Increasing plant availability of selenium in rice soils under variable redox conditions

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This thesis is dedicated to the loving memory of my father

H.M. Premarathna

27th November 1947- 13th April 2005

May attain the great Nirvana

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ABSTRACT

This research focused on increasing plant availability of Se in Se-deficient paddy soils by agronomic biofortification. However, Se fertilization is complicated by the fact that the margin of safety between levels of Se compounds that will cause dietary deficiency and those that result in toxicity is small. The study was designed to understand the changes in Se availability in paddy rice soils under different moisture conditions and management practices. This knowledge was then used to develop an effective Se fertilization strategy. Because Se exists in several redox states and these can be transformed in soil by processes of oxidation or reduction, the study evaluated the effectiveness of different redox species of Se as fertilizers. In addition, methods of application and times of application were also evaluated. As the speciation of Se in rice grain also affects bioavailability to humans, Se speciation in rice grain was also evaluated.

Isotopic dilution techniques were used to understand the potential availability of selenite (SeO_3^{-2}), selenate (SeO_4^{-2}) and elemental Se (Se (0)) applied to soils subjected to different water regimes – field capacity or submerged soil conditions. The availability of fertilizer Se (0), as measured by concentrations of labile Se species in soil, was low because of limited oxidation to SeO_3^{-2} or SeO_4^{-2}. Elemental Se is therefore not suitable for pre-plant Se fertilization of lowland rice because it is not readily oxidized in paddy rice soils. In the submerged soils, concentrations of labile SeO_3^{-2} and SeO_4^{-2} were also low. More than, 80% of the Se added as either SeO_3^{-2} or SeO_4^{-2} was fixed into non-labile pools, likely through reduction to Se (0). Rates of oxidation of Se (0) will play a critical role either in determining whether reduced Se (0), which likely formed in

submerged soils after fertilization, will contribute to plant Se uptake through oxidation during field drainage before harvest or in the rice rhizosphere.

Kinetics of Se transformations occurring when a paddy soil is fertilized, flooded, and then re-oxidised were investigated. The results showed applied SeO_3^{-2} was very quickly and completely transformed to non-labile pools under flooded soil conditions, with no detectable oxidation to SeO_4^{-2}during the drainage period (7 days). Applied SeO_4^{-2} was much more labile than SeO_3^{-2}, but the lability also decreased with time under submerged conditions and did not increase markedly during the drainage phase. These results indicate that SeO_3^{-2} would not be an effective pre-plant fertilizer for rice production. Selenate is likely to be more effective, but losses to non-labile forms during the submerged phase of production also means that efficiency of pre-plant SeO_4^{-2} fertilization is also compromised.

The results of the first pot study indicated that most accumulation of Se in the grain occurred with SeO_4^{-2} fertilizer when applied at heading; SeO_4^{-2}-enriched urea applied at heading increased grain Se concentrations 5 to 6 fold (450 to 600 µg kg^{-1}) compared to the control (no Se fertilizer) in all three moisture treatments. Foliar SeO_3^{-2} at heading and fluid SeO_4^{-2} applied at heading in field capacity treatments increased grain Se 3.5- and 6-fold respectively compared to the control. The majority of Se in rice grains was identified in all treatments to be selenomethionine (SeM) which comprised over 90 % of total grain Se. Selenate-enriched urea was the most effective Se fertilization strategy for paddy rice.

A rice-growth experiment carried out with ^{75}Se radioisotope spiked fertilizer granule confirmed that Se-enriched urea granules applied at either tillering or heading produced significantly higher grain Se concentrations compared to any other Se application method. There were also higher concentrations of SeO_4^{-2} in flood and pore water samples following the co-application of Se and urea compared to the other treatments. These results showed that urea applied in combination and co-located with SeO_4^{-2} had a significant effect on the uptake and accumulation of Se in rice grains. Selenate-enriched urea granules applied to floodwater sank to the well-developed mat of adventitious roots on the soil surface. Oxygen release from the rice roots promotes production of NO_3^- from applied urea, which may have inhibited SeO_4^{-2} reduction. The ability to maintain high concentration of SeO_4^{-2} in soil solution with minimum speciation changes could be the reason for the higher uptake and accumulation of Se in rice grains in SeO_4^{-2}-enriched urea treatment. Farmers well adapted to applying urea at tillering or heading are in a position to quickly and easily adopt these management practices. Future research should be conducted to verify these results under field conditions with other rice varieties and on different soil types.

DECLARATION

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LIST OF PUBLICATIONS

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CHAPTER 1

1. GENERAL INTRODUCTION

Humans and animals require an adequate supply of micronutrients for their normal physiological and biological functions, and selenium has been identified as an essential micronutrient in human and animal biology. Selenium (Se) deficiency occurs in both humans and animals (Rayman 2000; Reilly 1996). It is listed as a risk factor for certain human cancers, and conditions related to low immunity and oxidative stress (Changsheng 2007; Combs 2001a; Rayman et al. 2006). For farm animals the common Se-deficiency related conditions are white muscle disease, exudative diathesis, and liver necrosis (Reilly 1996). Selenium enters the food chain through plants that accumulate Se from soil. Selenium deficiency in soils has been reported in many countries, for example China, Siberia, North Korea, Sri Lanka, Finland, New Zealand (Combs 2001a; Fordyce et al. 2000; Fugii et al. 1988; Kang et al. 1991; McLaughlin et al. 1999).

The main source of Se for the majority of people living in Asian countries is rice, their staple diet, but concentrations may be insufficient to maintain human health (Cao et al. 2001a; Chen et al. 2002; Williams et al. 2009). According to new research findings, most of the rice in the global market fails to provide the daily minimum requirement of Se for an average adult (Williams et al. 2009). Numerous efforts have been made to increase Se concentrations in cereal crops growing on Se-deficient soils, including the use of genetically improved cereal crops with higher uptake of Se (Lyons et al. 2004) and the application of Se into soil crop systems, called agronomic biofortification (Cubadda et al. 2010; Lyons et al. 2004).

Agronomic biofortification has many advantages over direct supplementation, as inorganic Se absorbed by the plant is transformed into an organic form having a higher bioavailability. In addition, plants act as a barrier to prevent uptake of excess or toxic concentrations, which could happen in direct supplementation (Hawkesford et al. 2007). Application of Se fertilizer to rice fields is an easy and cost-effective method to increase grain Se concentration. However, the application of fertilizers containing Se is complicated, mainly by the fact that concentrations giving rise to deficiency, sufficiency or toxicity are separated by a narrow margin. Therefore, precise application rates, and efficient methods and time of application to maximise the uptake while minimising environmental contamination, are essential. Although successful Se fertilization programs have been developed and commercialized in Finland and New Zealand to increase Se concentration in pasture and upland crops, there is no agronomic biofortification strategy for lowland rice crops.

The optimal type of Se fertilizer and the best fertilizer management strategy for Se-deficient paddy cultivation has not yet been identified. Research on Se fertilization of rice has focused on foliar applications (Chen et al. 2002). Chen et al. (2002) found that foliar application of SeO_4^{-2} fertilizer was 30% more effective than foliar SeO_3^{-2} fertilizer. In a rate-response trial, an application rate of 20 g ha^{-1} to rice increased grain Se concentrations to 0.47- 0.64 mg kg^{-1}. However, foliar application has practical difficulties because Se is hard to apply uniformly on rice heads, especially under difficult climatic conditions such as rainfall and wind. In a field trial using wheat plants, Se application to soils resulted in higher accumulation of Se in grain compared to foliar sprays (Lyons et al. 2005).

The concentrations of Se in rice grain is a function of plant-available Se in soils. The speciation, partitioning and lability of Se in rice paddy soils are influenced by the soil oxidation-reduction potential, pH, microbial activity, mineralogy and organic matter content, and the presence of other competitive anions (e.g. phosphate- PO_4 and sulphate - SO_4) (Dhillon and Dhillon, 2000; Goh and Lim, 2004). The behaviour of Se has been extensively investigated in anaerobic soil systems (Sposito et al. 1991; Tokunga et al.1991; Tokunga et al.1996; Zhang and Frankenberger 2003; Zhang et al. 2004). These studies found the availability of soil Se to be reduced during the submerged phase of paddy rice growth, so that pre-plant fertilization of rice soils with SeO_4^{-2} may not improve Se uptake by rice during growth under flooded conditions. However, rice paddies are generally drained prior to harvest for 1-2 weeks, and this aerobic phase may have a significant influence on the partitioning and lability of fertilizer Se added to soils. It is uncertain if reduced forms of Se (either added or formed *in situ* in soil) formed following submergence could be sufficiently oxidized during pre-harvest drainage of rice paddies to increase the labile pool of Se and therefore improve Se uptake into rice grain.

A series of experiments were undertaken in this thesis to investigate the role of soil physical and chemical properties on the solubility and lability of Se fertilizers in submerged and drained soils, and the use of this knowledge to develop an efficient fertilizer program for the biofortification of Se in rice grain. The thesis is organised into seven chapters that include one published manuscript (Chapter 3) and three manuscripts submitted to international referred journals (Chapters 4, 5 and 6). The literature review (Chapter 2) outlines the current understanding and knowledge gaps in determining the fate and behaviour of Se in submerged and drained soils, availability to rice grains and potential impact on human health. Chapters 3 and 4 present research findings into the solubility, lability and hence the potential availability of Se fertilizers in submerged and drained rice paddy soils. Chapter 5 and 6 presents research findings from two pot experiments undertaken using rice plants to examine the plant availability and plant accumulation of Se fertilizers (SeO_4^{-2} and SeO_3^{-2}) following different methods of application, time of application and soil water regimes. The final chapter (Chapter 7) outlines the major findings and conclusions of the thesis and potential directions for future research.

CHAPTER 2

2. LITERATURE REVIEW

2.1 INTRODUCTION

This review summarises literature on the importance of selenium (Se) in the environment. This includes: the role of Se in human health and strategies to increase Se intake; the acquisition and role of Se in plants; Se in rice and the agronomy of rice production; and the chemistry and behaviour of Se in soils. Later sections discuss biofortification strategies and the use of soil fertilization to increase Se concentrations in rice grains.

2.2 SELENIUM: AN IMPORTANT ELEMENT IN THE ENVIRONMENT

Selenium is an element that has metalloid and non-metal properties, is in group VI of the periodic table with an atomic number of 34, atomic weight 78.96 and is chemically similar to sulfur (Neal 1995). Selenium has several chemically different oxidation states: selenide (Se^{-2}), elemental Se (Se(0)), selenite (Se(IV)), and selenate (Se(VI)) (Masscheleyn and William 1994). The fully oxidized selenate is found in solution as biselenate ($HSeO^{-4}$) (pK_{a1} = -2.06) or selenate (SeO_4^{-2}) with a pK_{a2} = 1.8 (Séby et al. 2001). Selenate is the dominant species at high redox potentials, e.g. in alkaline soils, and it is considered very soluble with low adsorption and low precipitation capacities (Fordyce 2007). Selenite is a weak acid that can exist as H_2SeO_3, $HSeO_3^{-1}$, or SeO_3^{-2}, depending upon the solution pH (pK_{a1} = 2.70 and pK_{a2} = 8.54) (Séby et al. 2001). Selenite is the dominant species in the moderate redox potential range, and its mobility is mainly governed by sorption/desorption processes on various solid surfaces such as metal oxy-hydroxides (Balistrieri and Chao 1987). Selenide (Se^{-2}) is stable under strongly reducing conditions in a variety of Se^{-2} precipitates and in a high number of organic compounds (Alejandro and Laurent 2009). Di-hydrogen selenide (H_2Se) is present in nature as a product of microbial processes (Oremland et al. 1994). Elemental selenium, Se(0), is an insoluble form having at least 11 different allotropes including two isomorphic forms (Alejandro and Laurent 2009).

2.3 ACQUISITION AND ROLE OF SELENIUM IN PLANTS

Plant uptake of Se depends on the availability of Se in soils (Martinez et al. 2009; Wang and Gao 2001). Plant uptake of SeO_4^{-2} from soil is much higher than that of SeO_3^{-2} (Gissel-Nielsen and Bisbjerg 1970a). It has been suggested that plants can take up SeO_4^{-2} (Shrift and Ulrich 1969) and selenomethionine (SeM) through their roots via active transport (Abrams et al. 1990) by which ions accumulate against a concentration gradient. This is supported by previous research where a metabolic inhibitor (hydroxyl amine) applied to plants growing in nutrient solution caused an 80% inhibition of SeO_4^{-2} uptake by plant roots (Zayed et al.1998). A similar mechanism has been reported for the uptake of SO_4^{-2} by plant roots, and it has been suggested that both anions have similar transporters at the root membrane (Shrift and Ulrich; Zayed 1998).

Acquisition of SeO_3^{-2} by plants is typically less than acquisition of SeO_4^{-2}. However, in the lower redox environment of submerged rice soils, SeO_3^{-2} is the dominant form of Se; and therefore rice takes up more Se as SeO_3^{-2} than any other Se form (Wang and Goa, 2001; Zhang, 2006). It has been suggested that SeO_3^{-2} can be absorbed by plants through passive transport (Zayed et al.1998). Foliar application of Se also been tested and applied widely in the world (Broadley et al. 2006; Chen et al. 2002; Fang et al. 2008)

Acquisition of Se by plants can also occur when Se is applied as Se(0) but to a more limited extent than SeO_3^{-2} or SeO_4^{-2}. Gissel-Nielsen and Bisbjerg (1970) found that the uptake of Se from Se(0) was low compared to SeO_3^{-2} and SeO_4^{-2} for both clover *(Eugenia caryophyllus)* and mustard (*Brassica juncea*). Mustard plants had a higher (0.8 to 0.4 mg kg^{-1}) Se uptake from Se(0) than clover.

Different rates of translocation and transport mechanisms of SeO_4^{-2} and SeO_3^{-2} can be inferred from the shoot-root ratios of Se. Zayed et al., (1998) investigated SeO_4^{-2}, SeO_3^{-2} and SeM transport in broccoli (*Brassica oleracea var. botrytis L.*), Indian mustard (*Brassica juncea L.*), sugar beet (*Beta vulgaris L.*) and rice (*Oryza sativa L.*). The authors found that shoot-root ratios ranged from 1.44 to 17.2 for SeO_4^{-2}, 0.58 to 0.98 for SeM and 0.24 to 0.47 for SeO_3^{-2} showing more SeO_4^{-2} accumulated in shoots than SeM and SeO_3^{-2}. When considering the total Se uptake (both roots and shoots) by rice plants, the following order was observed: SeM > SeO_3^{-2} > SeO_4^{-2}. After translocation, Se can be stored as organic Se in various parts of the plant such as leaves, shoots, seeds, fruits, or roots.

The translocation of Se in plants is influenced by the Se species taken up by the roots (Arvy 1993; Terry et al. 2000). Selenate is translocated directly to leaves from roots via xylem with no chemical modifications, and inside the chloroplasts of the leaves selenate can be converted into organic forms such as SeM (Arvy 1993). A large proportion of SeO_3^{-2} taken up by plant roots is converted to SeM in the roots before it is translocated to other tissues in the plant (Arvy, 1993). Arvy (1993) found SeO_3^{-2} taken up by the roots of the bean plant (*Phaseolus vulgaris*) was converted to organic Se species (e.g. SeM) before being translocated in the xylem to other plant tissues. Similar to Arvy (1993), Li et al. (2008) also found SeO_3^{-2} taken up by the roots of the wheat plant (*Triticum aestivum*) was converted to organic Se species (e.g. SeM, SeOM) before being translocated in the xylem. However, Beath (1937) measured Se in cereals grown on seleniferous (Se-rich) soils in Wyoming and found the same amount of Se in roots and shoots at young age, and less in shoots at maturity. In a hydroponic experiment, in the SeO_4^{-2} treated wheat plants, SeO_4^{-2} was the most dominant Se species in roots, xylem and leaves with little assimilation into organic forms in 1 d. By contrast in SeO_3^{-2} treated plants most of the uptake was transferred into organic forms in 1 d (Li et al. 2008).

2.4 SELENIUM AND HUMAN HEALTH

Selenium is responsible for various physiological functions in the human body. There are more than 25 seleno-proteins that have been identified, most of which protect the body tissues against oxidative stress, maintain defences against infection, and regulate growth and development (Rayman 2000; WHO 2001).

2.4.1 ROLE OF SELENIUM IN THE HUMAN BODY

Selenium was first identified as an essential micronutrient for humans during the 1950s (Schwarz and Foltz 1957). After identifying the Se as an essential micronutrient, scientific communities became interested in the role of Se in the human body. In the 1970s Se was found to be an essential constituent of the enzyme glutamine peroxidise (GPX), which has an antioxidative role in the human body (Combs 2001a). All Se forms absorbed by humans are incorporated into proteins and several types of Se-containing proteins exist in the human body (Reilly 1996). For example, Se is the key element in the amino acid selenocysteine, a key component of seleno-protein enzymes (Burk and Hill 1993; Reilly 1996). The major metabolic function of Se in these proteins is gained through redox activity, and the replacement of Se with S has been shown to bring about a reduction in their activity (Pyrzynska 2002; Reilly 1996). Other major Se-binding proteins are selenomethionine-containing proteins which are the major Se form in cereals and other crop based foods. Although they do not have Se-specific activities in the body, once the protein breaks down and Se is released, it can then be incorporated into other metabolic pathways (Reilly 1996). At present at least 15 seleno-proteins have been identified; these proteins, their tissue distribution (WHO, 2001) and specific roles (Rayman 2000) are listed in Table 1.

2.4.2 SELENIUM DEFICIENCY DISEASES/DISORDERS

Keshan disease and Kaschin-Beck disease are the most common Se-deficiency diseases recorded in the literature, and were recorded where people received a daily intake of Se below 7 to 11 μg d^{-1} (WHO, 2001). Keshan disease is mostly found in

China, in children aged 2 to10 years and to a lesser extent in women of child-bearing age. Keshan disease is an endemic cardiomiopathy disease involving the enlargement and weakening of the heart muscle. The incidence of the disease has been lowered in problem areas by providing either oral tablets containing Na_2SeO_3 (0.5 to 1 mg Se child^{-1} wk^{-1}) or SeO_3^{-2}- fortified table salt, 10 to 15 mg Se kg^{-1} (Combs 2001b).

Kaschin-Beck disease involves osteoarthropathy, affecting growth plates of growing bones and causing swollen painful joints. It specifically affects joints in fingers, toes and knees, and in severe cases it can cause dwarfism (Combs 2001b). This disease has been detected in young children in Se-deficient areas in China, Siberia and North Korea (McLaughlin et al. 1999).

Table 1-Seleno-proteins and their nutritional functions contd. (Rayman 2000)

Seleno-protein	**Function**
Glutathione peroxidases (GP×1, GP×2, GP×3, GP×4)	Antioxidant enzymes that remove hydrogen peroxide, and lipid and phospholipids hydroperoxides (thereby maintaining membrane integrity, modulating eicosanoid synthesis, modifying inflammation and likelihood of propagation of further oxidative damage to biomolecules such as lipids, lipoproteins, and DNA).
(Sperm) mitochondrial capsule seleno-protein	Form of glutathione peroxidase (GPx4) that shields developing sperm cells from oxidative damage and later polymerises into structural protein required for stability/motility of mature sperm.

Iodothyronine deiodinases	Production and regulation of level of active thyroid hormone, T3, from thyroxine, T4
Thioredoxin reductases	Reduction of nucleotides in DNA synthesis; regeneration of antioxidant systems; maintenance of intracellular redox state, critical for cell viability and proliferation; regulation of gene expression by redox control of binding of transcription factors to DNA
Seleno-phosphate synthetase, SPS2	Required for biosynthesis of seleno-phosphate, the precursor of selenocysteine, and therefore for seleno-protein synthesis.
Seleno-protein P	Found in plasma and associated with endothelial cells. Appears to protect endothelial cells against damage from peroxynitrite
Seleno-protein W	Needed for muscle function
Prostate epithelial seleno-protein	Found in epithelial cells of ventral prostate. Seems to have redox function (resembles GPx4), perhaps protecting secretory cells against development of carcinoma
DNA-bound spermatid seleno-protein	Glutathione peroxidase-like activity. Found in stomach and in nuclei of spermatozoa. May protect developing sperm
18 kDa seleno-protein	Important seleno-protein, found in kidney

In addition to the above two diseases, Se deficiency may have adverse effects on thyroid-related disorders (goitre), because Se is vital for the production of thyroid hormones (Arthur et al. 1993; Combs 2001b). New research findings have provided valuable information on the severity of Se deficiency in vitamin E-deficient people; it can increase the risk of mutation rates in RNA-viruses (Combs 2001a), which may cause not only Keshan disease but also other diseases for which the causal agent is an RNA virus, such as influenza, hepatitis and HIV aids. In addition to the above diseases, Se deficiency has negative impacts on the immune system, and on male and female fertility (function and development of sperm and also on female miscarriage) (Martin et al. 2006). Selenium also has anti carcinogenic effects on some types of cancers in the human body (Combs 2001a).

Selenium can also be toxic if consumption is higher than a threshold value. An increased incidence of nail dystrophy has been associated with consumption of high Se foods supplying more than 900 mg Se d^{-1}. These foods were grown in Se-rich soil from specific areas in China. A positive association between dental caries and urinary Se output under similar circumstances has also been reported (WHO 2001).

2.4.3. SELENIUM STATUS OF THE WORLD POPULATION

The concentration of Se in the human body varies depending on the location, agronomic practices, type of foods available and also on food preferences. For example, the information in Table 2 illustrates the daily intake of Se by adults in different geographic locations. Selenium daily intake varies from 3 to 11 µg Se d^{-1} in Se-deficient areas in China to 1338 µg Se d^{-1} in seleniferous areas in the same country. According to these data, millions of people in the world may consume Se levels far below the levels that are required to prevent those diseases related to Se deficiency.

The occurrence of Se deficiency or toxicity is dependent on the amount and form of Se intake. Levels of Se in the human body have been studied by many scientists; in so doing, different biochemical tests have been employed, such as the Se concentration in blood, nails, hair and also urine. Blood Se concentration is considered to be a useful measure in understanding Se intake and Se status in the body, whereas hair Se is more strongly related to long-term Se exposure. Urinary Se is closely correlated with plasma Se and therefore it is used for estimating recent Se intakes (Thompson 2004). Selenium concentrations in the blood and serum plasma of healthy adults in 69 countries have been listed by Combs (2001a) and, according to those mean values of whole blood, Se concentrations can vary from 9 µg L^{-1} in Keshan disease areas in China to 3480 µg L^{-1} in seleniferous areas in the same country.

Humans and animals take up the majority of their Se requirements through food, most of which is derived directly from plants, including staple foods such as rice, wheat, maize and sorghum (Lyons et al. 2004). However, concentrations of Se in cereals vary according to the Se status of soils in which they are grown. Soils deficient in Se are likely to produce grain also deficient in Se, and consumption of this grain will likely lead to Se deficiency symptoms in humans if it is their primary source of food. Understanding the chemistry of Se in soils is therefore an important part of managing Se in the food chain, and of developing management practices to improve the Se nutrition of humans.

Table 2-Geographic differences in Se intakes (µg d^{-1}) of adults (WHO 2001)

NOTE:
This table is included on page 14 of the print copy of
the thesis held in the University of Adelaide Library.

2.4.4. SUFFICIENCY AND DEFICIENCY LEVELS

Thompson (2004) established some useful guidelines with currently available data: the minimum Se intake for the prevention of Keshan disease is considered to be 20 µg d^{-1}, and the Se intake to achieve maximum production of seleno-proteins in plasma is between 45 to 50 µg d^{-1}. However, the Se intake needed to protect against cancer has been established as 120 µg d^{-1} (Thompson, 2004). The recommended Se intake for adults has been established in a few countries using a number of parameters, such as the Se concentration needed to make optimum serum seleno-protein levels, and the average weight of an adult in each country: these values are summarised in Table 3.

2.4.5. SELENIUM CONCENTRATIONS IN FOOD AND PLANT AVAILABILITY

Humans and animals take up Se through foods; therefore, Se intake is dependent on the type of food consumed. For example cereals, poultry, eggs, fish and red meats are generally rich in Se but concentrations depend on the region in which they are produced (Table 4). The majority of people living in developing countries cannot afford Se-rich protein foods so most of their Se daily intake comes from cereals, their staple food.

Table 3- Recommended Se intake for adults (Thompson 2004)

Country/organisation	Gender	Se intake (μg d $^{-1}$)
Australia	Men	85
	Women	70
USA and Canada	Men	55
	Women	55
United Kingdom	Men	75
	Women	60
World Health Organisation	Men	40
	Women	30
Europe	Men	55
	Women	55
Germany, Austria, and Switzerland	Men	30-70
	Women	30-70

Table 4. Typical Se contents (mg kg^{-1}) in selected foods from several countries (Combs 2001b).

NOTE:
This table is included on page 17 of the print copy of
the thesis held in the University of Adelaide Library.

The bioavailability of Se in the body is determined by the form of Se in foods. Selenomethionine is more effective at increasing Se concentrations in human blood than inorganic forms provided in supplements because it can easily be incorporated into human proteins such as haemoglobin and albumin. Organic Se species must be catabolised into inorganic forms such as selenate or SeO_3^{-2} before being reduced into Se^{-2} which is the form that can help to make seleno-proteins.

2.4.6. DIFFERENT METHODS FOR INCREASING SELENIUM INTAKE

Different strategies have been tested worldwide in order to achieve the optimum Se levels in the human body. The most common methods found in the literature are increased consumption of high-Se foods through education, individual supplementation, Se supplementation to livestock (Lyons et al. 2005) and biofortification of food crops (Martin et al. 2006).

2.4.6.1. Education

Eggs, poultry, kidney, liver, fish products, brazil nuts and crab meat are rich in Se (Broadley et al. 2006). Therefore, the consumption of a mixed diet including all of the above protein sources with cereals is recommended to increase daily Se intake. The United States recommended dietary allowance (RDA) for Se of 70 and 55 $\mu g\ d^{-1}$ for adult men and women respectively is achievable through eating a mixed diet (Duffield et al., 1999). Therefore, it should be possible to ensure a sufficient daily intake of Se through providing education to the general public on eating a balanced diet including a few of the above listed foods in meals every day. However, the foods listed above are not typically eaten in large quantities and are not affordable for the majority of people living in countries where Se deficiency occurs.

2.4.6.2. Individual supplementation

Supplementation, using both organic and inorganic forms of Se, has been practiced in many countries (Lyons et al. 2005). This method has been employed to increase Se intake in Se-deficient Keshan disease areas (Combs 2001a). Supplementation of Se through oral tablets having Na_2SeO_3 (0.5 to 1 mg Se child $^{-1}$ week^{-1}) or supplementation of Se through Se-enriched table salt (10 to 15 mg kg^{-1}) has effectively reduced the incidence of disease. Selenium-rich yeast and garlic also have been tested in Se-supplementation trials and it has been shown that they have the ability to reduce overall cancer morbidity by 50%; the yeast and garlic contained organic Se in γ -glutamyl-Se-methylselenocysteine (73%) and SeM (85%) forms, respectively (Clement et al. 2000). In addition to the above Se-rich foods, Se-enriched tea (Hu and Ding 1998), high-Se mussels (Mao et al. 1997), mushrooms, onions and Se-fortified beer have been produced (Combs 2001a).

2.4.6.3. Supplementation of livestock with selenium

Selenium supplementation has been carried out in order to increase the Se concentration in livestock products such as meat, milk and eggs, and also to minimise the risk of livestock suffering from Se-deficiency disorders. Selenium supplementation to livestock is commonly achieved through direct administration of Se with other injections and/or slow release recticulum-rumen bullets (Loganathan and Hedley 2006).

The fertilization of pasture lands with Se-enriched NPK fertilizer at a rate of 10 g ha^{-1} has also been identified as an effective method to increase Se levels in livestock in countries such as New Zealand, Finland and Australia.

2.4.6.4. Selenium biofortification

Biofortification is defined as the increase of the plant available concentrations of elements of interest in the edible portion of crop plants through fertilization (agronomic biofortification), through crop selection, or breeding (genetic biofortification) (Broadley et al. 2006; Lyons et al. 2005).

2.4.6.5. Genetic biofortification

Some crop species may have the ability to take up more Se than other species, even when grown in low Se soils. Even within species, genetic variation can be used to increase the uptake of Se so that it is more available for human consumption. Selection of existing high Se varieties for Se-deficient areas may be a good management option and for the long term these crops can be used for breeding in order to produce new varieties with a high Se-accumulation ability.

There are few data concerning increasing Se accumulation in crops through genetic breeding studies (Lyons et al. 2005). One study using soybeans showed that the ability to accumulate Se varies 15 fold within a variety, and therefore breeding technology can be employed to enhance the ability of soybean to take up more Se from soils (Combs 2001a). Brown rice varieties have been shown to absorb less Se than other crops, but across brown rice varieties there are also great differences in Se uptake; therefore breeding technology can be employed to improve the Se uptake of rice plants (Lianhe et al. 2006).

2.4.6.7 Agronomic biofortification

Application of Se fertilizer in order to increase the Se concentrations in crops is employed in Se-deficient areas across the world (Broadley et al. 2006). Successful Se-fertilizer programs for forages and cereal crops have been developed and effective results have been obtained in animal and human populations in Finland and New Zealand. For example, studies in Finland found that Se intake (per capita) increased from 25 $\mu g\ d^{-1}$ to 124 $\mu g\ d^{-1}$ after a Se-fertilizer program was employed (Broadley et al. 2006).

2.4.7 REASONS FOR THE STUDY OF SELENIUM IN RICE GRAINS

Rice is the second most important cereal crop in the world and 90 % of global production is concentrated in Asian countries (Table 5). China and India are the major rice producers and consumers in the world, as they account for more than one-third of the world population. World rice consumption has increased by 40 % in the last 30 years, from 61.5 kg per capita to about 85.9 per capita. According to one data source of Asian consumption of rice, China consumes 90 kg $person^{-1}\ year^{-1}$, Indonesia consumes 150 kg $person^{-1}\ year^{-1}$, and Myanmar consumes 200 kg $person^{-1}\ year^{-1}$, whereas average consumption is lower than 10 kg $person^{-1}\ year^{-1}$ in western countries (France: 4 kg, US: 9 kg) (UNCTAD 2004).

Table 5- World rice production and percentage calorie supply in each country.

Major rice producing and consuming countries	Rice production [a] kt	% caloric supply [d]
China	123,200	30
India	80,000	30
Indonesia	32,500	50
Bangladesh	26,000	72
Vietnam	20,500	66
Thailand	16,500	44
Philippines	8,300	42
Japan	8,200	23
Brazil	7,600	13
United States	6,457	2
Korea, South	5,300	31
Pakistan	3,500	5
Australia	965	3
Others	45,385	
World Total	384,407	

a- USDA, Foreign Agricultural Services (FAS), Aug 2002

b- IRRI world rice statistics 2000

2.4.7.1 Selenium concentrations in rice grains

Concentrations of Se in rice will vary according to the Se status of soils in which rice is grown. Soils deficient in Se are likely to produce Se-deficient rice grain, and consumption of this grain will likely lead to Se-deficiency symptoms in humans who consume this staple food (Table 6). For example in China, provinces such as Heilong-jiong, Schuan and Shaan-xi are deficient in Se, and Keshan disease and livestock white muscle disease have been observed. Selenium concentrations in the soils ranged from 0.03 to 0.10 mg kg^{-1} (Changsheng 2007). In 2004, areas under rice cultivation in those provinces were 1588, 2064 and 146 thousand ha respectively (IRRI 2007). Deficiency of Se also occurs in many other rice growing soils in the world, including Sri Lanka (IRRI 2007). Additionally, although the total Se concentration may be high in a particular soil, the available fraction may be low due to many soil factors. Therefore, in order to increase Se in rice plants a fertilizer strategy should be developed with careful attention paid to the soil properties.

Table 6-Total Se concentrations in paddy soils and rice grains of different countries

Country /Area	Total soil Se ($\mu g\ kg^{-1}$)	Rice grain Se ($\mu g\ kg^{-1}$)	Reference
China			
North High Se region	44.8	34	(Cao et al. 2001a)
Central moderate Se region	35.1	31	(Cao et al. 2001a)
Northwest adequate Se region	200-400	63	(Wang and Gao 2001)
South Low Se region	25.5	27	(Cao et al. 2001a)
Extremely low Se region	>100	17	(Wang and Gao 2001)
India			
Se rich area in Hoshiarpur district	2400	9900	(Dhillon and Dhillon 2000)
Sri Lanka	113-663	45-58	(Fordyce et al. 2000)
Kandy, Mathale	310-5258	25-40	(Fordyce et al. 2000)
Kaluthara, Warakapola			
Horana, Mathugama	276-3947	25	(Fordyce et al. 2000)

2.5 RICE PRODUCTION

Rice is consumed by a large proportion of the world's population and it is the staple food in the diet of the population of Asia, Latin America, and Africa (Fageria 2007; Sun et al. 2010). Rice provides 35–60 % of the dietary calories consumed by nearly more than 3 billion people and globally it is the second most cultivated cereal after wheat (IRRI 2007).

Rice is grown in both dryland and wetland conditions and over a wide range of latitudes (Fageria 2007). For example, rice is cultivated in north eastern China at 53°N latitude, in central Sumatra on the equator, and in New South Wales, Australia, at 35°S latitude (Mae 1997). *Oryza sativa* and *Oryza glaberrima* are the two main species of cultivated rice in the world (Fageria 2007). *Oryza sativa*, the main rice species cultivated throughout the world, is divided into three groups based on geographical distribution. These groups are japonica, indica, and javanica (Takahashi, 1984). Japonica is mainly grown in the temperate regions and indica is grown in the tropical and sub-tropical regions. Rice can be grown on irrigated lowlands, rainfed lowlands or on uplands. Irrigated lowland paddy cultivation accounts for 55 % of the total world rice cultivation areas and contributes 76 % of the world rice production (Fageria 2007).

Rice can be grown under different soil water conditions ranging from poorly drained and waterlogged in lowland soils to well drained in upland soils, but the majority of rice cultivation is on poorly drained waterlogged soils. Rice cultivation takes place all over the world in different climatic conditions, and soils are also variable in their physicochemical characteristics. During this review I will mainly focus on rice growing under lowland soil conditions because the majority of rice cultivation takes place in submerged soils. Lowland rice soils are managed for rice production in a different way to the soils under upland cultivation. The most important characteristic of a submerged soil is the presence of standing water during the greater part of the rice growing season (Sahrawat 2005). Normally soils are levelled to make levees to impound water, the levees are then puddled, and a depth of 5 to 10 cm water is maintained during the first few months until grain filling. Following grain fill, the land is drained and allowed to dry prior to harvest. After harvest, the land is abandoned until the next season, or is cultivated in upland areas, or re-flooded for the next crop; this is a regular cycle (Ponnamperuma 1972). The physiochemical and microbiological characteristics of a submerged soil are markedly different to the same soil in upland conditions. Care must be taken when designing a Se-fertilizer program for lowland rice, such that the physicochemical changes in rice soils during rice cultivation are understood and taken into account.

2.6 SELENIUM IN SOILS

Selenium is listed as among the most widely distributed elements in the Earth's crust, and on average occurs at a concentrations of about 0.05 to 0.09 mg kg^{-1} (Neal 1995). The concentration of Se in topsoils ranges from less than 0.1 mg kg^{-1} to more than 100 mg kg^{-1} (Graham et al. 2003). The source for soil Se is the rocks (e.g. sedimentary rocks) from which the soil was formed. The Se concentration in soils depends on the Se concentration in the parent material and the intensity of weathering and the degree of leaching of this parent material. Volcanic rocks, shales and some phosphate rocks in the USA and silicic rocks in Russia, Canada, China, the USA and New Zealand have been found to contain high concentrations of Se (Neal, 1995). Some anthropogenic sources can add Se to soils; for example, the combustion of fossil fuel and sulphide ore mining can raise Se to toxic levels in soils (Hartikainen 2005). In addition, Se is added to soils as a fertilizer in many countries in order to increase plant

Se content, while irrigating land with water having high concentrations of Se is a major source of Se in the western United States (Engberg et al. 1998).

Whereas some soils of the world are seleniferous, most are considered deficient. Selenium-deficient soils can be found in north-east to south-central China, New Zealand, Finland and Denmark, whereas seleniferous soils can be found in the semiarid regions of western USA (Neal 1995), the Great Plains of the USA and Canada, Enshi County, Hubei Province, China, and parts of Ireland, Colombia and Venezuela (Combs 2001a). The total and available Se concentration in soil is very important because it greatly affects the amount of Se in the crops grown on these soils. For example, rice grown in soils with Se concentrations of 4 mg kg^{-1} was found to result in chronic selenosis, whereas in China a Se concentration of 0.007 mg kg^{-1} was found to result in Keshan disease (WHO 2001).

Selenium in soils can be found associated with solid phases or in soil solution, as free ions, or adsorbed to soil colloids. The soluble and exchangeable fractions are the fractions mostly related to plant uptake. Once Se is added to soil as a fertilizer (solid or liquid) the soluble Se species will reach an equilibrium with the solid and soil solution, depending on the soil properties and the speciation of the Se applied into soil. The availability of Se for plant uptake depends on its speciation in the soil solution. In order to increase the potential availability of Se applied to submerged rice soils, it is necessary to understand the mobility and plant availability of Se in rice soils. Selenium availability is affected both by adsorption-desorption mechanisms and precipitation-dissolution mechanisms, depending on soil properties such as redox, pH, the concentration of other metals in soil, and the mineralogy. For example, adsorption-desorption mechanisms may control the speciation in oxic to sub-oxic soils, whereas precipitation and dissolution may control the speciation under anoxic conditions.

2.7 PHYSICAL AND CHEMICAL PROPERTIES OF SUBMERGED SOILS: EFFECTS ON AVAILABILITY OF SOIL SELENIUM TO RICE

Availability is very important when considering trace metal uptake by plants. In the following discussion, the term phytoavailability will be used to describe the portion of soil metal available for plant uptake. This term should not be confused with plant bioavailability, which is defined as the fraction of soil metal available for organisms (Pierzenski et al. 2000). In order to be plant available, trace metals should come in contact with the plant (physical accessibility). Moreover, trace metals need to be in a particular form (chemical accessibility) to be able to enter the plant root (Adriano et al. 2004).

The most available fraction of an element for a plant is usually represented by the soluble component, occurring as either free ions or soluble complex ions in soil solution. Ions that are weakly adsorbed onto an exchange surface in soils are also potentially available for desorption should soil solution Se be depleted through uptake. The combined soluble and adsorbed fraction is collectively known as the potentially bioavailable or labile fraction (Adriano et al. 2004).

There are many factors that can affect plant availability of Se in rice soils: soil pH; Eh; the type, amount and presence or absence of certain minerals; microbial activities; amount and type of organic matter; and management practices. These physical and chemical properties of soil will ultimately determine the availability of Se in soils.

2.7.1 DEPLETION OF OXYGEN AND ACCUMULATION OF CARBON DIOXIDE AND OTHER GASES

When the soil undergoes submergence, the exchange of gases between the soil and the atmosphere is restricted. Therefore oxygen is depleted because of microbial respiration, and the presence of CO_2 and other gases which accumulate in the flooded layer (Datta 1981; Ponnamperuma 1972; Sahrawat 2005). The degree of accumulation of CO_2 and CH_4 is due to anaerobic microbial decomposition of organic matter. In addition to CO_2 and CH_4, H_2, oxides of N and N_2 gas can also be present in rice soils (Datta 1981). Even though submerged rice soils are deficient in O_2, rice plants have adapted to the anaerobic soil conditions by supplying O_2 to the respiratory tissues by internal pathways, and the rice plant keeps the rhizosphere in an oxidized condition by pumping air into the root zone (Kirk et al. 1993). The ability of rice roots to pump air into the rhizosphere helps to maintain an oxidized condition in the few millimetres around the roots (Figure 1).

Figure 1 - Oxidized rice rhizosphere.

The rice plant has a special ability to form aerenchyma in roots. Aerenchyma can be defined as any tissue that contains large air-filled intercellular spaces or lacunae (Jackson et al. 1985). In the roots of some species these spaces are connected longitudinally. The resulting porous structure provides a pathway for the diffusion of oxygen from aerial plant parts to the roots, where it is released and maintains high redox potentials in the rhizosphere (Armstrong 1971). In flooded soils, rhizosphere oxidation is an essential adaptation to prevent the accumulation of Fe^{+2}, Mn^{+2}, and H_2S to phytotoxic levels around the roots. This oxidation is reflected in the precipitation of Fe^{+3} hydroxide plaques at the root surface. Aerobic conditions in the rhizosphere could oxidize Se(0) to SeO_3^{-2} and SeO_4^{-2}, thereby potentially increasing the plant availability of Se. These oxidised Se species can be adsorbed to Fe^{+3} hydroxide plaques, potentially reducing the plant available pool of Se for plant uptake (Zhou et al. 2007). Organic acids released by rice roots can solubilise Fe^{+3} hydroxide plaques, causing mobilisation of adsorbed SeO_3^{-2}. It has been suggested that Fe^{+3} hydroxide plaques can act as a buffer for Se in the rhizosphere. Zhou et al (2007) found that increasing amounts of Fe^{+3} plaque increased Se accumulation on the root surface, but decreased Se concentrations in rice shoots. The translocation of Se from roots to shoots was reduced when there were increasing amounts of Fe^{+3} plaque. It was also shown that the transport of Se in the xylem was sharply decreased by increasing amounts of Fe^{+3} plaque on the roots. The above results were based on a solution culture study and, as factors affecting the development of Fe^{+3} hydroxide plaques in the soil environment can be different from solution culture conditions, more research is needed to determine the significance of these results.

2.7.2 REDOX POTENTIAL AND SOIL PH

The redox potential of soils is a major characteristic that differentiates an upland soil from a submerged soil. Upon soil flooding the redox potential declines and stabilises at near zero to negative values, depending on factors such as submergence time and organic matter content and, upon aeration, increase to positive redox potentials. Depending on the redox potentials found in rice soils, soils can be divided into well-drained to highly-reduced soils (as shown in Table 7 below).

Redox potential is the measurement of oxidized or reduced conditions in soil. Oxidation is the loss of electrons and reduction the gain of electrons. The oxidised component is the electron accepter or the oxidant, and the reduced component is the electron donor or the reductant (Donald 1987). Redox conditions in soil systems can be expressed using two parameters; pe, which is the negative log value of electron activity and/or Eh, the voltage difference between platinum and standard hydrogen electrodes. Large positive values for pe and Eh indicate oxidised conditions or oxidised species in the soil solution, and negative values indicate reduced conditions. Values of Eh can be converted into pe using the following equation:

$$Eh\ (mV) = 59.2\ \mathrm{pe}$$

In the absence of oxygen in submerged soils, microbes use other oxidized soil components as electron acceptors e.g. NO_3^-, $Mn^{+3/+4}$, Fe^{+3}, PO_4^{-3} and organic matter (Masscheleyn and William 1994; Ponnamperuma 1967). The Eh and pH diagram of Se can be found in Figure 2.

Table 7- Oxidation- reduction potential in rice soils from well-drained to highly-reduced conditions (Sahrawat 2005)

NOTE:
This table is included on page 32 of the print copy of the thesis held in the University of Adelaide Library.

Figure 2 -Eh and pH diagram for Se (MacNeal and Balistrieri 1989)

Selenium can also act as an electron accepter for microbial respiration. Selenate can be reduced under anaerobic conditions to SeO_3^{-2} at Eh values of +200 to +300 mV, which is similar to the redox potential at which nitrate is reduced in soil (Masscheleyn and William, 1994). When NO_3^- is present in soil, SeO_4^{-2} reduction does not occur (Neal 1995). The redox potential for the reduction of elemental Se(0) to Se^{-2} is -10 to -40 mV, and SeO_3^{-2} to Se(0) or Se^{-2} is below +50 mV (Tokunga et al. 1996; see Table 8).

The quantity of oxy (hydr)oxides present in soils and their adsorptive capacity tends to change with redox potential (Alloway 1995). In paddy soils, alternate aerobic and anaerobic conditions change both pH and redox potential (Ponnamperuma 1972).

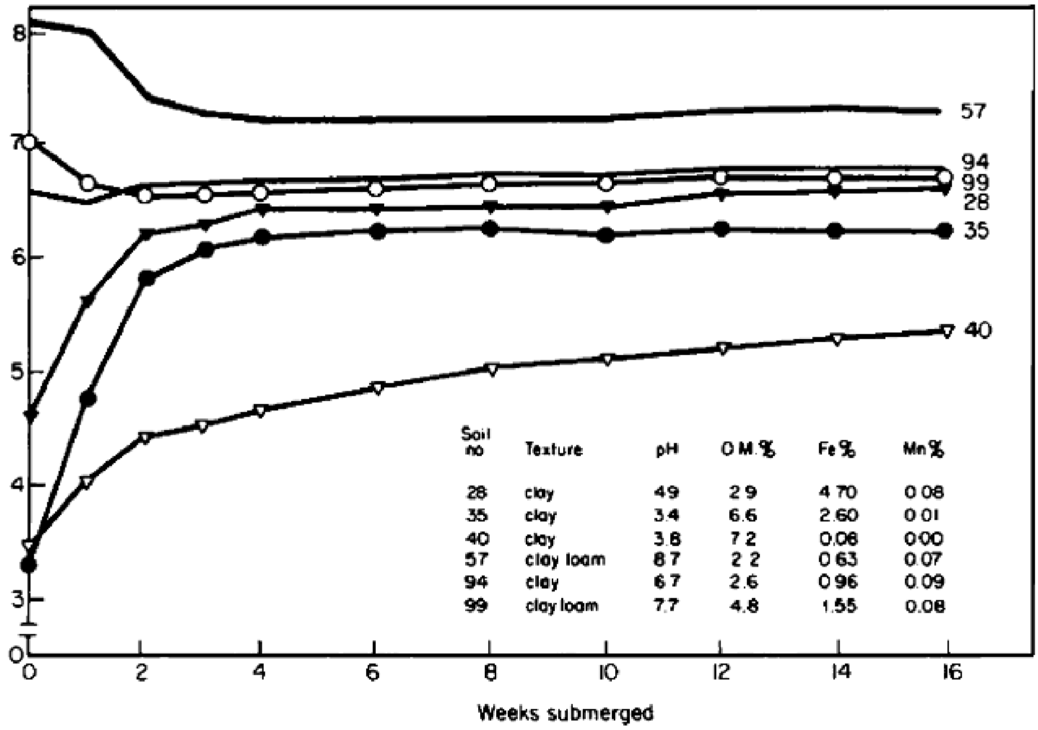
Table 8 –The electrochemical reactions in soil and the Eh levels at pH 7

Electrochemical reaction	Eh (volts) at pH 7	Reference
O_2/H_2O	0.83	(Ponnamperuma 1972)
NO_3/NO_2	0.54	(Neal 1995)
SeO_4^{-2}/SeO_3^{-2}	0.44	(Neal 1995)
MnO_2/Mn^{+2}	0.41	(Ponnamperuma 1972)
SeO_3^{-2}/Se^0	0.27	(Neal 1995)
$Fe(OH)_3/Fe^{+2}$	0.13	(Ponnamperuma 1972)
SO_4^{-2}/HS^-	-0.16	(Neal 1995)
CO_2/CH_4	-0.24	(Neal 1995)
N_2/NH_4	-0.28	(Neal 1995)

This can lead to reactions such as: adsorption/desorption of Se onto the different solid components and onto hydrous oxides of Al, Fe, and Mn; formation or decomposition of soluble and insoluble metal organic complexes; and precipitation as insoluble Se^{-2} under highly reducing conditions and oxidative dissolution or reduced Se as SeO_3^{-2} and SeO_4^{-2} under aerobic conditions. In addition, under sub-oxic conditions (250 to 50 mV) SeO_4^{-2} can be reduced to Se^{-2} by Fe^{+2} in the soil solution (Myneni et al. 1997). The overall reaction is as follows:

$$SeO_4^{-2} + 8Fe^{+2} + 9H^+ \longrightarrow 8Fe^{3+} + HSe^- + 4H_2O$$

The nature of Se species in the soil solution can be predicted using pe and pH diagrams, developed by Elrashidi et al. (Elrashidi et al. 1989). The Se speciation changes with the soil pH and redox conditions are illustrated in Figure 2. Selenate is the predominant species of Se under aerobic conditions, but as anaerobic conditions increase and redox potentials decrease in submerged soils the presence of SeO_3^{-2}, Se(0) and Se^{-2} species play an increasing role (Elrashidi et al. 1989).



Soil no	Texture	pH	O M.%	Fe %	Mn %
28	clay	49	2 9	4 70	0 08
35	clay	3.4	6.6	2.60	0 01
40	clay	3.8	7 2	0.08	0 00
57	clay loam	8 7	2 2	0 63	0.07
94	clay	6 7	2.6	0 96	0.09
99	clay loam	7.7	4.8	1.55	0.08

Figure 3 - Changing pH in soils with different soil properties during submergence.

Iron, Mn, silica and phosphate become more mobile when soils are submerged (Ponnamperuma 1972). Dhillon and Dhillon (2004) found that in acidic soils containing a high content of Fe and Al oxides, SeO_3^{-2} was strongly bound under reduced conditions. These authors further suggested that when plants remove Se or whenever Se is removed from the system by any mechanism, less soluble forms of Se adsorbed to organic matter or oxide minerals and clay are converted into soluble forms, making them available for plant uptake, even under submerged conditions.

With submergence the pH of acidic soils tends to increase while the pH of alkali soils decreases (see Figure 3 above) and in most situations pH stabilises around 6 and 7 i.e. tend toward neutrality. The decrease in the pH of alkaline soils occurs due to accumulation of CO_2 in flooded soils, because the standing water level restricts the diffusion of CO_2 from the soil and subsequent release to the atmosphere. Therefore CO_2 accumulates in flooded soils which neutralizes the alkalinity (Sahrawat 2005) as shown in the following equations:

$$CO_2 + H_2O = H_2CO_3$$

$$H_2CO_3 = H^+ + HCO^{-3}$$

The increase in pH of acid soils is mainly due to the reduction of Fe^{+3} phases in soils. Under anaerobic systems Fe^{+3} is used as an electron acceptor in decomposition of organic matter (Sahrawat 2005) as shown in following equation:

$$2Fe_2O_3 + CH_2O + 8H^+ = 4Fe^{+2} + 5H_2O + CO_2$$

2.7.3 DECOMPOSITION OF ORGANIC MATTER

Decomposition of organic matter (OM) is much slower in flooded soil compared to well drained upland soil (Sahrawat 2005). The incomplete decomposition of OM in flooded soils causes the formation of CH_4, NH_4, N_2 and its oxides, S^{-2}, organic acids, and H_2, changing the chemistry of Se in soils.

The availability of Se is affected by OM content and microbial activity. The addition of OM in flooded soils results in lower redox potentials than unamended soils (Ponnamperuma 1972). Mikkelsen et al. (1989), using a seleniferous soil from western San Jaoquin Valley California spiked with 1.5, 3, or 6 mg kg^{-1} Na_2SeO_4, observed that the addition of OM increased the rate of SeO_3^{-2} removal from soil solution. Even though the authors observed that the addition of OM removed the SeO_3^{-2} from the soil solution they were unable to observe the fate of the removed SeO_3^{-2} from the soil solution. Under the low redox conditions transformation of SeO_3^{-2} into Se(0) and/or Se^{-2} renders the Se unavailable for plant uptake. With the addition of OM to soils, Se in rice plants at the mid tillering, panicle exertion and maturity stages was lower compared to the treatment without OM (Mikkelsen 1989). This could be due to the formation of a strong complex of Se with OM or lower redox potentials in soils receiving OM. According to the above study, rice plants accumulated toxic concentrations of Se in all parts when they grow in soils with high Se and on upland conditions and/or without OM in lowland conditions.

In addition to the effects of OM on changing soil redox conditions, soil OM is also quite effective in retaining cations/anions depending on soil pH. Humus and other organic compounds can chelate metals and form stable complexes. Humic substances may be classified according to their solubility. Humic acids (HA) are soluble only in alkaline media, while fulvic acids (FA) are soluble in alkaline and acid media. Both HA and FA play different roles in controlling plant uptake of nutrients. Fulvic acid forms soluble complexes with metals over a wide range of pH. In soluble complexes, metals can be available for plant uptake, whereas in solid complexes they are immobilised. In rice soils with time, organic matter decomposition decreases, and soluble organic compounds and total dissolved metals stabilise at lower values and availability of metals diminishes (McBride 1995), while slowly the soluble organic complexes change to insoluble ones in soil solution (Schmidt 1997). Selenium may occur in organic complexes where organic matter accumulates (Dhillon and Dhillon 2004). One study in California showed that Se was enriched in soil organic matter mostly in the hydrophobic fulvate fraction (Neal 1995).

The rice rhizosphere is rich in carbon from root exudates and the decomposition of organic matter (Armstrong 1967 ; Flessa and Fischer 1992a). This provides an environment that enhances microbial activity leading to the transformation, degradation and biomethylation of Se (Azaizeh et al. 2003). It has been suggested that the biomethylation of Se is a detoxification mechanism of microorganisms to avoid Se toxicity in seleniferous environments because it leads to volatilisation of Se from the system (Azaizeh et al. 2003). One recent study using constructed wetlands showed that volatilisation of SeO_3^{-2} was higher than that of SeO_4^{-2}, and also that the volatilisation was always high when the soil was high in C sources (Azaizeh et al. 2003). This volatilisation process may cause a greater loss of Se in rice fields where Se under anaerobic conditions will be present as SeO_3^{-2} in soil solution.

2.7.4 CHANGING MINERALOGY AND OXIDES OF IRON, MANGANESE AND ALUMINIUM

During the 1970s scientists identified that plants grown in soils with high clay contents had a lower concentration of Se (Christensen et al. 1989; Mikkelsen 1989). Christensen et al. (1989) showed that the clay fraction had the highest percentage of adsorbed Se in soils, using an adsorption study where the adsorbed Se was calculated as the difference between applied Se to the soil and Se found in the soil solution.

Clay minerals and hydrous oxides combine with humic substances to form organo-mineral complexes that may control Se availability in soils (Mayland et al. 1989). Selenium combined with organo-mineral complexes was examined in another study where the Se adsorption was measured in clay fractions of soils with and without H_2O_2 treatments. The results showed a 5 to 10 % reduction in Se adsorption in H_2O_2-treated clays, demonstrating the formation of organo-clay complexes with Se (Christensen et al. 1989). Iron oxides (oxides and oxyhydroxides of Fe) play an important role in adsorption reactions in soils. The mineral species, and the concentration and crystal properties of the iron oxide vary greatly (Nakamaru et al. 2005b; Nakamaru et al. 2006; Peak 2006; Peak and Sparks 2002). The most frequent and stable iron oxides in soils are goethite (ά-FeOOH) and hematite (ά -Fe_2O_3). Goethite and hematite are important constituents of the highly weathered soils of tropical and subtropical regions that contain kaolinite as the major clay mineral (Singh and Gilkes, 1992). The oxide surface charge is pH-dependent, determined by the H^+ and OH^- concentrations in the solution. Iron oxides are considered to be the main material responsible for producing a positive surface charge in variable charge soils because their Fe-OH groups can absorb H^+ from the solution when the pH is lower than the zero point of the charge (pH at which the positive charges of soil equals to negative charges; (Zhang and Zhao, 1997). Therefore, at low pH iron oxides have the greatest ability to adsorb SeO_3^{-2}. According to Kirk (2004), Fe is the most abundant redox species in the rice soils of Asia, and 20 to 80 % of the Fe was in free Fe^{+3} forms; this free Fe could be reduced to soluble Fe^{+2} during submergence.

Under anaerobic conditions (low redox) there is less oxygen in the soil and most of the soil Fe is in a soluble form. Thus Se adsorbed to Fe oxides will be desorbed into the soil solution. If plant roots pump air into the rhizosphere, this can cause Fe^{2+} to be oxidised to Fe^{3+} and be precipitated on the root surface and in the rhizosphere as oxides. On the roots, this coating of Fe-oxides is called Fe plaque. Hansel (2001) showed that ferrihydrite, goethite and siderite were the major components of Fe plaque. Resorption of SeO_3^{-2} to Fe oxides in the Fe plaque (strong bidentate bonds) may limit the availability of Se to rice roots. A recent study revealed that Fe plaque acts as a buffer for SeO_3^{-2} in the rice rhizosphere, where the Fe plaque was shown to possess an ability to remobilize Se when roots released organic acids to the rhizosphere (Zhou et al. 2007). As Flessa (1992b) pointed out, rice roots can keep the rhizosphere oxidised even under reduced conditions. He further showed that the extent of the oxidation zone and the maximum redox potential reached in the rhizosphere depended on the reducing capacity of the soil, which in turn depended on the reducing substances and microbial respiration in the soil solution. There is no clear information on the behaviour of Se(0) with Fe plaque.

2.7.5 OTHER FACTORS GOVERNING THE AVAILABILITY OF SELENIUM IN PADDY SOILS

Plant uptake of Se will be determined by the availability of Se in the soil solution which in turn is affected by many soil processes and interactions with many other ions in the soil solution. The presence of ions that adsorb onto metal oxides via the same mechanism as Se (e.g. PO_4) results in more available Se in the soil solution.

Interactions between SeO_3^{-2} and PO_4^{-3} on the uptake of Se in plants have been observed by many authors, but the findings from these observations are contradictory (Carter et al. 1972; He et al. 1994,; Mikkelsen 1989). For example, Singh and Malhotra (1976) observed increased concentrations of Se in berseem clover (*Trifoli*um *alexandrinum)* when a fine sandy soil was amended with different rates of P fertilizer (0, 50 and 100 mg kg^{-1}). Ylaranta (1990) found no effects on Se uptake by ryegrass after adding increased levels of phosphorus to three soils in a pot trial. On the other hand, Hopper and Parker (1999) observed a 30 to 50 % reduction in SeO_3^{-2} uptake by ryegrass shoots and roots following a 10 fold increase of P in solution. These studies were undertaken in solution culture and demonstrate competition between Se and P for plant uptake at the root surface. These results cannot be compared directly with those found in soils as SeO_3^{-2} and PO_4^{-3} in soils compete for adsorption sites and if soil has more PO_4^{-3} ions, SeO_3^{-2} may be displaced from binding sites and made available for plant uptake. In addition, the changes in Se uptake may be due to other changes, such as pH changes, in the soil solution upon application of P fertilizer (Mikkelsen 1989).

Thus there are two possible mechanisms for the influence of PO_4 on uptake of Se in submerged soils; SeO_3^{-2} ions may be displaced from sorption sites by HPO_4^{-2} and/or P applied to the soil may increase the ability of roots to access Se (Mikkelsen, 1989; Nakamaru and Sekine, 2008). The above interaction was investigated by measuring the K_d values of Se in soils after application of different amounts of HPO_4^{-2} into soils. This experiment was conducted using two Japanese agricultural soils in an investigation of the desorption of soil-sorbed Se using a batch process with ^{75}Se as a tracer (Nakamaru et al. 2006; Nakamru and Sekine. 2008). The authors observed lower K_d values when HPO_4^{-2} concentrations increased in soils from 0.1 to 10 mM HPO_4^{-2}.

This suggests that PO_4^{-2} and SeO_3^{-2} compete for sorption sites, and the availability of SeO_3^{-2} in soils will be affected by the concentration of PO_4^{-2} in the soil solution.

The competition effect for adsorption of Se is not limited to PO_4; for instance, Zayed et al. (1998) observed a reduction in plant uptake of Se when solutions were supplied with SO_4^{-2}. Broccoli, Indian mustard, sugar beet, and rice were hydroponically in growth chambers and treated with 20 µM Se as Na_2SeO_4, Na_2SeO_3 or SeM, as well as SO_4^{-2} as $MgSO_4$ at three levels (0.25, 1.00 and 10 µM). The increase in SO_4^{-2} from 0.25 to 1.00 and 10 µM inhibited SeM uptake by 33 % and 15 to 25 % respectively. There was a 90 % reduction of SeO_4^{-2} uptake during this study.

The application of gypsum to soils has been shown to reduce the uptake of Se to plants by Dhillon & Dhillon (2000). These researchers applied increasing levels of gypsum (0.2 to 3.2 t ha^{-1}) to soils in the first year of the growing season in a wheat (*Triticum aestivum* L.) – rice (*Oryza sativa* L.) cropping sequence. Gypsum application at a rate of 0.8 t ha^{-1} significantly reduced the Se accumulation in wheat and its residual effect was evident in the following crops for 2 years. The reduction in Se accumulation varied from 53 to 64 % in wheat grain, 46 to 49 % in wheat straw, 35 to 63 % in rice grain and 36 to 51 % in rice straw with an application of gypsum at 0.8 t ha^{-1}. Similar results have been observed by Mikkelsen (1990) by adding different concentrations of SeO_4^{-2} and SO_4^{-2} in solution culture studies with rice. Selenium concentrations in the plants were found to increase with increased concentrations of Se in the solution culture, but increased SO_4^{-2} concentrations reduced the uptake of Se. The reduction of Se in plants when SO_4^{-2} is applied to the solution culture may be due to competitive inhibition, since SO_4^{-2} and SeO_4^{-2} use the same transporter (Terry et al. 2000).

2.8 CHEMICAL PROCESSES GOVERNING THE AVAILABILITY OF SELENIUM

The concentration of Se in soil solution has been suggested to be mainly governed by adsorption reactions (Elrashidi et al. 1989; Nakamaru et al. 2005a; Nakamaru et al. 2005b; Neal 1995). Adsorption, defined as the accumulation of a substance or material at an interface between the solid surface and the bathing solution, is thought to be the most important chemical process controlling the behaviour and bioavailability of trace metals in soils (Alloway, 1990; Sparks, 1995). As a consequence of adsorption, trace metals are removed from the soil solution and retained on soil surfaces. Many mechanisms are involved in metal adsorption, including cation/anion exchange or non-specific adsorption, and specific adsorption. Physical (van der Waals and electrostatic ion exchange) and chemical (inner-sphere complexation or specific adsorption) forces are involved in the adsorption of metals in soils (Sparks, 1995). The dominant reaction depends on the quantity and characteristics of the sites in the soil's solid phase, the concentration of metals and ligands capable of forming organo-mineral complexes, soil pH, EC and redox potential (Kiekens, 1983).

In non-specific adsorption, also known as cation exchange, metals are bound by electrostatic forces resulting in the formation of outer-sphere complexes (Essington 2004). The ions in the soil solution are in equilibrium with counter-ions that balance the surface charge. According to the principle of electroneutrality, the non-specific adsorption of metals should be followed by desorption of the stoichiometric quantities of counter-ions (Harmsen and Vlek 1985; Ji and Hi 1997). Non-specific adsorption is a reversible, diffusion-controlled, stoichiometric process, and there is some selectivity or preference of ions by the adsorbent, depending on their valence and degree of hydration. Both organic and inorganic surfaces are involved in electrostatic adsorption (Essington 2004).

The non-specific adsorption of anions is directly controlled by a positive charge, but not necessarily by the net surface charge of the soil. For instance, a soil which may have a negative net charge in the B horizon can still adsorb anionic metals when the pH is lower than the isoelectric point of charge (the isoelectric point is the pH at which a molecule or surface carries no net electrical charge) (Silveira et al. 1999; Dias et al. 2001a).

The adsorption properties of SeO_3^{-2} and SeO_4^{-2} on solid phases have been reported to be different. Selenate adsorbs onto solid surfaces as non-specifically adsorbing ions in outer-sphere complexation, where at least one water molecule is between the solid surface and the SeO_4^{-2} ion (Kirk 2004; Peak 2006; Peak et al. 2006). Selenate forms outer-sphere bidentate bonds with hydrous ferric oxide (Harrison and Berkheiser 1982; Peak and Sparks 2002). Furthermore, Su and Suarez (2000) observed bidentate inner-sphere complexation of SeO_4^{-2} with an amorphous-Fe(OH_3) solution interface using *in situ* attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy.

Table 9- Some reported K_d values for Se in the literature.

Soil information	Se species	No. Samples		K_d	References
		upland	lowland	(L kg^{-1})	
Andosol	SeO_3^{-2}	25	15	88-1616	(Nakamaru and Sekine 2008)
Fluvisol	SeO_3^{-2}	22	47	12-1606	
Cambisol	SeO_3^{-2}	22	7	19-2130	
Regosol	SeO_3^{-2}	3	0	4-20	
Clay loam top soil	SeO_3^{-2}			740	(Ashworth et al. 2008)
Highly organic top soil	SeO_3^{-2}			1800	
Sandy loam top soil	SeO_3^{-2}			490	
					(Collins et al. 2006)
Se-rich soil from California	SeO_3^{-2}	1		8.6-66	
	SeO_4^{-2}	1	5	1.5-5.1	
	SeO_3^{-2}	1	5	40	
Se-rich soil from Colorado	SeO_4^{-2}	1		2.9	
	SeO_3^{-2}	1		255	
Se-rich soil in Wyoming	SeO_4^{-2}	1	15	6.4	
	SeO_3^{-2}			3420-9520	
Sediments from France	SeO_4^{-2}		8	1.1-14.4	
Andosol	SeO_3^{-2}	20		200-600	(Nakamaru et al. 2005a)
Gray Lowland soils	SeO_3^{-}			100-20	

Table 9 contd.- Some reported K_d values for Se in the literature

Soil information	Se species	No. Samples upland	K_d lowland	References ($L\ kg^{-1}$)	
Gley lowland	eO_3^{-2}			100-400	(Nakamaru
Yellow soils	SeO_3^{-2}			100-500	2005a)
Red soils				200-210	
Calcareous	SeO_3^{-2}			21.5-34.8	(Wang and 2005)
Simbly	SeO_3^{-2}	1	5	2.7-28.9	Dhillon and
Brawa	SeO_3^{-2}	1	5	5.0-62.4	Dhillon, 19
Ladhowal	SeO_3^{-2}	1		1.0-8.3	
Palampur I	SeO_3^{-2}	1		1.3-14.0	
Teligi	SeO_3^{-2}	1	15	1.7-110.9	
Gurdaspur	SeO_3^{-2}	1		3.8-363.8	
Patancheru	SeO_3^{-2}	1	8	5.5-570.2	
Palampur II	SeO_3^{-2}	1		5.2-241.1	

Selenite is adsorbed by inner-sphere complexation (Peak et al. 2006; Su and Suarez 2000) onto solid surfaces of hydrous sesquioxides more strongly than SeO_4^{-2}, with no involvement of a hydroxyl group or metal hydroxyl group and is therefore directly bound to surface functional groups. This inner-sphere complexation is not readily reversible and has much slower desorption kinetics than outer-sphere complexation. It is also not affected by either the net charge of the solid surface or the ionic strength of the solution (Neal 1995; Peak et al. 2006).

Selenium adsorption behaviour can be predicted by the soil to soil solution distribution coefficient (K_d) (Dhillon and Dhillon 1999; Nakamaru et al. 2005b; Nakamru and Sekine. 2008) (see Table 9). The K_d is the ratio of adsorbed to solution phase material/element/ion of interest. During adsorption studies, the concentration of Se remaining in soil solution is measured following the addition of a known amount of Se into soil suspensions. These measurements are then used to plot adsorbed Se versus solution Se for a sequence of increasing additions of Se into the soils; this involves the inclusion of solution Se concentrations for each addition (Essington 2004). The linear range relationship can then be used to estimate the K_d value. However, there are a few disadvantages to this method, i.e., amounts of Se must be added to the soil that can be detected by the analytical instruments, rather than amounts of Se that give a real picture of Se-deficient soils. In order to overcome the above problem an isotopic dilution technique can be used to measure SeO_3^{-2} or SeO_4^{-2} K_d values in soils. A known small amount of radiotracer (either $^{75}SeO_3^{-2}$ or $^{75}SeO_4^{-2}$) is added to the soil instead of the known amount of stable Se. It is assumed that the added radiotracer acts in the same manner as the other isotopes of the same element and does not perturb the equilibrium of the stable metal in soil solution or sorbed on the solid phases. After equilibration the K_d values for SeO_3^{-2} and SeO_4^{-2} can be calculated using the following equation:

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

where r is the activity of $^{75}SeO_3^{-2}$ or $^{75}SeO_4^{-2}$ remaining in solution after equilibration (Bq); R is the total activity of $^{75}SeO_3^{-2}$ or $^{75}SeO_4^{-2}$ spiked into samples (Bq); and v is volume in L and m is mass in kg.

The K_d values for Se were measured for 58 Japanese agricultural soils by adding $^{75}SeO_3^{-2}$ to evaluate Se adsorption (Nakamaru et al. 2005b). The authors found K_d values using $^{75}SeO_3^{-2}$ to range between 12 to 1060 indicating 55 to 99% (mean = 97%) of added SeO_3^{-2} was adsorbed. The K_d values for SeO_3^{-2} were significantly correlated with the Al oxide and Fe oxide contents of the soils. These results showed that SeO_3^{-2} is strongly adsorbed to Al and Fe oxides in soils, a process that can restrict plant uptake by reducing the available fraction in the soil. In this study, K_d values were determined in mainly acidic soils under aerobic conditions and may not reflect the actual situation in rice soils at neutral pH and under anaerobic conditions. A mixture of SeO_3^{-2} and SeO_4^{-2} can be present in the soil depending on the soil's chemical and microbial properties. Therefore, measurement of K_d values for both Se species will give a more accurate estimation of the partitioning and hence the potential availability of Se present in the solid-solution phase.

2.9 MEASUREMENT OF SELENIUM AVAILABILITY IN SOILS

2.9.1 CHEMICAL EXTRACTION METHODS

Various chemical extraction methods have been used to either measure or estimate the available pool of Se in soil: hot water, ammonium bicarbonate-diethylene tri-aminepentaacetic acid (AB-DTPA), saturate paste extract, orthophosphate (PO_4) and

Na_2CO_3 are the most common extractants recorded in the literature (Dhillon and Dhillon 2004; Hettiarachchi and Gupta. 2007). These extraction solutions are used to estimate the plant available pools and should be able to determine the Se potentially available to plants; i.e. the Se present in the soil solution and readily available on the solid phases.

In addition to single extraction methods, sequential extraction methods have been used to fractionate solid-phase Se and hence estimate the potentially available pool of Se in soils (Dhillon and Dhillon 2004). The sequential extraction technique assumes each extracting agent determines a specific form of Se in soil. There are different operationally defined fractions in different sequential extraction methods developed by different scientists. Soluble, exchangeable, oxide bound, sulphide/organic matter bound, and residual or siliceous material associated Se are the main fractions in the method of Chao and Sanzolone (1989) while there are nine fractions in the method developed by Lipton (1991): soluble, ligand exchangeable, carbonates, oxidisable, easily reducible oxides-bound, amorphous-oxide bound, crystalline-oxide bound, alkali-soluble Al/Si bound, and residual. The conventional sequential extraction methods commonly used to fractionate trace metals in soils (Tessier, 1979) may not be suitable for Se as they can lead to changes in Se oxidation states which could cause reabsorbtion by an undissolved surface during extraction (Tokunaga et al. 1991). Another fractionation scheme has been developed to fractionate Se in seleniferous soils in order to extract Se in soluble, adsorbed, organic, and insoluble fractions (Martens and Donald 1997). Extractants used for fractionation studies can change the chemistry of the soil system causing significant redistribution of elements of interest among different geochemical pools (Hamon et al. 2008a). For instance, Dhillon and Dhillon (2004) measured available pools of Se in field capacity and submerged soils using both single and multiple extraction techniques. In alkaline soils, a 0.25M KCl method gave significant differences between single and multiple extractions depending on soil pH. In alkaline soils, multiple extractions gave a higher available fraction of available Se compared to single extractions. One of the reasons given for the higher availability of Se in multiple extractions was that more SeO_4^{-2} was desorbed each time that fresh KCl was added to the soil, and when the soil suspension was shaken, the centrifuged and supernatant solution was separated while SeO_3^{-2} should have been oxidized due to exposure of samples to air. Therefore fractionation studies may not provide accurate data regarding the Se distribution in rice soils.

2.9.2 POTENTIAL AVAILABILITY OF SELENIUM (*E* VALUES)

The isotopic dilution technique can be used to measure the exchangeable pool of Se in soils, which is known as the *E* value (Figure 4, below). Here the potential availability is the combination of the quantity of Se in the soil solution plus that on the solid phase which is in rapid equilibrium with the soil solution. The principles of isotopic dilution, measuring potential availability using the isotopic dilution technique, and the advantages and disadvantages of the method have been discussed in the literature (Hamon et al. 2002; Hamon et al. 2008a). Equations used for measuring potential availability have been developed based on important assumptions: that the small quantity of introduced radioisotope does not perturb the equilibrium of the system and that the introduced radioisotope does not have any access to non-labile pools; that the introduced isotope behaves exactly as the natural element; that all metal species measured in the solution are isotopically exchangeable, and that the introduced isotope is physically mixed with the entire labile metal pool (Hamon et al. 2008b)

The isotopic dilution technique has been used successfully in estimating the availability of metals in soils (Collins et al. 2006; Hamon et al. 2004; Lombi et al. 2003; Wendling et al. 2008). However, when there are a few oxidation states of an element in the labile pool at same time, trying to estimate one oxidation state may give erroneous results. Each oxidation state in soil solution should be measured rather than the total soluble element, so that the *E* value of each oxidation state can be determined. For instance Se is mainly present as SeO_3^{-2} or SeO_4^{-2} in soils, and therefore measuring the E values of both species is necessary.

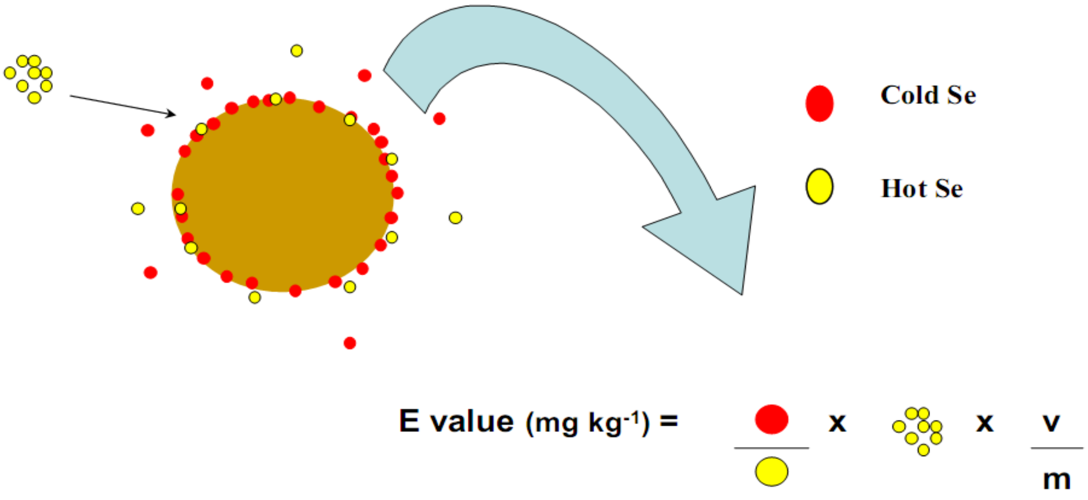


Figure 4 - Schematic diagram of the measurement of E values in soil.

The isotopically exchangeable pool of SeO_3^{-2} ($E_{SeO_3^{-2}}$ value) or SeO_4^{-2} ($E_{SeO_4^{-2}}$ value) in the soil can be calculated using the equation:

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

where S is the concentration of SeO_3^{-2} or SeO_4^{-2} in solution after equilibration (mg L^{-1}); r is the activity of $^{75}SeO_3^{-2}$ or $^{75}SeO_4^{-2}$ remaining in solution after equilibration (Bq); R is the total activity of $^{75}SeO_3^{-2}$ or $^{75}SeO_4^{-2}$ spiked into samples (Bq); and v is volume in litres and m is mass in kilograms.

It is essential to avoid speciation changes of the added tracer during pre-equilibration to avoid under or over estimation of *E* values, and if speciation changes occur then modification to the equation is essential to correct for this. This is because the adsorption patterns of $^{75}SeO_4^{-2}$ and $^{75}SeO_3^{-2}$ are different. Therefore, care should be taken to estimate the equilibration time where fast equilibration is achieved (Hamon et al. 2008a). When measuring the potential availability of Se in rice soils under flooded conditions it is essential to monitor the tracer, as a combination of low redox and high pH in submerged soils can cause redox changes not only to the added tracer but also to the other isotopes already in the soil system during sample handling and the equilibrium period. Stable isotopes have been used to assess the self exchange kinetics of SeO_4^{-2} to SeO_3^{-2} in soil during the equilibrium time, where soil is spiked simultaneously with ^{78}Se labelled SeO_3^{-2} and ^{76}Se labelled SeO_4^{-2}. After the equilibration time, the two oxidation states can be monitored by measuring the $^{78}SeO_4^{-2}/^{80}SeO_4^{-2}$ and $^{76}SeO_3^{-2}/^{80}SeO_3^{-2}$ ratios in soil suspension. If any speciation change of ^{78}Se labelled SeO_3^{-2} occurs then an increase of $^{78}SeO_4^{-2}/^{80}SeO_4^{-2}$ should be observed. This stable isotope technique has been successfully used to assess the self exchangeability of Se species in soils, and it has been found that speciation changes are slow in terms of the time taken to measure labile Se pools (Collins et al. 2006).

2.10 SELENIUM FERTILSATION FOR AGRONOMIC BIOFORTIFICATION

As an essential micronutrient for humans and animals, Se plays a major role in human and animal diets. Because of the low concentration of Se reported in staple foods such as rice, developing a Se fertilizer strategy would play a key role in increasing Se levels in the human body. There are a number of important ideas discussed in the next section to provide a better understanding of potential Se fertilization programs for lowland rice.

2.10.1 RATES, METHOD AND TIME OF APPLICATION OF EFFECTIVE SELENIUM SPECIES

The difference between essential and toxic concentrations of Se in humans and animals is very small and therefore fertilization of soils with Se is problematic. Selenium fertilization to increase the Se content in crops and thereby improve the Se nutrition of grazing animals and humans has only been supported by governments in a few countries. Different species of Se such as SeO_3^{-2}, SeO_4^{-2} have been applied to soils to increase the Se concentration in various crop plants (Mikkelsen, 1989; Gupta and Gupta, 2002). In order to increase Se concentrations in grazing animals, most of the research to date has focused on attempts to increase the Se concentration in pastures. Gissel-Nielsen (1970) found the application of SeO_4^{-2} fertilizers increased Se concentrations in red clover, mustard, sugar beet, and alfalfa plants up to 20 to 50 times more than SeO_3^{-2} fertilizers, likely because of strong non-reversible sorption of the latter (Section 2.8).

Slow-release Se fertilizers may be a viable strategy as small amounts of Se can be released in synchronization with plant absorption. There are commercial Se fertilizers available for application to soils in many countries e.g. Selcote Ultra (Nufarm NZ, Auckland, New Zealand). Selcote Ultra was marketed in 1989 to 1990 as the first Se fertilizer to replace earlier Selcote and Selcote Two Year which came to the market in the early 1980s for improved animal health (Broadley et al. 2006). Different countries have their own guidelines for Se application rate to soils: New Zealand has a recommended maximum of 10 g Se ha^{-1} yr^{-1}, United Kingdom a rate of 6.6 g Se ha^{-1} yr^{-1}; in Finland the rate for cereals is 16 g ha^{-1} yr^{-1} and for fodder crop and hay production 6 g Se ha^{-1} yr^{-1} is the maximum recommended dosage. Most of the studies to date on slow release Se fertilizers have been for pastures and not for soils under paddy cultivation. Application of Se at a rate of 10 g ha^{-1} supplied as slow-release Selcote Two Year (10 g Se kg^{-1} as 1:1 Na_2SeO_4:$BaSO_4$) supplies enough Se for three years but 3 to 5 g Se ha^{-1} yr^{-1} supplied as Selcote (with 10 g Se kg^{-1} as Na_2SeO_4) provides an adequate supply of Se for pasture for only one year (Broadley et al. 2006). This shows that the application of slow-release fertilizers into soils can supply Se to plants for longer periods than normal Se fertilizers such as Se containing salts. Slow-release Se fertilizers have not yet been tested with rice crops.

The chemical form of Se fertilizer, its solubility under different pH and Eh conditions during rice growth, and the rate of fertilizer applied are the key factors determining the amount of Se absorbed by plants. Most studies indicate that Se availability is low in acid and neutral soils due to the immobilisation of SeO_3^{-2} by strong adsorption or precipitation with hydrous sesquioxides (Balistrieri and Chao 1987; Blaylock et al. 1995; Duc et al. 2003). Dissolution and desorption are slow processes in these soils (Mayland et al. 1989). Therefore, strong retention of Se in those soils can be a major limitation for low Se absorption by rice plants given that SeO_3^{-2} is the main form of Se under the redox and pH levels that prevail in paddy soils.

Application of SeO_4^{-2} fertilizers to acid and neutral soils is risky because Se has a high bioavailability and plants can absorb large quantities that could cause Se toxicity. For example SeO_4^{-2} fertilizer application to pasture has been shown to result in toxic concentrations of Se in the first cut of pasture and then sharply decreasing concentrations with each subsequent harvest (Mayland et al. 1989). On the other hand, SeO_4^{-2} can be transformed to SeO_3^{-2} under the Eh and pH conditions prevailing in rice soils. There is no recommended Se fertilization strategy for rice production. Chen et al. (2002) tested 20 g ha^{-1} of $NaSeO_4$ and $NaSeO_3$ as foliar fertilizers for rice, and found a 20 to 25% higher Se concentration in grains when SeO_4^{-2} was applied (Chen et al. 2002). Assuming the 20 g ha^{-1} foliar application rate was optimal, then the rates of Se required for soil application are likely to be more than 20 g ha^{-1}, (considering that adsorption/precipitation reactions and possible changes in speciation reduce solubility).

In addition to the above Se species, Se(0) has also been examined as a potential fertilizer (Gissel-Nielsen, 1970). There are few forms of Se(0) found in nature. Amorphous red and black Se is readily oxidisable while the gray-hexagonal allotrope is inert (Geering et al. 1967). Geering et al. (1967) found that when Se(0) was added to soil, it precipitated as red amorphous Se(0). If the solution temperature was higher than 30 ^{0}C, red amorphous Se(0) slowly converted to the inert form. There are still gaps in our understanding of both Se behaviour in paddy soils and also of the optimal fertilization strategy for crops growing under these conditions.

As noted above, some research on Se fertilization of rice has focused on foliar applications (Chen et al. 2002). In a rate-response trial, an application rate of 20 g ha^{-1} to rice increased grain Se concentrations from 0.071 to 0.64 mg kg^{-1}. However, foliar application has practical limitations because Se is hard to apply uniformly on rice heads and leaves, often under difficult climatic conditions e.g. heavy rainfall and strong wind. Also, Se is toxic at high concentrations so fertilizer application rates must be precise.

As Se uptake is likely to be via a S uptake pathway, Se may also be absorbed during the vegetative phase and absorption may continue up until the reproductive phase. Application of Se fertilizer at the vegetative phase will likely be helpful in increasing Se levels in rice grain but it is likely that Se will be removed from the available pool with time as redox and pH changes in rice soils affect the speciation. Further study is required to identify the optimal time for Se application to maximize plant uptake and accumulation of Se in rice grains.

2.11 SUMMARY

Selenium is an essential micronutrient for humans and animals and therefore an important trace element in soils growing crops. A trend towards a decline in the available Se concentration of soils has been noted in some regions of the world; a trend that may be associated with the burning of fossil fuels and the release of sulphur (a Se antagonist), acid rain, soil acidification and use of high sulphur and phosphate fertilizers. In addition, the global demand for food requires that food often be produced in soils depleted in micronutrients, such as Se. Given the importance of adequate Se concentrations for animal and human health, the biofortification of Se in foods through fertilization, also known as agronomic biofortification, may be an effective strategy to improve nutritional requirements.

The efficient use of Se fertilizers can only be optimised when we understand the speciation of Se in soils and processes that control Se uptake by rice plants. The speciation of Se in soils depends on a complex mixture of processes and factors. In a paddy soil environment the main controls on Se speciation are redox and pH. Under the pH and redox levels prevailing in paddy soils, SeO_3^{-2} and Se(0) are the major forms of Se. Selenite is strongly adsorbed to Fe and Mn oxides, therefore availability is low. Elemental Se is reported to be a transitory compound in soils where SeO_3^{-2} reduces to Se^{-2} under anaerobic conditions. Elemental Se is reported to be unavailable for plant uptake because it is insoluble. Changes in Se(0) in paddy soils greatly affects its availability for plant uptake. The SeO_4^{-2} form of Se is highly plant-available and may also be formed in paddy soils when drainage occurs two weeks before harvesting. Selenium-deficient soils could therefore be fertilized with either of the three Se forms, namely SeO_3^{-2}, SeO_4^{-2} and Se(0), but their effectiveness will vary mainly depending on the redox and pH conditions in soil. The relationship between plant uptake of Se and the chemistry of Se in paddy soils during rice growth is not well understood. More studies are needed to understand the availability of Se in lowland rice systems.

2.12 THESIS AIM

The aim of the thesis was to understand the processes influencing the plant availability of Se in rice soils under variable redox conditions and then to select best fertilizer application time, method of application and selenium species to increase the accumulation of Se in rice grain.

2.13 THESIS OBJECTIVES

The objectives of this thesis were:

- To examine the partitioning of Se species in submerged soils following the application of Se fertilizers under upland and lowland conditions;
- To investigate changes in the potential availability of Se in soils, under submerged conditions and subsequent aeration, following the application of Se fertilizers;
- To examine the influence of Se fertilizers, application time and method on the uptake, translocation and accumulation of Se in rice plants (grains, roots, culms and leaves);
- To understand the mechanism of Se uptake in soils fertilized with SeO_4^{-2}-enriched urea; and

- To develop an effective fertilization strategy to increase Se concentrations in grains of lowland rice for improved human and animal health.

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CHAPTER 3

3. POTENTIAL AVAILABILITY OF FERTILIZER SELENIUM IN FIELD CAPACITY AND SUBMERGED SOILS

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CHAPTER 4

4. 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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CHAPTER 5

5. SELENATE-ENRICHED UREA GRANULES ARE A HIGHLY EFFECTIVE FERTILIZER FOR SELENIUM BIOFORTIFICATION OF PADDY RICE GRAIN

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CHAPTER 6

6. MECHANISMS RESPONSIBLE FOR THE EFFECTIVENESS OF SELENATE-ENRICHED UREA GRANULES AS AN EFFECTIVE SELENIUM BIOFORTIFICATION STRATEGY FOR LOWLAND RICE (O*RIZA SATIVA L.*)

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ABSTRACT

Selenium (Se) is an essential micronutrient for humans and animals but is deficient among more than one billion people in the globe. Rice is the staple food for more than half of the world's population. Over-reliance on rice for micronutrients such as Se causes health problems related to Se deficiency, such as thyroid dysfunction, cancer, viral diseases, and cardiovascular diseases. Increasing Se concentration in rice by the application of fertilizer Se is an agronomic approach that increases Se intake by humans and improves human health. In a previous study we found that SeO_4^{-2}-enriched urea granules applied at the heading stage to floodwater was a much more effective fertilizer than soil (pre-plant) or foliar applications. A rice-growth experiment was carried out with ^{75}Se-labelled fertilizer, to track the fate of added fertilizer Se and to follow the accumulation of fertilizer Se into rice plant parts. Five fertilizer treatments were applied at either tillering or heading: (i) SeO_4^{-2}-enriched urea granules broadcast onto floodwater; (ii) urea granules broadcast onto floodwater and fluid SeO_4^{-2} applied to floodwater; (iii) urea granules and fluid SeO_4^{-2} placed at the soil water interface (SWI); (iv) a urea and SeO_4^{-2} combined solution placed at the SWI; and finally, a granular urea only treatment (urea was broadcast to floodwater). Results showed SeO_4^{-2}-enriched urea granules applied at either the tillering or heading stage produced significantly higher grain Se concentrations compared to any other Se application method. There were also higher concentrations of SeO_4^{-2} in floodwaters and pore-water samples following the application of SeO_4^{-2}-enriched urea granules. Co-localization of urea and SeO_4^{-2} in the granules may be important in restricting the conversion of SeO_4^{-2} to SeO_3^{-2} in the floodwater and soil. The ability to maintain a high concentration of SeO_4^{-2} in soil pore water could be the reason for higher uptake and accumulation of Se in rice grains by using SeO_4^{-2}-enriched urea granules.

Keywords: *selenium, selenite, selenate, grain Se accumulation, rice*

INTRODUCTION

Selenium (Se) deficiency affects over a billion people in the world (Combs, 2001). An increase of Se in staple crops such as rice is a world nutritional goal, as rice is the staple for more than half of the world's population. Numerous efforts have been made to increase Se concentrations in cereal crops growing on Se-deficient soils, including the use of genetically improved cereal crops for higher uptake of Se (Lyons et al., 2004) and the application of fertilizer Se into soil crop systems, called agronomic biofortification (Cubadda et al. 2010; Lyons et al. 2004).

Agronomic bio-fortification has many advantages over direct supplementation, as inorganic Se absorbed by the plants transform into an organic form having a higher bioavailability (selenomethionine). In addition, plants act as a barrier to prevent uptake of excessive/toxic concentration which could occur with direct supplementation (Hawkesford and Zhao, 2007). Selenium fertilizer can be applied into soil or as a foliar spray. While soil application of Se has been successful ensuring appropriate levels of Se accumulation in wheat grains (Lyons et al., 2005), pre-plant soil applications are ineffective for paddy rice due to rapid sorption and or transformation of oxidised forms of Se into reduced forms during growth under flooded conditions (Premarathna et al. 2010). Furthermore, oxidation of reduced Se forms is slow, so that even pre-harvest drainage of soil releases little or no soluble Se for crop uptake (Premarathna et al. 2011). Foliar Se fertilization has been examined in China and found to be an effective

Se biofortification method (Hu et al., 2002). However, the disadvantages of foliar fertilization are the additional labour involved in separately applying Se from other fertilizers, as well as the lack of any residual effect for subsequent crops. Therefore, a simple and effective Se fertilization strategy for paddy rice is required to fulfil the goal for agronomic biofortification of this staple food crop.

In a previous study, we examined the accumulation of Se in rice grains with selenite (SeO_3^{-2}) and selenate (SeO_4^{-2}) fertilizers (applied at a rate of 30 g ha^{-1} at two different times, i.e. either at soil preparation or heading stage) where Se was applied using different methods (i.e. Se-enriched urea granules, fluid Se into floodwater, or as a foliar spray). We found that SeO_4^{-2} was the best Se species for fertilization of rice, while Se applied at the heading stage as SeO_4^{-2}-enriched urea granules was much superior to any other fertilization method (Premarathna et al. 2011). The mechanisms behind this effective Se fertilization strategy have not been elucidated.

In this study, we therefore examined different placement methods for Se and urea, and Se-enriched urea granules, in submerged rice soils. Furthermore, we examined the speciation of Se in floodwaters and pore waters to elucidate the possible mechanism for the increased uptake of Se in rice grains following the application of SeO_4^{-2}-enriched urea granules.

MATERIALS AND METHODS

Standards and reagents

All reagents and standards used in this study were made from trace metal grade reagent solutions. Ultrapure deionised water (Mill-Q, Millipore) was used for all chemical preparations and dilutions. Sodium selenate (Na_2SeO_4) and citric acid were purchased from Sigma (Australia). Carrier-free radioactive $^{75}SeO_3^{-2}$ was purchased from the University of Missouri research reactor, Colombia, MO, USA. The $^{75}SeO_3^{-2}$ stock solution was converted to radioactive $^{75}SeO_4^{-2}$ by the addition of 30 % hydrogen peroxide (Beauwens et al. 2005). The complete conversion of $^{75}SeO_3^{-2}$ to $^{75}SeO_4^{-2}$ was confirmed using high performance liquid chromatography (HPLC) and γ-spectroscopy (Wallac 1480, Wizard 3) (Premarathna et al. 2010).

Treatments and experimental design

The pot experiment was conducted in a light-, temperature-, and humidity-controlled growth chamber. Maximum and minimum temperatures inside the growth chamber were 30^0 C and 25^0 C, respectively; with a 12 h daylight and 12 h dark cycle at 53% humidity. There were 40 pots arranged in a randomized block design with four replications. Treatments were four SeO_4^{-2} plus urea application methods applied at either tillering or heading stages. The four treatments used in this study were: (i) SeO_4^{-2}-enriched urea granules broadcast onto the floodwater; (ii) urea broadcast onto the floodwater and fluid SeO_4^{-2} applied separately into the floodwater; (iii) urea granules broadcast onto the floodwater and fluid SeO_4^{-2} injected onto the soil-water interface (SWI); and (iv) a solution of urea and SeO_4^{-2} combined injected onto the SWI. A urea granule control treatment was also included.

Pot experiment

Rice plants (*Oryza sativa* sp. Amaroo) were grown in 3.5 L black plastic pots lined with plastic bags and filled with 2 kg of Hanwood loam (a Rodoxeralf (*37*)) collected near Griffith, New South Wales a rice growing area of Australia (Premarathna et al. 2010).

The soil had a pH of 6.4 (1:5 soil: water suspension) (Rayment and Higginson, 1992), electrical conductivity (EC) of 141.2 µS cm^{-1} (Rayment and Higginson, 1992), total carbon of 2 % (Matejovic, 1997), cation exchange capacity (CEC) of 21 $cmol^{+}$ kg^{-1} and a total Se concentration of 0.117 mg kg^{-1}. All pots received an equivalent of 150 kg N ha^{-1} (half applied at soil preparation and the other half at either the tillering or the heading stage), 25 kg P ha^{-1} and 100 kg K ha^{-1}, together with a micronutrient mix of 0.3 $ZnSO_4$, 0.3 $CuSO_4$, 0.1 H_3BO_3, 20 $CaSO_4$, 20 $MgSO_4$ and 0.01 $(NH_4)_6Mo_7O_24H_2O$ kg ha^{-1} (Gunawardena et al., 2003) applied during soil preparation. Soil in the pots was submerged for 14 d before two rice seedlings (approximately fifteen days old) were transplanted into individual pots. Two weeks before harvest, pots were drained and moisture content was thereafter maintained at field capacity (10Kpa).

Application of selenium fertilizer treatments

A $^{75}SeO_4^{-2}$ radioactive tracer technique was used in this study to directly determine the uptake and accumulation of Se from fertilizers, as this allowed a very low rate of Se application without compromising detection limits. The rate of Se application for all application treatments was 0.4 mg Se kg^{-1}. The fertilizers were added into pots at either the mid-tillering or the heading stage. Selenate solution was spiked with carrier-free SeO_4^{-2} to achieve an activity of 1 MBq per pot. Control pots also received one MBq of ^{75} SeO_4^{-2} at the same time urea broadcasted onto floodwater. The activities of $^{75}SeO_4^{-2}$ and total SeO_4^{-2} concentrations used for the preparation of different fertilizers were checked by γ-spectroscopy and inductively coupled plasma-mass spectrometry (ICP-MS), respectively.

Collection of floodwater and pore water samples

Floodwater samples were collected at one, three, seven and fourteen day after fertilizer application. Floodwater samples were collected using a pipette (~ 10 ml) approximately 2 cm from the SWI. The pore water samples (~10 ml) were collected using Rhizon samplers (Eijkelkamp, Giesbeek, the Netherlands) installed vertically approximately 2 cm deep into the soil before flooding. Immediately before pore water collection, a plastic syringe was attached to the Luer-lock connector and approximately 1 mL of solution was drawn through the tubing and discarded to avoid Se redox changes from the presence of air in the tubing. Samples were then collected by screwing a needle into the Luer-lock connector and pushing the needle into the rubber septum cap of an evacuated test tube.

Floodwater and pore water samples were transferred directly to an anaerobic chamber (modular atmosphere controlled system, DW Scientific, West Yorkshire, U.K.) for further processing and handling. Total Se concentrations in floodwaters and pore waters were determined using ICP-MS and separation of ^{75}Se species was performed by HPLC and measured by γ-spectroscopy (Premarathna et al. 2010). After measuring the pH, the remaining solutions were stored at -20 ºC before determination of ammonia (NH_4^+) and nitrate (NO_3^-) concentrations by ion chromatography (Dionex ICS-2500, Sunnyvale, CA, U.S.A).

Total selenium concentration in rice grains

At maturity, rice grains were harvested, fresh weight recorded and dried at 55°C for three days to a constant weight (dry mass). Husks were removed from harvested grain using a laboratory-scale hand operated de-hulling machine. The resulting unpolished rice grains were ground using a laboratory seed grinder to a fine homogeneous powder. The ground samples were stored in a desiccator until digestion and analysis.

The grain samples were digested using a closed vessel microwave procedure (Ethos E touch control, Milestone, North America) using a two-stage time program: 5 min at 300 W and 40 min at 500 W. Approximately 0.5 g of finely ground grain samples were weighed into a Teflon digestion vessels and 10 mL of concentrate HNO_3 acid (Aristar) added. After microwave digestion, the vessels were allowed to cool for 30 minutes at room temperature and then diluted to 50 mL with ultrapure deionised water (Milli-Q, Millipore). The digested samples were analysed for total Se by ICP-MS (Agilent 7500ce ICP-MS with H_2 gas added to the collision cell at a flow rate of 4 mL min^{-1}) and ^{75}Se activity by γ spectroscopy. The accuracy of the digestion and ICP-MS analysis procedure was assessed through the analysis of the certified reference material NIST 1568a rice flour. The total Se concentrations determined in the rice certified reference material were in close agreement with the certified value (this study 0.38 ± 0.03 mg Se kg^{-1} (n = 5); certified value = 0.38 ± 0.04 mg Se kg^{-1}).

Fertilizer Se concentrations in rice grains from different treatments were calculated using the $^{75}SeO_4^{-2}$ tracer and the following equation:

$$\text{Grain Se from fertilizers (mg kg}^{-1}\text{)} = \frac{1\times}{a}\,\frac{b \times 1000\times 1000}{c}$$

where, a is the specific activity of ^{75}Se (Bq µg^{-1}); b is the total ^{75}Se activity in the digested solution in Bq; c is the weight of digested rice sample in grams.

Fertilizer selenium concentrations in soils

The distribution of fertilizer Se in the soil profile was examined after rice maturity using a metal corer (~ 3 cm diameter) at 0 to 2, 2 to 5 and 5 to 10 cm depths. The soils were oven dried at 40 °C, homogenized using a mortar and pestle and sieved to < 2 mm. Fertilizer Se concentrations were determined in soils following strong acid extraction using a 30% H_2O_2 and *aqua regia* procedure (Bakhtar et al. 1989). The ^{75}Se activities in solutions were determined using γ spectroscopy. The fertilizer Se concentrations at different soil depths were calculated using the following equation:

$$\text{Fertilizer Se in soil (mg kg}^{-1}\text{)} = \frac{1\times}{a}\frac{b \times 1000\times 1000}{c} = \frac{1}{a} \times b \times \frac{1000}{c} \times 1000 = \frac{1}{a} \times b \times \frac{1000}{c} \times 1000$$

where, a is the specific activity of ^{75}Se (Bq µg^{-1}); b is the total ^{75}Se activity in the digested solution in Bq; c is the weight of digested soil sample in grams.

Statistical analysis

The significance of effects of fertilizer application time and application method on grain dry weight and Se concentrations in grains were determined using analysis of variance (ANOVA) in Genstat software (Genstat 10th ed, VSN International, Hempstead, UK).

RESULTS

Rice grain dry mass

The time of fertilizer application ($p \leq 0.001$) was found to have a significant effect on grain dry mass (Table 1). A significant higher grain dry mass was found in fertilizer treatments applied at the mid-tillering than heading stage (Table 1). The SeO_4^{2-} plus urea fertilizer treatments were found to have no significant ($P > 0.05$) effect on the rice grain dry mass when applied at mid-tillering, however, a small but significant ($P \leq 0.05$) effect on grain dry mass was observed when applied at the heading stage (Table 1).

Floodwater and pore water chemistry

The pH of floodwater and pore water samples 1 d after fertilizer application ranged from ~7.5 to ~8.5 (Figure 1). There was no significant differences ($P > 0.05$) found in NH_4^+ concentrations in pore waters collected for the different fertilizer treatments applied at the mid-tillering stage (Figure 3). The NH_4^+ concentrations in pore water samples were found to be below detection limits 3 d after the fertilizer application when applied at the mid-tillering stage (Figure 3). When fertilizers were applied at heading, the highest NH_4^+ concentrations in pore water samples were found when urea granules were broadcast with SeO_4^{2-} added into the floodwater, or injected into the SWI (Figure 3). One day after fertilizer application, NO_3^- concentrations in pore water samples were found to range from the instrumental detection limit (0.1 $\mu g\ L^{-1}$) to 0.4 $\mu g\ L^{-1}$ (data not shown), and thereafter were below detection in all treatments. There were no observable differences in NO_3^- concentrations in pore water samples between fertilizer treatments. Selenium concentrations in floodwater and pore water were extremely low (~5 $\mu g\ L^{-1}$) in the control (urea only) treatment (Figure 2). Concentrations of Se were increased by twenty to forty fold immediately after fertilization, but declined very rapidly with time so that 10 days after fertilization, floodwater and pore water concentrations had returned to control values. Generally, concentrations of Se were similar in floodwater and pore water, indicating that the added fertilizer Se penetrated the SWI quite quickly. There were no consistent or persistent effects of fertilizer treatment on total Se concentrations in floodwater or pore water

Fertilizer Selenium in rice grains

The concentration of Se in rice grains was found to be significantly ($p \leq 0.001$) influenced by the method of fertilizer application (Table 2). Fertilizer Se concentrations were found to be significantly higher in rice grains from the SeO_4^{-2}-enriched urea granule treatment applied at either growth stage (Table 2). Differences between the other treatments were insignificant.

Selenium speciation in floodwaters and pore waters

Recovery of Se from the HPLC column (from total radioactivity balance) was generally good, averaging 96%. The majority of fertilizer Se species in floodwater and pore water samples was identified as inorganic SeO_4^{-2} or SeO_3^{-2} species, through retention time comparisons to synthetic standards (Table 3). However, there were significant speciation differences between the fertilizer treatments, with the majority of Se in floodwaters and pore water samples from the SeO_4^{-2}–enriched urea granule treatment being predominantly SeO_4^{2-} (Table 3). For the other fertilizer treatments, the majority of Se in floodwaters and pore was predominantly SeO_3^{2-} (Table 3).

Fertilizer selenium concentrations in soil profiles

Fertilizer Se concentration measured at three different depths in each pot at the end of the experiment revealed that most of the fertilizer Se remained in the top few centimetres of the soil (Table 4).

Table 1. Fertilizer application method and time of application on dry weight of rice grains (g pot^{-1}) (means ± SD, n=4)

Application time	Fertilizer application method[1]	Dry weight[2] (g pot^{-1})
Mid-Tillering	SeO_4^{-2}-enriched urea granules onto floodwater	15.13±0.58^{d}
	Urea granules and fluid SeO_4^{-2} separately onto floodwater	15.19±0.69^{d}
	Urea granules onto floodwater and fluid SeO_4^{-2} into SWI	15.78±0.15^{d}
	Urea and SeO_4^{-2}combined solution into SWI	15.14±1.00^{d}
	Urea granules onto floodwater	14.83±0.70^{d}
Heading	SeO_4^{-2}-enriched urea granules onto floodwater	14.03±0.98^{cd}
	Urea granules and fluid SeO_4^{-2} separately onto floodwater	12.55±0.74^{bc}
	Urea granules onto floodwater and fluid SeO_4^{-2} into SWI	11.45±0.54^{ab}
	Urea and SeO_4^{-2}combined solution into SWI	11.70±0.58^{ab}
	Urea granules onto floodwater	9.84±0.35^{a}

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[1] SWI- soil water interface; [2] analysis of variance letters are such that where treatments share a letter they are not significantly different.

Table 2. Effect of fertilizer application method on the accumulation of fertilizer Se (mg Se kg^{-1} DW) in rice grains (means ± SD, n=4,)

Fertilizer application method[1]	Mid-Tillering (mg kg^{-1})	Heading (mg kg^{-1})	Average Stage[2] (mg kg^{-1})
SeO_4^{-2}-enriched urea granules onto floodwater	13.2±5.2	7.5±3.2	10.5^b
Urea granules and fluid SeO_4^{-2} separately onto floodwater	5.7±1.8	4.1±1.4	7.0^a
Urea granules onto floodwater and fluid SeO_4^{-2} into SWI	5.4±0.9	6.0±0.6	6.0^a
Urea and SeO_4^{-2}combined solution into SWI	3.3±1.3	5.4±0.9	5.4^a

SWI- Soil water interface; [2]analysis of variance letters are such that where treatments share a letter they are not significantly different (at P = 0.05).

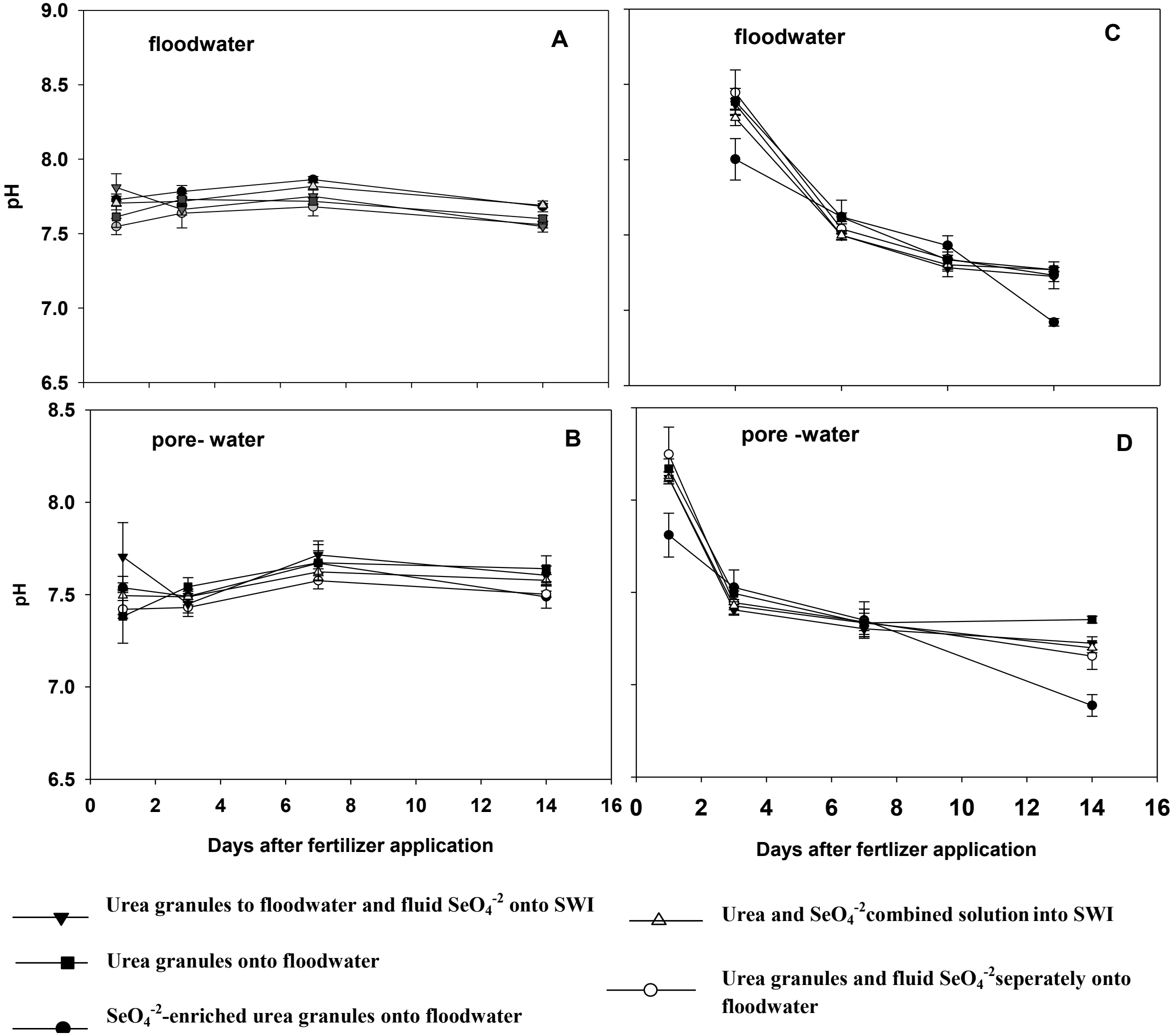

Figure 1-Floodwater and pore water pH after fertilizer application at mid-tillering (A and B) and heading (C and D) (n =4).

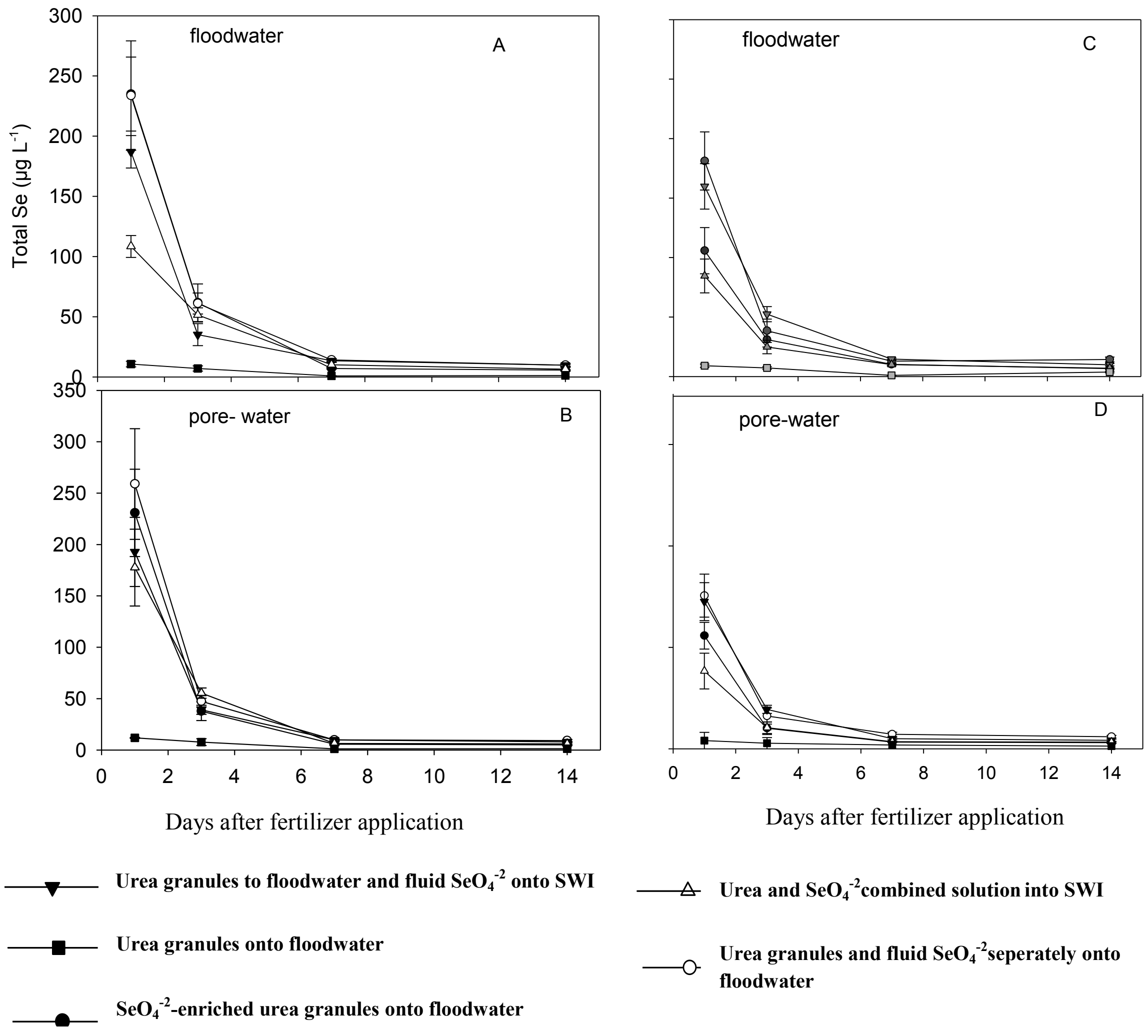

Figure 2- Total selenium concentrations in flood and pore water samples at mid-tillering (A and B) and heading (C and D) (n =4).

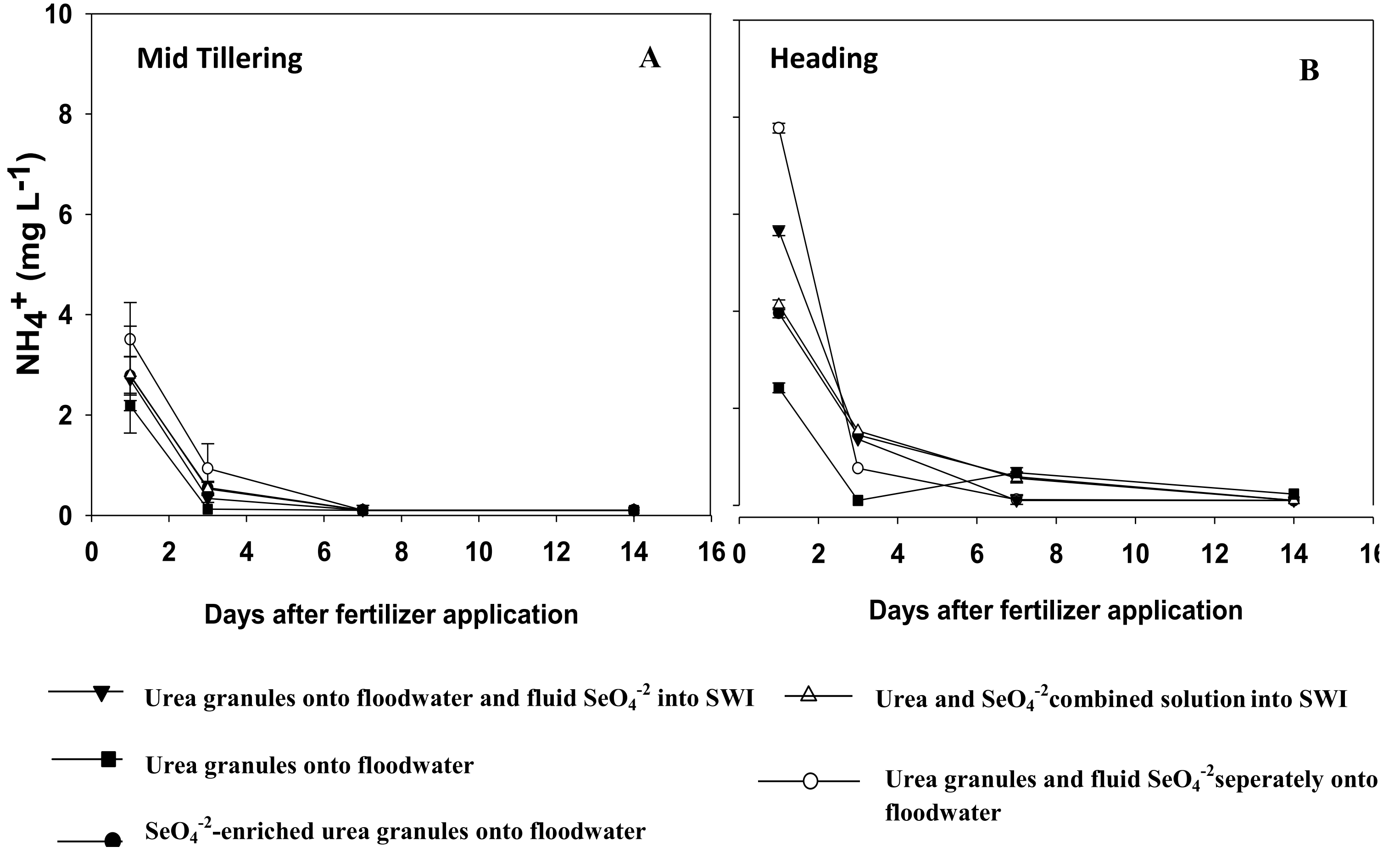

Figure 3- Ammonium concentration in pore water samples when fertilizers were applied at (A) mid-tillering, and (B) heading (n =4).

Table 3. Selenium speciation in floodwater and pore water 1 d after fertilizer application.

Application Time	Sample location	Fertilizer application method[1]	^{75}Se recovered (% ^{75}Se added)[2]	SeO_3^{-2} (%)[3]	SeO_4^{-2} (%)[3]
Tillering	Flood water	Selenium enriched urea granules	88	15	85
		Urea granules and fluid SeO_4^{-2} into floodwater	72	15	85
		Urea granules and fluid SeO_4^{-2} into SWI	68	68	32
		Urea and fluid SeO_4^{-2} combined fluid into SWI	110	61	39
	Pore water	Selenium enriched urea granules	95	1	99
		Urea granules and fluid SeO_4^{-2} into floodwater	96	92	8
		Urea granules and fluid SeO_4^{-2} into SWI	81	62	38
		Urea and fluid SeO_4^{-2} combined fluid into SWI	110	71	29
Heading	Flood water	Selenium enriched urea granules	105	21	79
		Urea granules and fluid SeO_4^{-2} into floodwater	113	92	8
		Urea granules and fluid SeO_4^{-2} into SWI	118	88	12
		Urea and fluid SeO_4^{-2} combined fluid into SWI	107	79	21
	Pore water	Selenium enriched urea granules	109	9	91
		Urea granules and fluid SeO_4^{-2} into floodwater	95	89	11
		Urea granules and fluid SeO_4^{-2} into SWI	85	89	11
		Urea and fluid SeO_4^{-2} combined fluid into SWI	89	64	36

[1] SWI- Soil water interface; [2] recovered ^{75}Se as a percentage of ^{75}Se added to HPLC column; [3] Se species as a percentage of column recovered ^{75}Se.

Table 4. Fertilizer selenium (mg Se kg^{-1}) in soils with depth at the end of the study (n=4; means ± SD).

Application time	Fertilizer application method[1]	Soil Depth 0-2 cm	 2-5 cm (mg Se kg^{-1})	 5-10 cm
Tillering	Selenium enriched urea granules	0.63 ± 0.27	0.08 ±0.12	0.03 ± 0.03
	Urea granules and fluid SeO_4^{-2} into floodwater	0.48 ± 0.00	0.04 ±0.07	0.04 ± 0.02
	Urea granules and fluid SeO_4^{-2} into SWI	0.70 ± 0.00	0.06 ± 0.11	0.05 ± 0.03
	Urea and SeO_4^{-2} combined fluid into SWI	1.12 ± 0.00	0.10 ± 0.11	0.05 ± 0.03
Heading	Selenium enriched urea granules	1.24 ±0.00	0.13 ±0.01	0.04 ± 0.04
	Urea granules and fluid SeO_4^{-2} into floodwater	1.10 ± 0.00	0.21 ± 0.08	0.11 ± 0.03
	Urea granules and fluid SeO_4^{-2} into SWI	0.9 ± 0.26	0.20 ± 0.06	0.05 ± 0.02
	Urea and SeO_4^{-2} combined fluid into SWI	1.03 ± 0.00	0.11 ±0.01	0.06 ± 0.06

[1] SWI- Soil water interface.

DISCUSSION

The adequate supply of nitrogen in submerged soils to plants is required to promote rapid growth, increase the size of leaves and grains, increase the number of spikelets per panicle and the percentage of filled panicles and increase the protein content of grains (Datta, 1981). In this study, a significant higher grain dry mass was found in rice grains when urea was applied at mid-tillering compared to application at heading stage (Table 1). This suggests nitrogen fertilization before heading is important for the maximising grain yields (Bahmanyar and Mashaee, 2010; Fageria, 2007). This higher dry mass when fertilizers were applied at the mid-tillering stage may be due to increased number of tillers. Tillering number is associated with panicle number, hence, panicle number is an important yield component in rice and is determined during the vegetative growth stage (Datta, 1981) and protein synthesis and grain filling (Datta, 1981).

Fertilizer Se concentrations in floodwaters and pore water samples were found to decrease very rapidly after fertilizer application to soils (Figure 1). Selenium concentrations have previously been observed to decrease with time in submerged soils following SeO_4^{-2} addition (Jayaweera and Bigger, 1996; Li et al., 2010; Mikkelsen et al., 1989). Selenate is only weakly sorbed by soils (Barrow and Whelan 1989; Premarathna et al. 2010; Premarathna et al. 2011), so the reduction in water concentrations was likely not a result of strong SeO_4^{-2} adsorption to soil particles. The rapid disappearance of fertilizer Se from floodwater and pore water was likely due to reduction of added SeO_4^{2-} to SeO_3^{-2}, followed by strong sorption of this Se species and/or further reduction into non-labile Se pools such as elemental Se or selenides

(Mikkelsen et al. 1989; Myneni et al. 1997; Premarathna et al. 2010). The presence of SeO_3^{-2} in the floodwater and pore water (Table 3) after fertilizer application is proof that this reduction occurred quite rapidly for all treatments except the SeO_4^{2-}-enriched urea treatment. The rapidity of the disappearance of Se from both floodwaters and pore waters indicates that SeO_4^{2-} can apparently be quite quickly reduced (with days) in a rice paddy system - redox reactions of Se species are therefore not always slow (Collins et al. 2006). Fertilizer SeO_4^{2-} can therefore be regarded as a very transient redox species in this system. The SeO_3^{-2} produced by reduction of fertilizer SeO_4^{2-} can also be regarded as a transient species, at least in terms of plant availability, as it strongly sorbs to the sold phase and transforms quickly into non-exchangeable forms in the soil (Barrow and Whelan, 1989; Premarathna et al. 2010). Hence penetration of Se into the soil profile was minimal (Table 4).

Selenate-enriched urea granules were found to be the most effective fertilizer to supply Se to the rice plants and to biofortify the grains (Table 2). We considered that the enhancement of Se uptake by rice due to co-application of urea could be related to interactions between N nutrition and uptake of micronutrients. Plant roots exposed to high NH_4^+/ urea have the potential to absorb more anions from solution (Beusichem et al. 1988; Rayar and Tang, 1977; Thien and McFee, 1970) due to (i) cation and anion balance within the cell; (ii) pH changes during the transfer of anions across the root symplast; (iii) changes in root morphology (Rehm and Caldwell, 1970; Riley and Barber, 1971; Thien and McFee, 1970); and (iv) positive effects of amino acid and high transporter protein activities that help in absorption, translocation and loading of nutrients to the grains (Erenoglu et al. 2010) . However, the other treatments which we used applied similar amounts of urea, and the benefit of Se-enriched urea granules therefore appears to be a result of co-placement of urea and SeO_4^{2-}.

The effectiveness of SeO_4^{2-}-enriched urea granules could be related to the ability of this formulation/placement to maintain higher concentrations of Se in floodwater or soil pore water. Certainly it appears from the data in Table 3 that reduction of fertilizer SeO_4^{2-} in a rice paddy is inhibited when co-applied in a urea granule, and therefore SeO_4^{2-} concentrations in floodwater and soil pore water were much higher with this formulation, for at least 1 day after fertilizer application (Figure 2 and Table 3). The significantly higher Se concentrations in rice grain fertilized by SeO_4^{-2}-enriched urea may be due to the lower solid phase partitioning and increased lability (i.e. potential bioavailability) of the SeO_4^{-2} species in submerged soils (Premarathna et al. 2010). However, this inhibition of reduction is evidently not sufficient to maintain higher SeO_4^{2-} concentrations in floodwater over time.

The higher supply of fertilizer Se to rice grains from the SeO_4^{-2}-enriched urea granule treatment (Table 3) suggests co-localisation of SeO_4^{-2} in or on the urea granule is important in restricting (short-term) the conversion of SeO_4^{-2} to SeO_3^{-2} in paddy soils. After application of urea granules to floodwater, they sink to the SWI and quickly dissolve and are hydrolysed, so the area around them becomes a zone of high pH and ammonia concentration; the reaction is catalysed by the enzyme urease (Kirk 2004). This zone generally becomes neutralized in most submerged soils as the ammonia converts to form NH_4^+ (Vlek and carter 1983). Rachhpal-Singh and Kirk (1993a,b) suggested following broadcast applications of urea on rice field floodwater the spread of urea and NH_4^+ into the soil is typically only a centimetre or two in a week. Ammonium is a toxic compound to microorganisms that mediate redox reactions in rice paddies. The reduction of SeO_4^{-2} may be restricted near urea granules due to the toxic effects of NH_4^+ on microorganisms. This possible influence of NH_4^+ on the reduction of SeO_4^{-2} might not have been as strong for the other fertilizer treatments as the Se and urea (granule or fluid) were either not co-located or in the case of the combined urea-Se solution, were more dilute, limiting the toxic effect of ammonium.

Another possibility explaining the prevalence of SeO_4^{2-} over SeO_3^{-2} in the waters of the granular SeO_4^{-2}-enriched urea treatment could be the effect of NO_3^- acting as a substitute electron acceptor. Rice can actively pump air into its roots resulting in a rhizosphere that can be more oxidized relative to the bulk soil (Lin et al. 2003). Ammonium generated from the hydrolysis of urea may have been converted to NO_3^- by nitrification in the rhizosphere (Kirk et al. 1993). As NO_3^- was produced from NH_4^+, microorganisms may have substituted this NO_3^- as an electron accepter in preference to SeO_4^{-2}, limiting its reduction to SeO_3^{-2} and/or other reduced species. However, the effect would likely be ephemeral as when NO_3^- production from NH_4^+ was complete, reduction of SeO_4^{-2} would resume. Murse and Kimura (1997) reported the addition of NO_3^- into submerged soils can oxidise elemental sulfur to sulfate. Further research needs to be undertaken to elucidate the mechanisms(s) for the short-term restriction of SeO_4^{-2} reduction in soils following application of SeO_4^{-2}-enriched urea granules.

In conclusion, application of SeO_4^{-2}-enriched urea granules to the floodwater was the best placement for Se fertilizer tested for the biofortification of Se grains. Co-localisation of SeO_4^{-2} with urea in soils near roots may be important in restricting (short-term) the conversion of SeO_4^{-2} to SeO_3^{-2} in the floodwater and at the SWI.

Coating or incorporation of SeO_4^{-2} onto urea is simple and inexpensive, and as farmers often apply a side dressing of urea to floodwaters during crop growth, the practice is a simple and extremely effective way to supply Se to crops.

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CHAPTER 7

7. CONCLUSIONS AND FUTURE DIRECTIONS

7.1 CONCLUSIONS

The oxidation of Se(0) in soils in laboratory-based incubation experiments was found to be slow and would not provide sufficient bioavailable Se to rice plants during growth during the submerged and subsequent aerobic phases (Premarathna et al. 2010). Added Se(0) did not readily oxidize and provide labile SeO_3^{-2}, even after incubation for 60 d under aerobic conditions. A maximum of 22% of the added Se(0) was oxidized to SeO_3^{-2} and remained labile in the MI soil, with corresponding values in the other two soils of <10%. These low oxidation rates for rice soils are in line with those reported in the literature for other soils (Dowdle and Oremland, 1998; Losi and Frankenberger, 1998; Zawislanski and Zavarin, 1996). Therefore, Se(0) is not suitable for fertilization of lowland rice soils and should be excluded from further studies.

The lability of SeO_3^{-2} was low in all submerged soils, probably due to continue non-reversible sorption reactions ("fixation") in soil as shown by Barrow and Whelan (1989b), and/or reduction of the added Se (as SeO_3^{-2} or SeO_4^{-2}) to Se(0) under the low-redox conditions favouring this reaction. These results support those of Mikkelsen et al. (1989), who found that added SeO_4^{-2} disappeared quickly from a soil solution under flooded rice cultivation and formed SeO_3^{-2}, which subsequently also disappeared from solution. Mikkelsen et al. (1989) suggested that this could have been due to (i) reduction to Se(0), (ii) sorption of SeO_3^{-2} by soil minerals or organic matter, or (iii) incorporation of SeO_3^{-2} into the microbial biomass. Incorporation into the microbial biomass is an unlikely explanation because the disappearance of oxidized Se forms was not affected greatly by the addition of OM, which would have stimulated microbial activity (Mikkelsen et al., 1989). Our data on labile Se pools, which take account of sorbed Se species, indicate that non-reversible sorption or reduction to Se(0) were the most likely causes. However, the disappearance of SeO_3^{-2} in a short time after application, as shown in Chapter 4, was mainly due to strong non-reversible sorption in the BT soil. Data from the supporting experiment, explained in the fourth chapter, suggest that added SeO_3^{-2} partitioned strongly onto the soil solid phase and this is likely to have occurred within a few minutes, as observed by Neal and Sposito (1989). According to the study of Saha et al. (2004a), SeO_3^{-2} adsorption is a very fast reaction and the adsorption process involved two steps: the first is complete in 0.083 -0.5 hr, and then there is a relatively slow second reaction. 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 (Barrow and Whelan 1989c; Essington 2004; Mandal et al. 2009). Therefore, our data suggests that use of SeO_3^{-2} to fertilize paddy rice is not advisable as the lability of applied SeO_3^{-2} reduces rapidly, especially in soils with a high amounts of Al, Fe, and Mn oxides and organic matter.

The dominant form of labile Se in the aerobic (FC) soils was generally SeO_4^{-2}, as would be expected from the prevailing pH and redox conditions in FC soils (Ashworth et al. 2008; Blaylock and James 1994; Dhillon and Dhillon 2004; Tokunaga et al. 1998). Under submerged conditions, changes in the lability of SeO_4^{-2} were soil specific. In the BT soil, labile SeO_4^{-2} disappeared relatively rapidly under submerged conditions. In the MI and KK soils, Se added as SeO_4^{-2} initially remained in the labile pool but declined to low values by 60 d. As the lability of Se decreased over time, this study highlighted the importance of Se application time. According to the above studies, application of Se prior to planting is not recommended as the Se availability will decrease rapidly and be low by the time plant roots are ready for Se uptake.

The effectiveness of applied Se (SeO_4^{-2} and SeO_3^{-2}) fertilizers, time of application, application method and soil water regime on the accumulation of Se in grains was examined using a laboratory pot trail with rice (Chapter 5). Selenium applied at the heading stage led to higher grain Se concentrations than Se applied at soil preparation (Chapter 5, Table 2). Perhaps, at heading stage, plants are more physiologically active and mobilizing nutrients to fill the grains with photosynthetic products faster. Also, by heading stage, plant roots were well developed and well distributed ready for nutrient uptake. Furthermore, Se applied at soil preparation had two weeks without plant growth and, by the time plants were introduced into the pots, Se added as SeO_3^{-2} would have been sorbed (likely non-reversibly) onto/into soil colloids/minerals and also SeO_4^{-2} may have been reduced (Chapter 3 and 4). By the time the root system of transplanted rice was ready for nutrient uptake, most of the added Se may have been converted to unavailable forms such as selenide (Se(-II)) or elemental selenium (Se(0)).

The SeO_4^{-2} fertilizer treatments were found to have the highest Se concentrations in grains while SeO_3^{-2} treatments led to the lowest grain Se concentrations (Chapter 5, Table 2). The highest grain Se concentration was found for SeO_4^{-2}-enriched urea granules applied at heading stage for all water treatments (Chapter 5, Table 2). Selenomethionine was found to be the major Se species present in grain tissues from the SeO_4^{-2}-enriched urea granule treatment (Chapter 5, Figure 1). This Se species has previously been reported to be the major Se species present in rice grains (Chapter 5).

Hence, the SeO_4^{-2}-enriched urea treatment did not cause the accumulation of different Se species (e.g., inorganic Se species) than those expected with a foliar or fluid SeO_4^{-2} fertilizer.

The higher Se accumulation in rice grain of the SeO_4^{-2}-enriched urea granule treatments may be due to several possible mechanisms. The presence of N fertilizer (urea, mostly in the form of NH_4^+ in rice soils) may have influenced absorption of Se from the root mat at the SWI, as after application to floodwater the Se-coated urea granules sank onto the exposed rot mat at the SWI. Plant roots exposed to high NH_4^+ or urea have the potential to absorb more anions (Kirkby and Mengel 1967; Thien and McFee 1970). Higher leaf Se concentrations in the submerged treatments receiving Se-enriched urea granules, compared to those receiving fluid Se, lends support to the idea of efficient translocation of Se when Se is co-applied and co-located with urea. It should be noted however, that the fluid Se treatments also received urea, although in this case the Se and urea were not co-located. A third hypothesis was that Se applied with urea may have reacted to form SeU in the granules or at the soil/floodwater interface, and this enhanced the absorption of Se by the roots.

We tested the hypothesis that SeU could be taken up by rice plants (Chapter 5, experiment 2), where pure SeU was applied to flood waters and persistence in floodwater determined, as well as translocation to the xylem of rice plants growing under submerged conditions. While addition of selenourea resulted in higher concentrations of Se in floodwater (compared to SeO_4^{-2}-enriched UAN and urea) 1 day after application, it did not persist and Se was not detectable in floodwater 10 days after fertilizer application (Chapter 5, Table 6). Concentrations of Se in rice xylem sap was highest with selenourea 1 day after fertilizer application, but 9 days later Se concentrations in rice xylem sap were highest with SeO_4^{-2}-enriched urea granule treatment.

The effectiveness of urea treatments on the accumulation of added fertilizer Se in submerged and drained soils was further examined in Chapter 6. A significant higher grain dry mass was found in rice grains after the application of nitrogen fertilizers at mid-tillering than heading stage (Chapter 6, Table 1). There were no signs of nitrogen deficiency observed on rice plants following fertilizer treatments applied at the mid-tillering or heading stages (e.g. yellow discolouration of leaves). The significant higher grain dry mass found in the urea control treatment applied at mid-tillering compared to heading suggests nitrogen fertilization before heading development was important for the increased dry mass of grains (Bahmanyar and Mashaee 2010; Fageria 2007).

The SeO_4^{-2}-enriched urea granule treatment was found to supply the highest amount of Se directly from the fertilizer to rice grains when applied at the mid-tillering or heading stages (Chapter 6, Table 2). The total Se concentrations in rice grains following the application of SeO_4^{-2} fluid in the presence of urea granules or urea fluid was found to be 33-49% lower than when applied as the SeO_4^{-2}-enriched urea granule (Chapter 6, Table 2). The majority of Se in floodwater and pore water samples was identified as SeO_4^{-2} 1d after application of the SeO_4^{-2} enriched urea granule fertilizer treatment (Chapter 6, Table 3). For other fertilizer treatments the majority of Se in floodwater and pore water samples was identified as SeO_3^{-2}. The significant higher Se concentrations in rice grain from use of SeO_4^{-2}-enriched urea granules may have been due to the lower solid phase partitioning (i.e. higher total SeO_4^{-2} concentrations in floodwaters and pore waters) and increased lability (i.e. potential bioavailability) of the SeO_4^{-2} species in submerged soils (Premarathna et al. 2010a).

The high efficiency of SeO_4^{-2}-enriched urea granules suggests co-localisation of SeO_4^{-2} with urea in soils near roots may be important in restricting the conversion of SeO_4^{-2} to SeO_3^{-2} in soils. After application of urea granules to soil they dissolve and are hydrolysed so that the area directly around them becomes a zone of high pH and ammonia concentration. This may restrict microbial activity and hence restrict reduction reactions close to the granule. In addition, nitrate produced from the NH_4^+ through nitrification may have substituted around the granules as a microbial electron acceptor for SeO_4^{-2}, thus allowing higher concentrations of SeO_4^{-2} in floodwater/pore water (Chapter 6, Figure 2 and Table 3). Higher concentrations of nitrate were not detected in floodwaters of treatments fertilized with SeO_4^{-2}-enriched urea granules (Chapter 6, Figure 3), but the effect may have been localised to around the granule and not detectable in bulk solutions. However, the maintenance of higher SeO_4^{-2} concentrations in floodwater were ephemeral, and Se concentrations in floodwater and pore water declined rapidly to background concentrations (Chapter 6, Figure 2).

It is evident further research needs to be undertaken to elucidate the mechanisms(s) for the short-term minimisation of SeO_4^{-2} reduction in soils following application of SeO_4^{-2}-enriched urea granules.

7.2 PRACTICAL IMPLICATIONS

Some practical implications for Se fertilization of paddy rice from the work described in this thesis are:

i. Oxidation of Se(0) was quite slow in the experiments, even under aerobic conditions, so it is unlikely that Se(0) would be able to provide useful amounts of Se to paddy rice. The addition of SeO_3^{2-} or SeO_4^{2-} to submerged soils also resulted in significant fixation of the added Se (>80 % of the added Se) during the experiment, again suggesting that pre-plant addition of SeO_3^{2-} or SeO_4^{2-} to soils may not be an effective way to fertilize paddy rice;

ii. Selenate was found to be the best Se species for enhancing Se accumulation in rice growing under submerged soil conditions. Selenate-enriched urea in a granular form was the most efficient Se fertilizer for increased Se accumulation in grain for lowland rice;

iii. Selenium application at the heading/tillering stage was the best time for Se application to lowland rice compared to Se application pre-planting; and

iv. Application of SeO_4^{-2}-enriched urea granules to the soil water interface was the best placement for Se fertilizer tested in this thesis for the biofortification of Se grains for improved human and animal nutrition. Coating or incorporation of SeO_4^{-2} onto urea is simple and inexpensive, and as farmers often apply a side dressing of urea to floodwaters during crop growth, the practice is a simple and extremely effective way to supply Se to crops.

7.3 SUGGESTIONS FOR FUTURE WORK

The following are suggested areas for future research:

i. As shown in the first incubation study, partitioning and lability and hence potential Se availability in soils is influenced by soil properties, such as redox, pH, clay mineralogy and OM content. Therefore, pot or field experiments need to be undertaken that examine the role of soil properties on the availability of added Se fertilizers to rice;

ii. Selenium speciation in flood and pore waters with time following fertilizer application needs to be examined to elucidate the role of short and long-term partitioning and redox reactions on the plant availability of fertilizer Se;

iii. Further research needs to be undertaken to elucidate the mechanisms(s) for the short-term restriction of SeO_4^{-2} reduction in soils following application of SeO_4^{-2}-enriched urea granules;

iv. The uptake of Se from fertilizers may depend on the variety of rice used locally throughout the world. Hence, further research should be undertaken to examine the influence of rice varieties on the uptake and accumulation of Se following application of SeO_4^{-2}-enriched urea granules to soils with varying physical and chemical properties;

v. The pot experiments were undertaken in growth cabinets with a controlled atmosphere with optimum temperature, R_H and water conditions. Results of the field studies cannot be expected to exactly replicate the pot studies, because pot study conditions are more specific to small soil volumes and may introduce artefacts due to enhancement of rhizosphere effects. Therefore, field experiments in various locations are essential for identifying the most appropriate fertilization rates before being recommended for commercial production and being made available to farmers; and

vi. More studies are required to gain a wide understanding of the positive and negative relationships of Se with other elements in fertilizers, floodwaters and soil pore waters.

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