this soil under aerobic conditions (Figure 5). Decreases in strength of Se(IV) sorption after submergence (Figure 5) were not associated with increases in lability, indicating that Se(IV) fixed into non-exchangeable pools is not released after submergence.

Conclusions

Once fertilizer Se is added to rice soils rich in Al and/or Fe oxides, submergence will cause oxidised species of Se to rapidly lose lability, particularly so for Se(IV). Strong non-reversible binding of Se(IV) to soil surfaces, or reduction to insoluble Se forms (Se(0) Se(-II)) are the likely causes of this loss of lability for Se(IV). For Se(VI), the loss of lability is slower and less pronounced, and may be due to reduction to Se(IV) and subsequent reactions as suggested above. Short term oxidation does not release any of this bound Se into the labile Se(IV) or Se(VI) pools. Short term oxidation (e.g. pre-harvest drainage) does not appear to release any of this bound Se into the labile Se(IV) or Se(VI) pools. Hence, application of Se as a pre-plant fertilizer is not advisable as availability of Se will decline with time and most of the added Se will not remain in the available pool when plants are in the active stage of Se accumulation. More studies are needed to determine optimum strategies for fertilization of rice to encourage Se biofortification.

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We thank Claire Wright for technical support with ICP-MS analyses, Gillian Cozens for assistance during the experiments and Jenifer de Livera for the help in preparation of reaction vessels. Technical assistance from Dr. Margaret Cargill in preparation of the manuscript is highly appreciated. We also thank to Dr. Srimathie Indraratne for supplying soil samples from Sri Lanka.

References


FIGURE CAPTIONS

Fig.1. Reaction vessel design.

Fig.2. Redox potential ($E_H$) (A) and pH (B) values in soil suspensions during anaerobic and subsequent aerobic phases.

Fig.3. Nitrate (A) and sulphate (B) concentrations in soil suspensions during anaerobic and subsequent aerobic phases.

Fig.4. Iron (II) (A) and dissolved sulphide (B) concentrations in soil suspensions during anaerobic and subsequent aerobic phases.

Fig.5. Solid-solution partitioning ($K_d$ values) for: (A) Se(IV) and (B) Se(VI) in soil solutions during the anaerobic and subsequent aerobic phases.

Fig.6. Labile pool of: (A) Se(IV) and (B) Se(VI) as a percentage (%) of added fertilizer Se during the anaerobic and subsequent aerobic phases. LSD values at 5% level for Se(IV) and Se(VI) are 7.8 and 10.6 respectively. * E value not calculated as solution Se concentrations were less than the limit of detection (0.36 µg L$^{-1}$)
Figure 1

[Diagram showing a setup with a pH electrode, Redox electrode, Valve with luer lock, stopper, Screw cap, Plastic container, Soil Suspension, Stirrer, Manifold, Gas inlet, Flow meter, and Nitrogen]
Figure 3

A

B

Days

NO₃⁻ (mg L⁻¹)

SO₄²⁻ (mg L⁻¹)

Reduced

Oxidized

anaerobic

aerobic

selenite applied

selenate applied

control soil

0

10

20

30

40

0

10

20

30

40

50
Figure 4

A

Fe (II) (mg L⁻¹)

0 20 40 60 80 100 120

aerobic anaerobic

Days 0 1 2 0 3 0 4 0

Se(IV) fertilizer

Se(VI) fertilizer

control

B

S (II) (mg L⁻¹)

0.0 0.2 0.4 0.6 0.8 1.0

anaerobic aerobic

Days 0 10 20 30 40

Aerobic vs. anaerobic conditions effect on Fe and S concentrations.
Figure 5

(A) Se(IV) Kd (L kg⁻¹) over time for different applied Se fertilizers:
- 0 d
- 14 d
- 30 d
- 37 d

(B) Se(VI) Kd (L kg⁻¹) for Se(IV), Se(VI), and Control soil:
- Se(IV)
- Se(VI)
- Control soil
Figure 6

(A) E value as a % of total Se(IV) applied

(B) E value as a % of total Se(VI) applied
Table 1. Physical and chemical characteristics of the selected Sri Lankan soil used in this study.

<table>
<thead>
<tr>
<th>Soil characteristics</th>
<th>Units</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay</td>
<td>%</td>
<td>17.7</td>
</tr>
<tr>
<td>Organic C</td>
<td>%</td>
<td>4.3</td>
</tr>
<tr>
<td>pH&lt;sub&gt;H₂O&lt;/sub&gt; (1:5 m/v)</td>
<td></td>
<td>5.4</td>
</tr>
<tr>
<td>WHC&lt;sup&gt;¥&lt;/sup&gt;</td>
<td>%</td>
<td>31.2</td>
</tr>
<tr>
<td>Se</td>
<td>µg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>117</td>
</tr>
<tr>
<td>Al</td>
<td>g kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>33.2</td>
</tr>
<tr>
<td>Fe</td>
<td>g kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>26.6</td>
</tr>
<tr>
<td>C</td>
<td>%</td>
<td>4.3</td>
</tr>
<tr>
<td>Mn</td>
<td>mg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.06</td>
</tr>
<tr>
<td>Oxalate extractable Fe</td>
<td>g kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>6.9</td>
</tr>
<tr>
<td>Oxalate extractable Al</td>
<td>g kg</td>
<td>1.6</td>
</tr>
</tbody>
</table>

<sup>¥</sup>Gravimetric water content measured at 80% of water holding capacity measured at -10kPa
Table 1 - Concentrations of solution Se(IV) and labile Se(IV) (E values) in the Sri Lankan and Mt. Compass soils as a function of time after addition of 1 mg Se(IV) kg⁻¹.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Time</th>
<th>Se(IV) (µg L⁻¹)</th>
<th>E values (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mount Compass soil</td>
<td>5 min</td>
<td>48 ± 1.6</td>
<td>1.04 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>6 hrs</td>
<td>42 ± 0.4</td>
<td>1.04 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>24 hrs</td>
<td>40 ± 0.2</td>
<td>1.07 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>30 hrs</td>
<td>40 ± 1.0</td>
<td>1.10 ± 0.03</td>
</tr>
<tr>
<td>Sri Lankan soil</td>
<td>5 min</td>
<td>1.0 ± 0.1</td>
<td>0.89 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>6 hrs</td>
<td>&lt;0.36</td>
<td>‡</td>
</tr>
<tr>
<td></td>
<td>24 hrs</td>
<td>&lt;0.36</td>
<td>‡</td>
</tr>
<tr>
<td></td>
<td>30 hrs</td>
<td>&lt;0.36</td>
<td>‡</td>
</tr>
</tbody>
</table>

Values are averages ± SE

‡ E values not calculated as Se(IV) concentrations below the detection limit (0.36 µg L⁻¹)
Figure 1- Change in specific activity of Se(IV) with time in Sri Lankan soil and Mount Compass soil. * Specific activity not calculated for Sri Lankan soil as solution Se concentrations were less than the limit of detection (0.36 µgL⁻¹)