Cadmium Enrichment in Paddy Rice Grain: Understanding the Effect of Soil Oxidation and Limiting the Risk through Soil and Foliar Treatments

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Abstract

Cadmium (Cd) is a non-essential element and agricultural soil contaminant (Friberg *et al.*, 1974). Cadmium accumulation in paddy rice grain is a human health risk, exacerbated by the fact that grains accumulate more Cd than the nutrients iron (Fe) and zinc (Zn), based on their relative soil concentrations (Chaney *et al.*, 1996), which enhances Cd bioavailability (McLaughlin *et al.*, 1999b). The enrichment of Cd in paddy rice grain occurs during soil oxidation, which accompanies pre-harvest drainage of the flooded paddy (Iimura, 1981b; Inahara *et al.*, 2007) and coincides with the grain-filling phase of rice growth.

The objectives of this thesis were to elucidate which soil processes are likely responsible for Cd accumulation and enrichment relative to Fe and Zn in paddy rice grain, and to determine whether soil amendments to paddy fields or foliar sprays to rice plants could be used to limit Cd accumulation and enrichment in paddy rice grain.

In Chapter 3 it is demonstrated that differential oxidation of sulfide minerals is not likely the process responsible for Cd enrichment in paddy rice grain. In Chapter 4 it is shown that when sulfide minerals form and then oxidize in a soil environment, this process in fact limits Cd solubility relative to Fe and Zn. This brings to light a new paradigm, that sulfide formation, prior to oxidation, is in fact the best-case scenario in terms of limiting Cd enrichment in paddy rice grain.

In Chapter 4 it is demonstrated that under conditions of sulfate-limitation (i.e. worst-case scenario for Cd enrichment), the Cd:Fe and Cd:Zn ratios in soil solution are lower for soil higher in Fe and/or Zn. However, in Chapter 5 it is shown that rice plants grown in soil amended with the same rates of Fe and/or Zn as in Chapter 4, produced grains with higher Cd:Fe and Cd:Zn ratios than controls. When Mn was added to soil, rice grains contained more Zn, and therefore the bioavailability of Cd was lower. When soil was amended with EDTA, rice grains had lower Cd:Fe and Cd:Zn ratios and therefore would pose a lesser risk to human health from Cd, whereas foliar sprays of EDTA or Zn had no effect on relative accumulation of metals in grains.

This thesis addresses major knowledge gaps in the area of Cd enrichment in paddy rice. It is suggested that the relative accumulation of Cd, Fe and Zn in paddy rice grain is most likely the net effect of the oxidation of sulfide minerals and concurrent cation competition during pre-harvest drainage. It is also shown that Cd accumulation in paddy rice grain may be greater for rice grown in soils higher in Fe and Zn in the presence of chloride, and that the risk may be lesser for soils high in Mn, or amended with EDTA. Further research is suggested to support these observations under a wider range of conditions.

Declaration

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- de Livera J, McLaughlin MJ, Hettiarachchi GM, Kirby JK, Beak, DG. Cadmium solubility in paddy soils: Effects of soil oxidation, metal sulfides and competitive ions. Science of the Total Environment 2011; 409: 1489-1497

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Thesis Structure

This thesis is organized into six chapters that include two manuscripts accepted for publication in international journals (Chapters 3 and 4). The introduction and literature review (Chapter 1) outline the current understanding and knowledge gaps in determining the fate and behaviour of Cd in soils, availability to rice grains and potential impact on human health. Chapter 2 contains the thesis aims and objectives. Chapters 3 and 4 present the research findings of investigations into the solubility, lability and hence the potential availability of Cd in submerged and drained rice paddy soils. Chapter 5 presents the findings of research into limiting Cd accumulation in rice grain through the addition of competitive ions (Fe, Mn and Zn) into soils, foliar applied Zn, foliar applied EDTA and soil applied EDTA. The final chapter (Chapter 6) outlines the major findings and conclusions of the thesis and potential directions for future research.

1 Introduction and Literature Review

1.1 Cadmium in soils

Cadmium is a naturally occurring element that falls within Group IIB of the periodic table. It is a non-essential element in the terrestrial environment (McLaughlin *et al.*, 1999b) that is known to affect human and animal health (Garrett, 1995), e.g. renal dysfunction, osteoporosis and cancer (Garrett, 1995; Nordberg, 1995). The Eh-pH species distribution diagram for Cd (Figure 1-1) shows that in sub-oxic to oxic environments at near-neutral and acidic pH values, Cd is found in solutions as the divalent cation, Cd(II). In sub-oxic to oxic environments at neutral to alkaline pH values, Cd can be present as cadmium carbonate ($CdCO_3$) and cadmium hydroxide ($Cd(OH)_2$) species. Under reducing conditions in sulfate-rich environments, Cd can be present as cadmium sulfide (CdS) (Brookins, 1986).

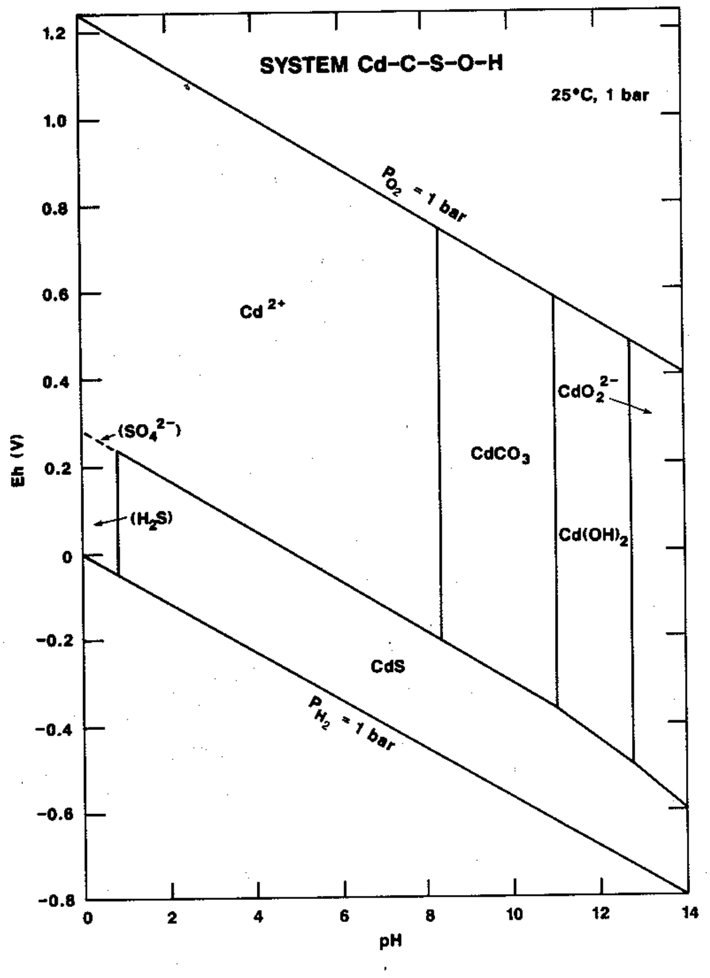

Figure 1-1. Speciation of Cd-S-C-O-H with Eh and pH (Brookins, 1986; Brookins, 1988).

Cadmium is naturally present in air, water and soil. Human beings are exposed to Cd from all of these sources, however exposure through soil (i.e. foodstuffs grown in contaminated soil) is the most significant pathway (Friberg *et al.*, 1974; Hutton, 1982; Nordberg, 1995). Cadmium accumulates in soil due to both natural (e.g. weathering) and anthropogenic (e.g. industrial) processes. Geogenic Cd in the earth’s crust is present at a concentration of 0.1 to 2.0 mg/kg (Garrett, 1995; McLaughlin *et al.*, 1999b). In soils the average concentration of Cd is 0.2 to 0.3 mg/kg (Garrett, 1995), but can range from 0.01 to 7.0 mg/kg (Bohn *et al.*, 2001).

Concentrations of Cd in a plant-bearing soil at pH 6.0 above 1.0 mg/kg dry weight are labeled contaminated by the UK (HPA, 2007) and Australia (Warne *et al.*, 2007).

Cadmium can be found at elevated concentrations in soils as a result of anthropogenic activities; for example, Thai paddy fields downstream of an actively mined Zn mineralization have been found to have soil Cd concentrations ranging from 0.5 to 284 mg/kg (Simmons *et al.*, 2005). Typical concentration of Cd in the soil solution of contaminated soils range from 300 to 400 μg/L (Kabata-Pendias and Pendias, 1984), whereas in uncontaminated environments typical Cd concentrations range between 0.2 and 6 μg/L (Bohn *et al.*, 2001). At low concentrations, pollutants disposed in soils may return to their natural biogeochemical cycles and be rendered benign by aging reactions to less available forms. However, when the rate of pollutant deposition to soil exceeds the rate at which this aging process can occur, Cd can accumulate in soil at potentially toxic concentrations (Bohn *et al.*, 2001). Atmospheric exposure of soils to Cd can arise from mining and smelting of metals, combustion of fossil fuels and waste incineration (Friberg *et al.*, 1974; McLaughlin *et al.*, 1999b). Cadmium can accumulate in soils due to contact with solid or liquid wastes from electroplating, plastics manufacturing, mining, alloy preparation, photography, rubber curing and use of fungicides and pesticides (Gimeno-Garcia *et al.*, 1996; Kirkham, 2006). Cadmium is also present in many man-made objects which, when discarded inappropriately, can contaminate soil. These include household appliances, automobiles, batteries, paint pigments, agricultural implements, airplane parts, industrial tools, hand tools, nuts, bolts, screws and nails (Kirkham, 2006).

In agricultural soils, the main routes of Cd contamination are via the application of commercial fertilizers (Cd is an impurity in phosphate (PO_4^{3-}) rocks), pesticides, manures, and sewage waste including effluents and biosolids (Friberg *et al.*, 1974). In many Australian soils receiving phosphatic fertilizers, more Cd is currently added than is removed in agricultural produce or by leaching (McLaughlin *et al.*, 1996). All of these anthropogenic activities can enrich Cd concentrations in soils, making soil the main source of exposure to Cd for animals and humans. Specifically, agricultural soils can provide a main exposure pathway to animals and humans, via the food that grows on them and accumulation of Cd into the edible tissues, see Figure 1-2.

Agricultural soils in Europe range in Cd concentration from 0.1 to 2.0 mg/kg (Hutton, 1982), while in New Zealand the range in Cd concentration has been found to be from 0.04 to 1.53 mg/kg (McLaughlin *et al.*, 1996). In Chinese cropping soils, the concentration of Cd ranges from non-detect (n.d.) to 1.47 mg/kg, whereas for paddy and uncultivated control soils the range is n.d. to 0.90 and n.d. to 1.76 mg/kg, respectively (Wong *et al.*, 2002).

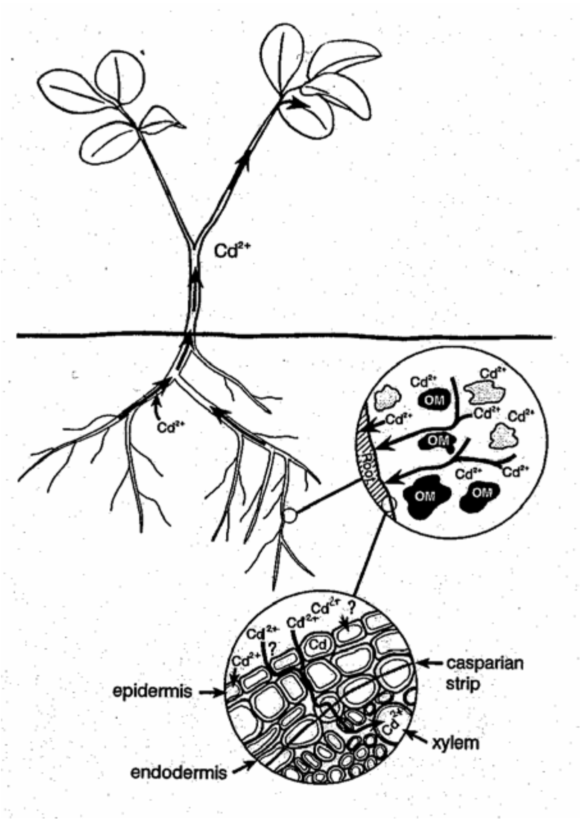

Figure 1-2. Plant uptake of Cd (McLaughlin and Singh, 1999).

Cadmium contamination of agricultural soils by addition of sewage waste, manures or by smelter emissions, is often associated with zinc (Zn) co-contamination. As a result, Cd in soil is mostly found with Zn as a co-contaminant, at a minimum ratio of 1:100 (Chaney *et al.*, 1996; Friberg *et al.*, 1974). This is illustrated by a soil survey conducted in the dominant rice producing area of Zhejiang Province (specifically the Hangzhou-Jiazing-Huzhou Plain) in South-East China (Liu *et al.*, 2006). The area is agricultural with rice as the dominant crop grown in paddy soils. It is known to be polluted by metals and it was found that Cd concentrations in the soil ranged from 0.01 to 0.65 mg/kg, and Zn from 13.51 to 246.44 mg/kg. In Australian agricultural soils, the regulatory limit for soil Cd concentration (following any biosolids amendment) is 1.0 mg/kg (Warne *et al.*, 2007). If Cd-contamination is derived only from addition of PO_4^{3-} fertilizers however, Zn will not accompany the Cd at such ratios, a factor which greatly influences plant uptake of Cd (Section 1.6.21).

1.2 Cadmium in the human body

The bioavailability of Cd in foods to humans depends upon its speciation within the food, dietary composition and nutritional status of the individual. There is a strong link between the presence of micronutrients (e.g. Fe, Zn, etc.) in plants and humans and the uptake and toxicity of Cd in the human body (McLaughlin *et al.*, 1999b). Human trials have shown that adequate Fe nutrition inhibits Cd absorption, and population studies have shown that adequate Zn nutrition can inhibit Cd absorption (McLaughlin *et al.*, 1999b). There is also evidence to suggest that protein and calcium (Ca) in foods can inhibit human Cd-absorption (Friberg *et al.*, 1974).

In western societies where such micronutrient deficiencies (e.g. Fe and Zn) are uncommon, Cd-related health effects are rarely seen. However, as Cd concentrations in agricultural soils increase over time with continued anthropogenic additions, health effects in western societies may be observed in the future. In Australia, potato tubers from some areas have recently been found to contain Cd in excess of the maximum permitted concentration of 0.10 mg/kg fresh weight (McLaughlin *et al.*, 1995). Cadmium may become a health problem in Europe, as modeling has shown that in the next 100 years, Cd levels in European soils could increase by up to 54% (Hutton, 1982). Rice is a major food source for a large proportion of the world's population and hence supplies a large proportion of their essential micronutrients and is a potential major route for human exposure to Cd. Those at greatest risk from Cd-contaminated rice are subsistence rice farmers (Chaney *et al.*, 1996). This is because subsistence on rice results in moderate to severe Ca, Fe and Zn deficiency, which greatly increases Cd absorption and retention by humans (Chaney *et al.*, 1996; McLaughlin *et al.*, 1999b).

The health problems caused by excess Cd in humans are well documented. The consumption of rice with elevated Cd concentrations has been shown to be responsible for 'Itai-Itai' disease which arose in Japan in the 1960s (Nordberg, 2004). Even today, rice is the leading source of Cd burden for the Japanese population (Tsukahara *et al.*, 2003). The ill-effects of Cd exposure have not only been seen in Japan. In 1998, Chinese population groups were studied with regard to Cd exposure through rice consumption, with the finding that long-term exposure of the metal gave rise to decreased bone mineral density with accompanying increased prevalence of fractures and renal dysfunction (Jin *et al.*, 2002). Control groups had consumed rice containing a mean concentration of 0.05 mg/kg Cd, which was compared with groups consuming rice having mean concentrations of 0.48 mg/kg and 2.4 mg/kg. Renal dysfunction is known to be the 'critical effect' – the adverse effect that occurs at the lowest exposure concentration (Nordberg, 2003). A World Health Organisation (WHO) evaluation indicated that renal dysfunction could result from lifelong exposure of 140-260 µg/day (cumulative > 2000 mg) (Nordberg, 2004). Cadmium and Cd compounds have been recognized as carcinogenic to humans (Nordberg, 1995). The current provisional tolerable monthly intake of Cd was recently re-evaluated by the Joint Expert Committee on Food Additives and Contaminants of WHO and FAO, and is now 25 μg/kg body weight per month (WHO/FAO, 2010).

1.3 Cadmium uptake by food crops

The main risk of soil Cd to animals and humans is through exposure from agricultural soils through the food chain (Chaney *et al.*, 1996). Cadmium is accumulated in plants more readily than most other metals (e.g. copper, nickel or lead), and can be translocated into the edible parts before any signs of phytotoxicity (Li *et al.*, 1994; Tudoreanu and Phillips, 2004). In the example of rice, high concentrations of Cd in irrigation water exceeding 1 mg/L will adversely affect plant growth and grain yield, but at relatively lower concentrations of Cd the plant can grow healthily and produce brown rice containing more than 1 mg/kg Cd, an unacceptable level in terms of human health (Kitagishi and Obata, 1981). It is for this reason that Cd is one of the most important elements to consider in terms of food-chain contamination (McLaughlin *et al.*, 1999b).

Growing crops in uncontaminated soils, most foodstuffs will contain less than 0.05 mg Cd/kg. When grown in contaminated soil however, foodstuffs can accumulate more than 1.0 mg Cd/kg (Friberg *et al.*, 1974). The regulatory limit for Cd content in polished rice is set at 0.1 mg/kg by Food Standards Australia New Zealand (FSANZ, 2005) and at 0.4 mg/kg by the Food and Agricultural Organization of the United Nations (FAO/WHO, 2009).

1.4 Cadmium in rice

Paddy rice is the focus of this study due to its status as is one of the most important human dietary crops in the world, especially in Asia (Xu *et al.*, 2006). World rice production in 2004 was just under 610 million tonnes. At least 114 countries grow rice and more than 50 have an annual production of ≥ 100,000 tonnes. Asian countries are responsible for about 90% of total world rice production, with two countries, China and India, growing more than half the total crop (IRRI, 2007). Cadmium has been found in rice grains in several countries including Japan and China at concentrations that exceed the WHO guideline value for Cd (> 0.1 mg/kg) (Kirkham, 2006; Koshino, 1973). Cadmium-contaminated rice that was grown in paddies polluted by Zn mine effluent in Japan was found to be the cause of the ‘Itai-Itai’ disease that surfaced in the 1960s (Koshino, 1973). A study of Cd concentrations in Japanese rice grains produced between 1800 and 2000 found that between 1912 and 1925 the content of Cd in grain increased by 1.5 to 3 times, compared with 1902 values (Muramoto and Aoyama, 1990). This increase in rice grain Cd corresponded to the introduction of phosphate fertilization in Japan. The overall trend was one of sustained increase in Cd content of rice grains over the entire 200 year period.

In Australia, rice farms are mostly located in the temperate climate of the Murrumbidgee valleys of NSW and the Murray valleys of NSW and Victoria. There are approximately 2500 rice farms in Australia producing around 1.3 million tonnes of rice per year (RGA, 2007). The PO_4^{3-} rock used for nutrient fertilization in Australia mostly came from Nauru, a source with a Cd concentration of approximately 57 mg/kg (and 518 mg/kg Zn) (Muramoto and Aoyama, 1990).

1.4.1 Rice paddy soil chemistry

Rice can be grown either in paddies or on dry land (upland). The use of paddies results in greater productivity as the rice plant is tolerant to excess water and is able to outgrow weeds, creating a good habitat for animals that will kill pests. Cadmium accumulation in rice grains is reported to be less for paddy rice than for upland rice (Kashem and Singh, 2001).

Soil characteristics that are markedly different between flooded and upland cultivation include redox and soil pH (Figure 1-3). In a flooded soil, the redox potential stabilizes at approximately -200 mV, whereas redox in a well drained soil is typically above +400 mV. In acidic flooded soils, the pH rises towards neutral due to hydrogen-consuming redox reactions, whereas in alkaline flooded soils the pH becomes more acidic and drifts towards neutral due to the increasing partial pressure of CO_2 which affects $CaCO_3$ equilibria (Ponnamperuma, 1964). The same soils would remain at their original pH values if kept well drained (Figure 1-4).

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the thesis held in the University of Adelaide Library.

Figure 1-3. Changes in redox potential of a well-drained and a submerged soil with time (Ponnamperuma, 1964)

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This figure is included on page 10 of the print copy of
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Figure 1-4. Changes in pH of four soils kept submerged (Ponnamperuma, 1964)

The ability of paddy cultivation to limit Cd uptake by rice plants however, is not effective when the paddy is drained (at least 2 weeks) in preparation for harvest. Drainage of flooded paddy fields prior to plant maturity is common practice as it facilitates harvest (Honma and Hirata, 1977). Drainage of the paddy causes soil oxidation, which can render Cd more available for plant uptake during the grain-filling phase of growth (Chaney *et al.*, 1996; Honma and Hirata, 1977).

1.5 Limiting Cd accumulation in paddy rice grain

This thesis is focused on the problem of Cd accumulation in paddy rice grain, grown under flooded cultivation with pre-harvest drainage.

Cadmium uptake and accumulation by rice, like any other crop, is affected by soil and plant factors. Soil factors known to affect Cd accumulation in rice include Cd concentration, Cd source, metal sorption capacity of soil (cation exchange and sorption by organic matter, clays and Fe/Mn (hydr)oxides), macronutrients (e.g. P), micronutrients (Cu, Fe, Mn, Zn), chloride/salinity, silicon, pH, redox environment and mycorrhizal fungi (Ito and Iimura, 1975;

Kirkham, 2006; Zhang *et al.*, 2005). Plant factors known to affect Cd uptake include species, cultivar, plant part (root, leaf, grain/fruit), age, watering regime and metal-membrane interactions (McLaughlin *et al.*, 1999b).

1.6 Factors affecting phytoavailability of cadmium in soil and translocation to rice grain

1.6.1 Cadmium concentration in soil

Cadmium concentration in soil strongly influences Cd uptake by rice plants, although the extent of uptake is not linearly related to the soil concentration (Fujimoto and Uchida, 1979). Four-hour solution culture experiments on excised roots (exposed to between 0.02 and 10.24 mg/L Cd spiked with the radioisotope ^{115}Cd) have shown that below 0.15 mg/L Cd in solution, the rice plant 'actively absorbs' Cd from solution, but above 0.15 mg/L the plant actively depresses Cd uptake. Furthermore, it was noted in flooded pot experiments that although root absorption of Cd increased as solution Cd increased from 0 to 6.25 mg/L, the percentage of absorbed Cd that was translocated to the shoot decreased. The Cd concentrations used in these studies were unrealistically high, however other workers have had similar findings at lower concentrations in 10-day solution culture experiments on rice seedlings (Ito and Iimura, 1976b). As the Cd concentration in solution was increased from 1.0 to 8.2 μg/L, Cd absorption by plants increased, although from 8.2 to 30 μg/L the plant-absorption did not change. This was taken to represent the turning point from active to passive absorption.

Cadmium concentration in soil not only influences plant-uptake, but translocation to grain as well. In a submerged field trial in China across two seasons, soil Cd concentration was varied from approximately 1.85 mg/kg to 77.55 mg/kg, by adding $CdNO_3$. At low soil Cd concentrations the maximum Cd concentration in rice grains was 0.371 mg/kg, whereas at high soil Cd concentrations, the Cd in grain increased to 2.19 mg/kg (Yu *et al.*, 2006).

1.6.2 Cadmium source

Geogenic Cd (and Zn) in soil was found to be less available to ryegrass than soluble Cd salt added to soil and aged for 1 year (Almås and Singh, 2001). Two different soils were amended with either 0 or 4 % (w/w) organic matter (from pig manure), and tested at either 9 or 20 ºC. Soils were spiked with radioisotopes in order to distinguish between plant uptake of metal added to the soils and metal naturally present in the soils. At the start of the experiment the geogenic Cd in bioavailable soil fractions was 15% less than the added Cd salt, but after 1 year the geogenic Cd was only 8% less than added Cd salt, showing the effect of aging of the added Cd salt.

Cadmium added to soil as cow manure compost has been found to be more bioavailable to rice plants than Cd added as manufactured fertilizer in a 2.5 year field trial using both flooded and non-flooded cultivation (Kikuchi *et al.*, 2007). The fertilizer contained about ten times more Cd than the compost, further illustrating the importance of Cd source to plant bioavailability. In addition, it has been shown that when Cd is added to soil in a soluble form such as metal salt, pH is the controlling factor for Cd lability (Degryse *et al.*, 2004). When Cd was added to soil in less soluble forms such as smelting waste or sludge-amendment, Cd lability could not be predicted well by pH or organic matter content.

This source effect is critical for focusing research into how Cd behaves in the environment. Laboratory experiments that use metal salts as the Cd source must be interpreted cautiously compared to a field situation where Cd is present in the more likely Cd-sources of P-fertilizers, biosolids and mine wastes.

1.6.3 Long term aging of cadmium in soils

Aging or “long-term fixation” reactions in soils have been reported to decrease the solubility and bioavailability of Cd (Kirkham, 2006). In reduced paddy soils, free Cd(II) concentrations have been found to decrease over 6 months of incubation time after addition of soluble Cd salt (Cui *et al.*, 2008).

Cadmium bioavailability was shown to decrease in soil over 10 years in a field experiment which had been amended with Cd-containing biosolids (Granato *et al.*, 2004). Corn (*Zea mays* L.) was grown at the site at the cessation of biosolids application, and again 10 years later. The results showed that the total Cd concentration in the soil did not change significantly after 10 years, but the organic carbon content decreased by 26%, and the amount of Cd in the corn leaves and grain reduced by 44 and 50% respectively.

Aging reactions have also been found to decrease Cd bioavailability to wheat in a long term field study (Hamon *et al.*, 1998). In this study, an acidic clay loam (Armidale, NSW, Australia) was treated with superphosphate (SSP) in the field from 1948 until 1975. In 1975 the plots were split so that on one half the soil had received continued fertilization until 1998, while the other half received no more fertilizer; i.e. Cd in the fertilized soil was not able to age due to continuous disturbance. Wheat was grown (28 days) in a growth chamber pot study on soil from each treatment, with the finding that at a given concentration of total Cd in soil, Cd was slightly less phytoavailable in the soil that received no fertilizer since 1975. However, in comparison with other trace metals (e.g. As, Hg, Pb), Cd availability to plants declines only slowly with time, if at all (Jensen and Mosbæk, 1990) . In fertilized Australian soils for example, more Cd is currently added than is removed in agricultural produce or by leaching (McLaughlin *et al.*, 1996).

1.6.4 Soil pH

The pH of soils has been found to influence the lability and availability of Cd in soils (Degryse *et al.*, 2004; Kashem and Singh, 2004; Li *et al.*, 2005; McLaughlin *et al.*, 2006). Degryse *et al*. (2004) found that increasing soil pH from 5.3 to 7.2 decreases the amount of labile Cd (pool of easily exchangeable/desorbable and free Cd) in soils. Other research supports the findings that Cd bioavailability is higher in soils of low to neutral pH compared with soils of high pH (Kashem and Singh, 2004; Li *et al.*, 2005; McLaughlin *et al.*, 2006). The effect of pH on the lability of Cd in soils may be due to a number of factors including soil surface charge influence on the affinity of Cd for sorption sites (McBride, 1989), precipitation of $CdCO_3$ and $Cd(OH)_2$ at high pH, as well as changes in solution speciation (Figure 1-1).

The observed effect of pH on labile Cd agrees with observed changes in plant availability, i.e. low soil pH increases Cd absorption by rice plants (Chino, 1981; Kikuchi *et al.*, 2008; Li *et al.*, 2005; Smith, 1994). In pot experiments, Li *et al*. (2005) found that as pH decreased, Cd uptake increased for rice plants growing in acidic red soils from Southern China. Cadmium uptake by rice was also observed to increase as pH decreased from 8 to 5 in soil suspension experiments over a range of redox potentials from -200 to +400 mV (Reddy and Patrick, 1977). Kikuchi *et al*. (2008) found that increasing the soil pH from 6.1 to 6.9 decreased Cd uptake into rice grain in a field study under both upland and flooded cultivation (Kikuchi *et al.*, 2008). The increase in soil pH was brought about by soil amendment with MgO materials. Similarly, Li *et al*. (2008) found that increasing the soil pH from 4.5 to 6.5 (by amendment with 2 g/kg limestone) in a greenhouse pot experiment, reduced Cd uptake into rice grain from 2.39 mg/kg to 0.96 mg/kg. The relationship ($r = -0.86$, $p < 0.01$) between soil pH and available Cd (0.01 M $CaCl_2$ extractable) was defined as available Cd = -0.486 x pH + 4.30. Cadmium in grain could also be predicted from soil pH with the following equation: $Cd_{grain} = 0.372 – 0.038 \times pH$ ($r = -0.55$, $p < 0.01$). Cattani *et al*. (2008) showed that the addition of lime to field-grown rice can decrease Cd accumulation in the grain by approximately 25 %, and was more effective under ‘dry’ cultivation than flooded. However, raising the pH of soils may not be an ideal solution to Cd uptake by rice plants, because although liming acidic soil decreases Cd uptake, it can also significantly decrease rice yield due to liming-induced Zn-deficiency (Xiong and Lu, 1993).

1.6.5 Redox potential

Soil redox potential is well known to influence Cd solubility, lability and uptake by rice plants (Balistrieri *et al.*, 1992; Contin *et al.*, 2007; Guo *et al.*, 1997; Jung and Thornton, 1997; Kashem and Singh, 2004; Swarup *et al.*, 1992). Indeed, the key processes influencing solubilites of Cd and other trace metals; sulfur biogeochemical cycling (0) and sorption to Fe and Mn oxyhydroxides (1.6.7), are strongly influenced by redox potential.

As the redox potential decreases from positive to negative values, i.e. below -200 mV, metals become less soluble. For example, Calmano *et al.* (1993) showed that for a constant pH, Cu, Cd, Pb and Zn were solubilised to a lesser extent from contaminated sediment under reduced conditions compared to oxidized conditions. Carbonell *et al*. (1999) found that Cd and Ni were removed from solution as Eh was lowered from 500 to -250 mV in controlled Eh experiments at pH 5.0 or 7.0. Cornu *et al*. (2007) showed that lowering the Eh from +590 mV to -236 mV resulted in a steady decrease in Cd solubility, in a rewetting incubation experiment over ~21 days. The decrease in Cd solubility occurred concurrently with an increase in Fe and Mn and a decrease in sulfate, and was attributed to the precipitation of Cd-bearing sulfide minerals, while Fe and Mn oxyhydroxides concurrently underwent reductive dissolution. Cadmium solubility was also seen to decrease under lower Eh conditions in 4-

week bench-top experiments using a variety of soils ranging in pH from 5.2 to 9.6 and ranging in organic matter content from 0.8 to 3.87% (Swarup *et al.*, 1992).

In another example of low redox potential causing a decrease in Cd availability, in 24-week pot experiments (Kashem and Singh, 2004), flooding gave rise to lower exchangeable Cd while increasing the Cd concentrations of the Cd bound to Fe and Mn-(hydr)oxides and organic matter. These findings are supported by results from field studies by Jung and Thornton (1997) that showed under anaerobic conditions (flooding), Cd was mainly associated with Fe and Mn (hydr)oxides, and moved to the exchangeable fraction during oxidation periods. Guo *et al*. (1997) also showed that Fe and Mn (hydr)oxide associated Cd decreased when Eh decreased in 60-day sediment suspension experiments, while carbonate, humic-associated Cd and sulfide-associated Cd increased. The same effect was observed in a seasonally anoxic lake, where Cd concentration in the bottom waters was lowest during anaerobic periods (Balistrieri *et al.*, 1992).

Cadmium solubility may be affected differently by redox conditions in different soils. Ge *et al*. (2007) showed that flooding lowered Cd solubility in some soils, but increased it in others when three paddy soils were tested in incubation experiments. This was likely due to the differing iron oxide contents of the soils, i.e. a higher amount of iron oxide buffers Eh at a relatively higher level, whilst more H^+ is consumed to achieve reduction thereby causing higher pH. The different pH and buffering capacity of each soil in turn influences Cd dissolution and precipitation processes.

When reduced soils/sediments are oxidized as occurs during pre-harvest drainage of rice paddies, Cd solubility increases. For example, when reduced river sediments were oxidized in a laboratory experiment, Cd solubility increased (Cappuyns and Swennen, 2005). The kinetics was such that it took more than 48 hours for substantial release of Cd and Zn to occur. Due to the positive correlation between concentrations of Cd, Zn and SO_4^{2-}, an association of Cd and Zn with sulfide minerals in the reduced sediment was inferred. Cadmium behaved differently to Zn, in that it was not removed from solution during oxidation because of co-precipitation and/or re-adsorption to precipitated Fe oxyhydroxides. This is to be expected as other workers have shown in laboratory experiments using a precipitated Fe gel, that Cd is adsorbed to a lesser extent than Zn (Kinniburgh *et al.*, 1976). In another study, Cd solubility was found to be unaffected by oxidation in a controlled stirred suspension study where anoxic lake sediments were incubated for 30 days under reduced conditions before controlled oxidation by addition of air (Miao *et al.*, 2006). Oxidation did not affect Cd solubility, whereas the solubility of other elements (Al, Ca, Mg, Pb, and Zn) increased. The different result in this study may be due to the prolonged oxidation period which occurred in 50 mV increments (1 week incubation at each level) from -200 mV to +500 mV.

The ability of flooding to decrease Cd solubility and extractability suggests that anaerobic conditions should also reduce Cd uptake by rice plants. Previous research has shown that Cd uptake by rice plants was greater for upland than flooded cultivation in field experiments (Iimura, 1981a). In soil solution laboratory experiments, Cd uptake into roots and shoots was less at a redox potential of -200 compared to +200 mV when rice was grown for 10 days at pH 5, 6, 7 and 8 (Reddy and Patrick, 1977). Other workers' pot experiments also found that the Cd concentrations of rice leaves and grains was lower under submerged conditions, attributing the effect to formation of CdS in the soil (Bingham *et al.*, 1976; Iimura and Ito, 1978; Iimura *et al.*, 1977; Ito and Iimura, 1975; Kashem and Singh, 2001). Ito and Iimura (1975) found that sulfide formation increased abruptly below -130 mV, coinciding with a decrease in available Cd. Bingham and Page (1975) also detected no SO_4^{2-} in soil solution at harvest in flooded pots as compared with 4 to 20 meq/L in non-flooded pots, indicating that sulfur was present mostly as reduced S (i.e., sulfide) in the flooded pots.

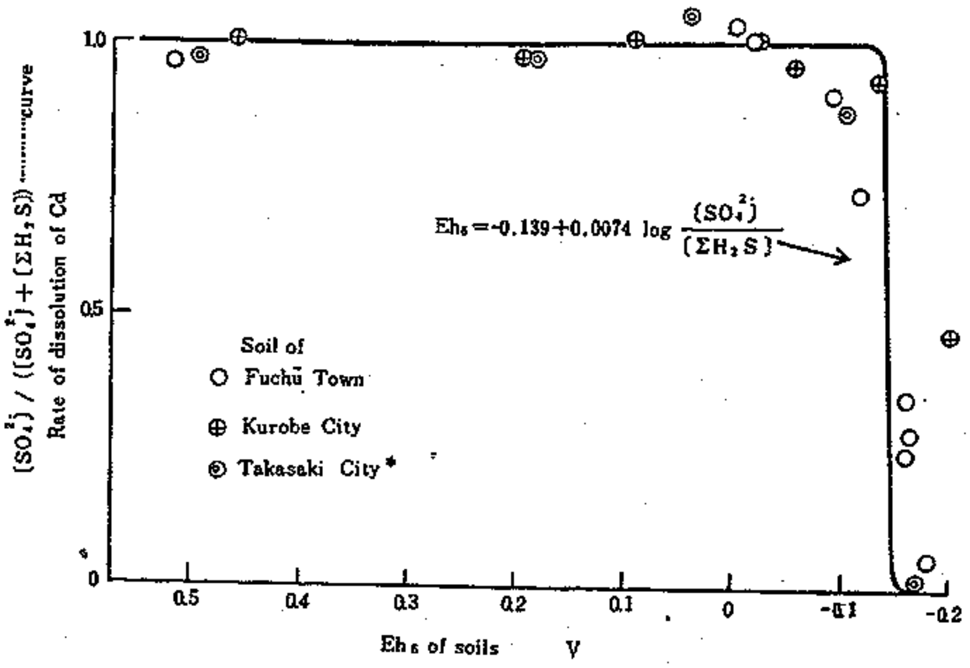

Figure 1-5. **Dissolution of Cd from soils as influenced by oxidation-reduction potential (Ito and Iimura 1975). Rate of Cd dissolution is indicated by circle symbols. The theoretical relationship between Eh and reduction of sulfates to sulfides is indicated by the solid line. * contaminated by the refinery in Annaka City.**

A contributing factor to the observations of lower Cd uptake under flooded conditions may be low root metabolic activity (Chino and Baba, 1981). When Cd and Zn were applied to subsurface soil layers in flooded pots, the metals were less readily translocated in rice plants than when Cd and Zn were applied to the surface soil. In both treatments, Cd and Zn were not precipitated in the soil, rather absorbed by the roots. Therefore, it was suggested by the authors that roots growing in the surface layer are active in the translocation of metals to the shoots, while the roots in subsurface layers are inactive due to their low metabolic activity induced by oxygen starvation.

Soil redox potential has been shown to affect Cd translocation to grain in the same way as total plant uptake, i.e. lower observed Cd concentrations in rice from paddy soils is attributable to the reaction of Cd with sulfide to form insoluble Cd sulfide under anoxic reduced conditions. For example, in pot experiments Cd was translocated less to rice grains under anaerobic flooded conditions compared to upland conditions (Arao and Ae, 2003). This was true for 5 rice cultivars grown to maturity in two soils, a Grey Lowland Soil and an Andosol (maximum Cd concentration in soil 0.9 and 7.4 mg/kg respectively). Under upland conditions the maximum Cd concentration in rice grains was 4.95 mg/kg compared to 0.157 mg/kg under submerged conditions. In a field study on rice grown on adjacent upland and paddy fields (Ae and Arao, 2002), Cd concentrations in rice grains were lower when soils were submerged (paddy - 0.02 mg Cd/kg; upland - 0.58 mg Cd/kg). Field trials in Italy on soil containing 0.96 mg Cd/kg have demonstrated that Cd accumulation in rice grain was at most 0.14 mg/kg when grown under submerged conditions relative to 0.25 mg/kg under irrigation only ‘dry’ conditions (Cattani *et al.*, 2008).

The influence of water management on Cd accumulation in rice grain is complicated by the fact that flooded soils are commonly drained before harvest. Pre-harvest drainage can markedly increase Cd uptake in rice grains (Daum *et al.*, 2001; Iimura and Ito, 1978; Kikuchi *et al.*, 2007; Minagawa *et al.*, 1974; Obata and Umebayashi, 1988; Tokunaga *et al.*, 1976). For example, Minagawa *et al*. (1974) found that Cd uptake by rice in field experiments increased significantly as a result of drainage, and Kikuchi *et al*. (2007) showed in field experiments that Cd accumulation in brown rice grain was less when flooded conditions were maintained throughout cultivation, at 1.7 μg/plant compared to7.2 μg/plant when pre-harvest drainage occurred. Iimura and Ito (1978) showed that draining their submerged rice plants at panicle initiation resulted in greater Cd concentrations. Tokunaga *et al*. (1976) compared Cd concentrations in rice grains between contaminated paddy fields that were either constantly flooded or drained before harvest, with the finding that eight times more Cd was translocated to the rice grain when the paddy field was drained before harvest. In field experiments by Daum *et al.* (2001), the concentration of Cd in rice grain was more than six times higher in fields undergoing a 19 day pre-harvest drainage as well as 10-days drainage during internode elongation, than in fields with standard 9-day pre-harvest drainage.

When pre-harvest drainage occurs, the timing of drainage can influence the extent of Cd accumulation in rice grain. Obata and Umebayashi (1988) found in pot experiments that Cd accumulation in brown rice could be reduced by keeping paddy soils submerged during the period from heading to milk-ripe growth stages. Takijima *et al*. (1973) found that when surface drainage occurred from panicle formation until ripening, up to 9.4 mg/kg Cd was translocated to grain, compared to 1.0 mg Cd/kg in the control where flooding was maintained throughout the growth period. Minagawa *et al*. (1974) found that the Cd concentrations in rice grain were minimized when drainage was delayed until 20 days after heading. Similarly, Arao *et al*. (2009) showed that Cd accumulation in rice grain was minimized when soil was kept flooded for three weeks before and after heading, as compared to drainage at heading, drainage three weeks before heading with re-flooding at heading, and drainage from two weeks after transplanting.

Cadmium and Zn uptake by rice are not affected in the same way by changing redox conditions. Bingham *et al*. (1976) compared flooded and non-flooded culture on the uptake of Cd and Zn from sludge-amended soil (Cd concentrations of 0.09 to 640 mg Cd/kg, Zn concentrations not reported) by mature rice plants in pot experiments, finding that Cd concentration in leaves was less under flooded culture whereas Zn concentration was unchanged. It must be noted however, that Zn was already present in the sludge, whereas Cd was added as $CdSO_4$ shortly before the experiment; metal source is known to influence the solubility and hence plant availability of metals (Degryse *et al.*, 2004). The findings of Tiller and Wasserman (1973) support the result for Zn, having found that flooding or not flooding did not affect Zn accumulation in rice shoots in any consistent manner across 4 soils, despite the fact that flooding significantly increased Zn solubility as determined by isotopic dilution.

Iimura and Ito (1978) had found similar results incubating soils at 30 °C under waterlogged anaerobic conditions; they found that Cd was less bioavailable under flooded culture but Zn was less affected by flooding. Specifically, as redox potential decreased to below -130 mV, the amount of soluble metals decreased abruptly. The effect was clearer for Cd than for Zn, which was attributed to a stoichiometrical shortage of sulfide to precipitate with the greater amount of Zn compared to Cd in the soils used. This would explain why Bingham *et al*. (1976) observed no change in Zn uptake between flooded and non-flooded culture. Other workers found that the decrease in Cd availability with flooding can be due to the formation of $CdCO_3$ rather than CdS, as was discovered by Cd K-edge X-ray absorption fine structure spectroscopy of limed Thai paddy soils (Khaokaew *et al.*, 2007).

Changes in the redox potential influence the way in which Zn interacts with Cd for translocation to rice grains. Under flooded conditions the presence of Zn at 110 mg/kg (relative to no Zn) decreased Cd translocation to grain, but under drained conditions Zn enhanced Cd translocation to grain when the soil Cd concentration was 1,140 mg/kg (Honma and Hirata, 1974). This result was obtained using high metal loadings at an approximate 10:1 Zn:Cd ratio. Other workers' findings contradict this, finding that under flooded conditions higher Zn concentrations in soil cause greater translocation of Cd to grain (Koshino, 1973). However, this result was obtained at a lower Zn:Cd ratio of 6:1, with Zn (soil concentrations of 150 to 600 mg/kg), Cu (50 to 200 mg/kg), nickel (Ni) (50 to 200 mg/kg) and Cd (2.5 to 10 mg/kg) were added as sulfates to sewage sludge which was then added to soil.

1.6.6 Sulfur biogeochemical cycling

The accumulation of Cd in paddy rice grain may be influenced by sulfur cycling in soils. It has been suggested that Cd is released from Cd sulfide phases present in flooded soils

(Iimura, 1981b; Inahara *et al.*, 2007) when oxidative dissolution occurs during pre-harvest drainage of the paddy fields (Iimura *et al.*, 1977; Ito and Iimura, 1975). Pure Cd sulfide (CdS) oxidizes more rapidly than pure Zn sulfide (ZnS), which may result in greater relative bioavailability, plant uptake and translocation of Cd during the grain-filling period of rice growth (Chaney *et al.*, 1996). However, this has not been verified in a field environment and a substituted series of Cd/Zn sulfides between CdS and ZnS with Fe substitution may be more likely to occur in nature than the end-members CdS or ZnS (Barrett and McBride, 2007; Duxbury *et al.*, 2005; Van den Berg *et al.*, 1998; Vaughan and Craig, 1978).

It has been found that pure CdS oxidizes more rapidly than pure ZnS (Barrett and McBride, 2007). In the field, Cd and other elements are unlikely to be present as pure phases but as mixed sulfide phases. For solid-solution (Cd, Zn) sulfide minerals, Cd and Zn have been found to be equally insoluble (when the Zn:Cd ratio in the starting solids was between 0 and 20), or Zn was preferentially released during oxidation (when the Zn:Cd ratio in the starting solids was between 20 and 100) (Barrett and McBride, 2007). Therefore, spiking soil with Zn to provide a greater proportion of Zn to precipitate as sulfide minerals may help to limit the solubility of Cd, and hence limit the concentration of Cd in rice grains.

However, further research is needed, as the employed range of Cd:Zn ratios by Barrett and McBride (2007) was not environmentally representative, nor was the effect of Fe substitution in the Cd-Zn solid-solution series considered, despite Fe being abundant in soil environments and known to enhance the rate of ZnS oxidation for ZnS present in a pyritic matrix due to galvanic interactions (Hita *et al.*, 2006).

The formation of metal sulfides in submerged soils is greatly dependent upon microbial reduction of SO_4^{2-} to dissolved sulfide (S^{2-}), a process which requires, and is often limited by, organic carbon availability (Du Laing *et al.*, 2009). When sufficient carbon is available, sulfide mineral formation may be limited by availability of sulfate. Sulfate reduction in flooded soils may be limited by an excess of Fe in the system (Du Laing *et al.*, 2009), which accepts electrons more readily than sulfate, during microbial respiration.

The formation of metal sulfide colloids in flooded soil solutions (Weber *et al.*, 2009) and in oxidizing sulfide suspensions (Barrett and McBride, 2007) has also been documented. This requires further investigation, as Cd, Fe and Zn present in colloidal metal sulfides would likely be unavailable for plant uptake due to particle size.

1.6.7 Iron and Mn oxyhydroxides

The affinity of metals for oxyhydroxides is controlled by metal properties such as oxidation state, ionic radius, tendency to hydrolyze, electronegativity and softness, as well as oxyhydroxide mineral properties such as degree of crystallinity, surface morphology and point of zero charge, as well as the soil pH (McBride, 1989). Zinc(II) has a greater affinity for Fe oxyhydroxides than Cd(II) in the absence of any other complicating factors. However, this may be different in the presence of organic matter, chloride and other soil and soil solution constituents.

The presence of adsorbent minerals in soil is known to affect Cd uptake by rice. Li *et al*. (2005) found that Cd bioavailability to rice in a pot study was less for soil with higher Fe-(hydr)oxide content. Other workers have found that Cd accumulation in wheat grain was less for soils of higher clay content (McLaughlin *et al.*, 2006). McLaughlin *et al*. (2006) found the relationship was consistent for twelve field sites across Australia, when Cd was added to soil as either biosolids, chloride or sulfate salts. The rationale for these observations is that clay minerals (layer silicates) and Al, Fe, Ti and Mn oxides are adsorbent materials for metal cations (Schwertmann and Cornell, 1991), and hence can remove Cd from the bioavailable pool. The adsorptive ability of these minerals is due to their high surface area and surface charge. Surface charges of soil minerals can be either positive or negative, and the charge can be either permanent or pH-dependent (Bohn *et al.*, 2001). Common Fe oxyhydroxides (goethite and hematite) and kaolinite start to develop negatively charged surfaces above the pH point of zero charge (PZC), which has been reported at 9.0 for goethite, 8.5 for hematite and 4.7 for kaolinite (Essington, 2004).

Iron and Mn oxyhydroxide minerals are well known adsorbents for metals (Jenne, 1968), and are likely the primary sink for Cd and Zn in oxidized soil environments (Du Laing *et al.*, 2009). Therefore, it may be expected that soils high in these minerals will provide less Cd for plant uptake during pre-harvest drainage of paddy fields.

Buekers *et al*. (2008) showed that aging of minerals between 1 and 70 days decreased metal concentrations in solution by factors of 1-20 for Zn and 1-4 for Cd, depending on the type of oxyhydroxide. Adsorption kinetics were determined in suspensions of synthetic hydrous ferric oxide (HFO), ferrihydrite (FH), goethite (GT) and hematite (HT) over 70 days at pH ranging from 3.8 to 7.0.

Chaney *et al*. (2006) found that addition of Fe and Mn oxides had no effect on the Zn:Cd ratio in lettuce leaves, however they note that the result may be different for species such as rice which use phytosiderophores to solubilize soil Fe. They postulate that increasing the availability of Fe through soil addition may work to reduce phytosiderophore secretion, and as phytosiderophores may also transport Cd, this may reduce Cd uptake (Ishimaru *et al.*, 2006).

1.6.8 Chloride

The presence of chloride in soils has been shown to increase Cd uptake by rice plants (Ohtani *et al.*, 2007). Cadmium concentrations in plant shoots and soil solution were higher when soils were spiked with an ammonium chloride salt, rather than ammonium nitrate, ammonium dihydrogen phosphate or ammonium sulfate. Plants were grown for 30 days in a greenhouse pot experiment and soil contained 2 mg Cd/kg and 120 mg Zn/kg.

The presence of chloride in solution is known to increase Cd uptake by other crops, i.e. potato, Swiss chard and wheat (McLaughlin *et al.*, 1999a; Ozkutlu *et al.*, 2007; Smolders *et al.*, 1998). For Swiss chard (*Beta vulgaris* L., cv. Fordhook Giant) grown to maturity, an increase in nutrient solution chloride concentration (as NaCl) from 0 to 6,960 mg/L was found to increase average Cd concentrations in shoot from 1.7 to 3.2 mg/kg plant dry weight (Smolders *et al.*, 1998). This was observed in pot experiments using agricultural soils ranging in Cd content from 0.20 to 0.31 mg/kg EDTA-extractable Cd.

Increasing the chloride concentration in leaf-applied solution (0, 50 and 167 mM NaCl) was also found to increase Cd translocation (but not Zn) to grains in durum wheat (*Triticum turgidum* L. *durum,* cv. Balcali-2000) plants grown in a greenhouse (Ozkutlu *et al.*, 2007). The increase in grain Cd concentration was 26% and 41% for the 50 and 167 mM Cl^- treatments respectively, when the soil Cd concentration was amended to 1 mg/kg. Chloride applied at 167 mM Cl^- also increased the grain Cd concentration when the Cd source was leaf-applied at 8.8 mM.

Weggler-Beaton *et al*. (2000) also found that increasing the chloride concentration in irrigation water (0, 400, 800, 1200, 1600 mg/L) gave higher Cd concentration in plant shoots for both Swiss chard (*Beta vulgaris* cv. Foodhook Giant) and wheat (*Triticum aestivum* cv.

Halberd) in a growth chamber. The Cd source was biosolids with a concentration of 15 mg Cd/kg, mixed with soil in a 1:24 ratio.

In potato (*Solanum tuberosum* L.), higher chloride concentrations in irrigation waters was found to correspond to higher Cd concentration in the tubers (McLaughlin *et al.*, 1999a). There was a high probability (> 50%) of producing potato tubers exceeding 0.05 mg Cd/kg when electrical conductivity (EC) of irrigation water was 1.4 dS/cm. Tubers were likely to exceed 0.10 mg Cd/kg when EC was 3.0 dS/cm. This finding was made in a survey of 134 potato-producing areas of Australia.

Studies comparing $NaNO_3$ treatments with NaCl treatments have found that only the NaCl treatment had the effect of increasing Cd uptake in Swiss chard (Smolders *et al.*, 1998) and in wheat (Khoshgoftar *et al.*, 2004). This shows that Na^+ competition with Cd for sorption sites is not the process responsible for the result, as was suggested by the earlier results of Bingham *et al*. (1983). The increased uptake of Cd is considered likely to be due to either plant uptake of Cd chloride complexes in solution or to chloride enhanced diffusion of Cd^{2+} through the apoplast to sites of Cd uptake within the root (Smolders *et al.*, 1998).

1.6.9 Organic matter

Organic matter can bind metals through adsorption, complexation and chelation, but can also mobilise metals through the formation of soluble metal complexes with low molecular weight organic ligands (Du Laing *et al.*, 2009).

Reductive release of organic matter has been shown to be the dominant mechanism (over Fe oxyhydroxide association) controlling trace metal (Ni, Pb) release in wetland soils (Grybos *et al.*, 2007). During anaerobic incubation, trace metals were released concurrently with DOM and Fe oxyhydroxide dissolution. However, the effect on Cd was not documented.

Organic matter has been shown to decrease Cd solubility/bioavailability in soils. Kashem and Singh (2004) found that addition of organic matter (derived from cow manure) at 4% (w/w) led to decreased solubility of Cd in pot experiments under both flooded and non-flooded conditions. Extraction of the soil was carried out using different reagents to determine Cd associations with operationally-defined solid phases (sequential fractionation). When organic matter was present, the Cd concentration was lower in the exchangeable and carbonate soil fractions but higher in the more the strongly fixed fractions. Cui *et al*. (2008) also showed that addition of rice straw to paddy soil decreased the free Cd(II) concentrations in soil solution in column experiments. However, it was noted that the effect was more likely due to the pH rise that the straw addition induced, rather than the provision of additional organic matter.

In rice-growing experiments with three different soils, total uptake of Cd in shoot, straw and polished rice was reduced by approximately 30% when organic matter was applied (Kashem and Singh, 2001). The effect was less pronounced in soils naturally rich in organic matter, but the same trend was observed whether rice was grown under flooded or non-flooded cultivation. Li *et al*. (2005) also found that increased organic carbon in soil caused a reduction in Cd uptake by rice plants in a pot study.

In contrast, a study using ryegrass (*Lolium multiflorum* Lam.) found with organic matter (derived from pig manure) application to soil at 4% (w/w) an increase in Cd uptake in pot experiments over 1 year (Almås and Singh, 2001). The increase in plant uptake with organic matter was observed for both geogenic and added Cd. The increase in uptake was attributed to soluble organic acids competing with sorption processes, thereby liberating more Cd for plant uptake.

1.6.10 Biosolids addition to soils

While biosolids may contain Cd themselves, in a Cd-contaminated soil, biosolids have a known ability to limit Cd uptake by rice plants; however, it is important to discover whether it is the organic or inorganic component of the biosolids that is responsible for this. If it is mostly the organic component that binds Cd in the soil, then there is the potential for release of metal from the organic matter as decomposition occurs with aging (Stacey *et al.*, 2001). The widely varying compositions of different biosolids is demonstrated by a study where three, five-year old biosolids were incubated for 100 days (between 15 and 25ºC); only one of the biosolids released metals to the soil despite signs of organic carbon degradation in all the biosolids (Stacey *et al.*, 2001).

Li *et al*. (2001) found that the biosolids-associated reduction in Cd availability is not dependent only upon the soil organic fraction. This was determined by producing adsorption isotherms for different soil samples at pH 4.5, 5.5 and 6.5 with biosolids-amended and control soils; each was also tested with organic carbon removed. The soil inorganic fraction was found to have a significant contribution to the total Cd-sorptive capacity of the soils, and was able to sorb more Cd after biosolids-amendment (same effect as with total soil). Other workers agreed that increased Cd sorption by biosolids-amended soil at pH 5.5 is due to a combination of organic and inorganic components of the biosolids (Hettiarachchi *et al.*, 2003). When desorption experiments were carried out, biosolids-amendment was also found to increase the retention of added Cd; the biosolids fraction mostly responsible for this effect was found to be Fe and Mn rather than organic matter (Hettiarachchi *et al.*, 2003). The use of synchrotron-based techniques; μ-XANES and μ-XRF, showed that Cd in biosolids was strongly associated with Fe and Mn phases (Hettiarachchi *et al.*, 2006). It could not be seen whether metals were specifically bound directly with Fe (hydr)oxides or as organic complexes sorbed to Fe (hydr)oxides. However, when 70-75% of the organic carbon was removed from the biosolids, there was little change to the association with Fe phases. This lends indirect evidence to the hypothesis that metals in biosolids are associated mainly with the inorganic fraction.

Other workers found that organic manure application depresses Cd uptake by rice plants but only for added Cd, rather than native Cd (Zhang *et al.*, 2002). It was also found that pig manure was a more effective depressant of Cd uptake than peat. This could be due to Zn-competition for plant uptake as in the pig's manure the Zn:Cd ratio was 120:1, but in the peat it was only 47:1.

1.6.11 EDTA addition to soils

There is some evidence to suggest that Cd uptake in rice grain could be affected by the addition of EDTA. Degryse *et al*. (2006) demonstrated the importance of metal complexation on plant uptake of Cd in experiments on spinach. Sixteen to 20 day-old plants were exposed to ^{109}Cd and one of six ligands tested; *N*,*N*-ethylenediaminediacetate (EDDA), nitrilotriacetate (NTA), *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N'N'*-triacetate (HEDTA), ethylenediaminetetraacetate (EDTA), ethylene-bis-(oxyethylenenitrilo)-tetraacetate (EGTA), *trans*-1,2-cyclohexyldiamine-*N*,*N*,*N'N'*-tetraacetate (CDTA) (in order of increasing stability constant); during four hours. Less stable ligand complexes resulted in more metal diffusion, giving rise to greater plant uptake despite the free ion concentration remaining constant (1 nM). The relevance of this finding to Cd accumulation in paddy rice grain is unclear, as no other metals were present in the experiments, and reduced conditions were not considered.

Karak *et al*. (2006) showed that uptake of Zn in rice grain was enhanced when Zn application to the soil was as Zn-EDTA rather than $ZnSO_4$. This may also be true for Cd, however it was not considered in the study. No details on the water management or Fe content of the soil were given however, so it is difficult to draw conclusions about the possible effect under variable redox conditions.

Cadmium accumulation in rice roots, shoots and grains was shown to be significantly reduced (> 67%) by the addition of Fe-EDTA at 0.09 g/kg in pot experiments with a Cd-contaminated (5.7 mg/kg total Cd) agricultural soil (Shao *et al.*, 2008). It is unclear whether Fe-EDTA induced a decrease in grain-Cd due to the addition of Fe or the addition of EDTA. It is possible that EDTA decreased Cd uptake in rice grain, as stability constants (log K) for CdEDTA and ZnEDTA are both 16.5, compared to 14.3 for Fe(II)EDTA (Martell and Smith, 1974), indicating that EDTA should complex Cd and Zn more strongly than Fe(II). This would prevent the uptake of Cd and Zn by the plant, and with increased free Fe(II), Fe would be more available for plant uptake. This assumes a kinetic limitation to Cd dissolution from soil to buffer the free Cd complexed by EDTA, so that a reduced Cd activity in solution results. In this scenario Zn uptake should be decreased along with Cd by the EDTA treatment, and the authors did observe that Fe-EDTA addition inhibited Zn accumulation in rice grains.

Because Fe was also added, one may expect that the increased soil Fe concentrations should have inhibited Cd accumulation in rice grain due to competition between Cd(II) and Fe(II). Additionally, better Fe nutrition should have inhibited the expression of the deficiency-induced Fe transport systems (which can also carry Cd(II)). The authors hypothesised that soil application of Fe-EDTA induced a decrease in grain-Cd by lowering the uptake capability of rice roots. Iron-EDTA provides Fe(II) to the soil, alleviating Fe-deficiency and inhibiting Fe transport systems, also reducing Cd uptake. This requires further investigation with respect to the possible effects of pre-harvest drainage; this would favour the oxidation of Fe(II) to Fe(III), with Fe(III) forming a complex with EDTA (log K = 25.1) of greater stability relative to Cd-EDTA or Zn-EDTA. This may result in Cd and Zn becoming more available than Fe for plant uptake during grain-filling, while Fe transport systems may be affected the transformation of Fe(II) to Fe(III).

In a study by January *et al*. (2008) the authors examined the addition of EDTA to Cd- and Fe-spiked soil in a greenhouse pot experiment to enhance the uptake of these metals in the hyperaccumulator Sundance sunflower. When plants were exposed to Cd, total uptake per plant was 0.66 mg without EDTA present, and 2.15 mg with EDTA present. Both root uptake and leaf translocation were greater with EDTA, with an increase in the Cd translocation factor (shoot concentration/root concentration) from 0.14 to 0.21. Plants were exposed to solutions containing 30 mg/L of Cd as sulfate salt and EDTA was present in solution either at 0 or 265 mg/L. However, the results require further investigation as the metal concentration used was far higher than typical Cd contaminated soils. Other workers also found that EDTA addition (varied from 0 to 5 mmol/L) to Cd (20 mg/kg), Pb (1000 mg/kg) and Zn (500 mg/kg) co-contaminated soils enhanced uptake of those metals in shoots of hyperaccumulator rainbow pink, grown for 50 days in a pot experiment. EDTA may have exerted toxicity to plant roots at these high concentrations, thus leading to high Cd uptake of Cd-EDTA complexes.

The effect of EDTA in enhancing metal uptake by plants may be due to direct uptake of the complex, rather than by enhanced diffusion. Schaider *et al*. (2006) showed that Cd-EDTA was taken up more readily than Fe-EDTA in mustard plants exposed to both at 400 μM concentrations, with the average xylem sap Cd-EDTA to Fe-EDTA ratio being 1.8. Plants were grown in solution and sand cultures, and exposed to metal-EDTA complexes and a dye tracer for apoplastic flow. Iron-EDTA was measured in solution by high pressure liquid chromatography (HPLC) separation and UV detection at 258 nm^{-1}, and Cd-EDTA was measured by HPLC separation followed by ion exchange to Fe-EDTA and UV detection at 258 nm^{-1}. Two phases of solute uptake were identified where xylem sap solute concentrations were < 0.5 % solution concentration under normal conditions and where xylem sap concentrations were > 3 % under conditions of stress. The finding of increased xylem sap metal-EDTA concentrations under conditions of stress was consistent with observations by other workers of increased bypass flow for other solutes such as sodium (Yeo *et al.*, 1987). Cd-EDTA^{2-} had higher xylem sap concentrations than PbEDTA^{2-} or FeEDTA^{-}. Metal-EDTA complexes dominated the metal speciation in xylem sap. Uptake of solutes was similar under both sand and solution culture.

1.6.12 Silicon

Silicon (Si) is a beneficial element to rice plants known to stimulate growth and yield, and is typically found in shoot tissue at a SiO_2 concentration of 10 to 15% dry weight (Marschner, 1995). Silicon can also alleviate Cd toxicity and translocation in rice plants. Marschner (1995) found that Si inhibits the translocation of Cd to shoots of rice; Cd concentration in the root was increased by 21% but decreased in the shoot by 24%. Rice seedlings were grown hydroponically in a growth-chamber for four weeks before being exposed to Si (0 or 50.4 mg/L) for a further two weeks. The plants were then treated with 5.62 mg Cd/L (toxic concentration) for 6 days before being harvested. Silicon also alleviated Cd-toxicity symptoms in the plants, resulting in greater plant weight and hence a dilution in Cd concentration of 67 and 35% in shoots and roots respectively. The Si concentrations used are realistic (typical concentrations in soil solutions range from 3.5 to 40 mg/L), however the Cd concentration used is far from the typical Cd concentration in soil solutions of 0.2 to 6 μg/L

(Bohn *et al.*, 2001; Kabata-Pendias and Pendias, 1984), and beyond even typical Cd concentration for contaminated soils of 300 to 400 µg/L (Kabata-Pendias and Pendias, 1984). Zhang *et al*. (2008) also found that Si inhibits Cd accumulation in shoots in a hydroponic experiment (105 days). This was true for the Si concentration range 0, 2 and 4 mmol/L and Cd concentration range of 0, 2 and 4 mmol/L. Again, Cd concentrations used were not environmentally relevant, being higher than those commonly encountered in contaminated soil environments, as described previously.

Silicon is thought to limit Cd uptake into rice shoots due to its incorporation into the structural cell walls (Shi *et al.*, 2005). Rice seedlings grown in solution culture were treated with 5.62 mg/L $CdSO_4$ and either 0 or 50 mg/L Si. The use of fluorescein tracers of different diameters showed that Si did not change the epidermal cell wall porosity of rice seedling roots. Energy dispersive X-ray analysis was used to examine the cell walls of the epidermis and endodermis; Si deposition was discovered mainly in the endodermis (inner root tissues). Silicon deposition in the endodermis may have served as a physical blockage to apoplastic Cd flow and introduction to the transpiration stream, restraining Cd-transport to shoots (Shi *et al.*, 2005).

The presence of Si at 0.6 mM was also shown to significantly decrease Cd accumulation in rice roots and shoots in solution culture experiments (Nwugo and Huerta, 2008). Cadmium exposure (2.5 mM) was initiated when plants were 6 days old, for 48 days, and the result was true for provision of Si nutrition from either 6 or 20 days of age. The result was likely due to Si alleviating the inhibitory effect of Cd on plant growth that occurred in the study; the Cd concentrations employed were far higher than would occur naturally even in highly contaminated soil solutions.

1.6.13 Temperature

An increase in temperature can enhance Cd (and Zn) translocation to rice shoots. Chino and Baba (1981) found that relatively low temperatures (20 ºC compared to 28 ºC) depressed the translocation, but not the overall uptake of Cd and Zn in rice plants.

Higher temperatures have been shown to increase Cd uptake in other plant species. In pot experiments run for 1 year using ryegrass grown at either 9 or 20ºC, metal content of aboveground plant parts was increased by at least 1.5 times at the higher temperature (Almås and Singh, 2001). This held true for two different soils, at both 0 and 4 % (w/w) organic matter (from pig manure).

1.6.14 Nutrients

Higher nutrient concentrations can decrease Cd bioavailability to plants (Brown *et al.*, 2004; Göthberg *et al.*, 2004; Hassan *et al.*, 2005a; Koshino, 1973; Saito and Takahashi, 1978b). The addition of PO_4^{3-} into soil has been found to have no effect on Cd uptake in rice plants, but pyrophosphate suppresses Cd uptake presumably via formation of an insoluble Cd-pyrophosphate complex (Koshino, 1973). In contrast, other workers found that either an excess or a deficiency of P increases Cd uptake by rice plants grown in solution culture (Saito and Takahashi, 1978b). It is thought that P-deficiency limits the ability of plant roots to exclude Cd. This result indicates that P must be delivered to the plant at nutritive concentrations in order to minimize Cd uptake. Phosphate also influences Cd availability in other species; 1% P-H_3PO_4 and 1 to 3.2% P-TSP decrease Cd availability to tall fescue grown in field experiments (Brown *et al.*, 2004), however these P concentrations are far above usual fertilizer addition rates and would likely have led to precipitation of Cd phosphates.

Nitrogen content of soils has been found to affect Cd translocation, with N-deficiency causing the greatest Cd translocation (Saito and Takahashi, 1978b). Manganese (Mn)- and Ca-deficiency also cause increased Cd uptake by rice plants (Saito and Takahashi, 1978b). The suggested mechanism for this is as described for P; deficiency limits the ability of roots to exclude Cd. An increase in sulfur concentration (from 6.4 to 25.6 mg/L) also decreased Cd uptake in rice roots and shoots when exposed to 0.112 mg Cd/L in hydroponic experiments (Hassan *et al.*, 2005a).

Copper (Cu) concentration in nutrient solution limits Cd translocation from roots to shoots in dark but not white rice varieties (Cui *et al.*, 2008). This was true for rice plants grown for 38 days in a growth chamber, subjected to Cu concentrations of 0 and 2 μmol/L and Cd concentrations of 5 and 10 μmol/L.

Nutrient concentrations can influence Cd uptake in other plant species. Water spinach grown for 7 days took up more Cd as nutrient concentrations increased, but the Cd was mostly retained in the roots (Göthberg *et al.*, 2004). Plants were treated with 1 to 100% Hoagland's nutrients (K^+, Ca(II), NH_4^+, Mg(II), Mn(II), Cu(II), Zn(II), Na(I), MoO_4^{2-}, Fe(III), $B(OH)_3$, NO_3^-, PO_4^{3-}, SO_4^{2-}, Cl^-, EDTA) and Cd concentration from 0 to 5.04 mg/L. This Cd concentration was much higher than typical soil solution concentrations for contaminated soils of 300 to 400 μg/L (Kabata-Pendias and Pendias, 1984).

1.6.15 Rhizosphere conditions

Rice plants pump air into their roots, resulting in a rhizosphere which is oxidized relative to the bulk soil (Lin *et al.*, 2003). This unique chemical environment can change metal speciation within the root zone (Lin *et al.*, 2003), potentially resulting in plant uptake that is modified from that expected from the conditions in the bulk flooded soil. It has been observed in greenhouse pot experiments (Lin *et al.*, 2003) that the extractable Cd concentration in the rhizosphere soil (ammonium acetate extractable) was significantly lower than the Cd concentration in the bulk soil. This was attributable to the lower pH in the rhizosphere, which is linked to higher Eh and greater oxygenation. The oxygenation of the rhizosphere is known to result in the formation of iron plaque on rice roots (Liu *et al.*, 2007a) and this may partly be responsible for reduced extractability of Cd.

1.6.16 Iron plaque on rice roots

Iron plaque is commonly formed on the root surface of rice (and other wetland) plants. An Fe plaque can form on roots when Fe(II) in the soil solution moves into the rhizosphere from the bulk soil and is oxidized to the less soluble Fe(III) and precipitates as Fe (hydr)oxide (Liu *et al.*, 2007a). Iron (hydr)oxides have a high adsorption capacity for metal cations (Schwertmann and Cornell, 1991), and could potentially decrease uptake of Cd by rice plants by adsorption or co-precipitation. Alternatively, Fe plaque on roots could increase plant uptake by acting as a reservoir for metals. Singh *et al.* (2003) found evidence that the plaque on roots can act as a barrier to metal uptake, finding that rice plants with iron plaque had greater resistance to Zn toxicity. When there was less Fe plaque under conditions of Fe-deficiency, root exudates (specifically phytosiderophores) were able to increase Zn uptake. Iron plaque on roots has been found to act as a barrier to Cd uptake and translocation to rice grains (Liang *et al.*, 2007). These authors found under field capacity conditions, where less Fe-plaque was formed, that grain concentrations of Cd reached a maximum of 1.7 mg/kg, but under flooded conditions of high Fe-plaque, Cd in the grain was only 0.4 mg/kg (Liang *et al.*, 2007). However, the presence of Fe plaque may not be specifically responsible for the reduced uptake of Cd, as solid and solution speciation was not determined; i.e. whether under flooded conditions Cd was unavailable for plant uptake as precipitated CdS in the soil, or if the more bioavailable solution Cd(II) was the dominant species under the saturated regime.

Contradicting these findings, other workers have found that Fe plaque on rice roots enhanced the deposition of Cd on root surfaces and uptake into shoots, in solution culture experiments over 72 hours (Liu *et al.*, 1999). The authors hypothesised that root exudates may have mobilized the plaque-sequestered Cd and therefore enhanced Cd-uptake in the rhizosphere. Under Fe-deficiency, the Cd uptake into shoots was slightly more than when plants were Fe-sufficient. This may be due to the Fe-acquisition system being stimulated in the Fe-deficient plants, and increasing the absorption of Cd as it could be bound by the same transport proteins.

There is also research challenging the findings that Fe plaque has any effect at all on Cd uptake. Observed reductions in Cd uptake by rice plants may be due to the presence of Fe in solution, rather than the formation of Fe plaque on roots (Liu *et al.*, 2007a). In solution culture experiments (solution Cd concentration of 0 to 1.0 mg/L) on rice seedlings with Fe plaque, it has been shown that Cd concentrations in Fe plaque, shoots and roots decreased with increasing Fe concentrations in the nutrient solution (0 to 100 mg/L). Liu *et al*. (2007a) suggested that the decrease in Cd concentrations was not due to adsorption of Cd by the Fe plaque, rather by the nutritional role of the increasing Fe in solution, a competitive ion effect. These authors found that the percentage of Cd in the Fe plaque was only 2 to 9% of the total Cd in plants, and an isotope experiment showed that when roots without plaque were exposed to ^{109}Cd, concentrations of the radioisotope in shoots were lower when Fe was present in solution. This clearly shows that in the absence of plaque, Fe in solution inhibits Cd uptake. It was also noted that less ^{109}Cd was translocated to the shoots as Fe concentration in solution increased, perhaps due to a nutritive effect, whereas there was no effect of Fe plaque on ^{109}Cd translocation. Further work using a greenhouse pot experiment by Liu *et al*. (2008a) supported the conclusions of Liu *et al*. (2007a), finding that although increasing the Fe supply from 0 to 2 g Fe/kg soil reduced Cd uptake and translocation in the plants, the percentage of total plant-associated Cd sequestered in the plaque (measured using a modified dithionite-citrate-bicarbonate extraction method on roots rinsed three times with deionised water) was at most 16%, compared with 72.8% in roots and 36.9% in shoots. Root Cd concentrations were negatively correlated with root Fe concentrations, indicating that this was more due to root sequestering processes rather than Fe plaque preventing Cd from entering the roots.

1.6.17 Root Exudates

Plant roots exude small amounts of organic material, which may include sugars, amino acids, nucleotides, organic acids, flavonones and enzymes (Rovira, 1969). These compounds assist the uptake of nutrients. These have been shown to enhance rice root uptake of Cd (Liu *et al.*, 1999) and Zn (Gao *et al.*, 2004a). For example, phytosiderophores have been shown to significantly enhance Cd uptake by rice roots in solution culture experiments (Liu *et al.*, 1999). Liu *et al*. (2007d) found in a pot experiment with two cultivars and soil Cd concentrations of 0, 10 and 50 mg/kg; that the organic acids secreted by rice roots (acids measured were acetic, formic, malic, tartaric, oxalic, citric) varied between cultivars and that the total acid concentration was highly correlated with Cd uptake.

1.6.18 Mycorrhizal fungi

Mycorrhizal fungi have been shown to suppress Cd translocation to rice grains in non-flooded pot experiments with contaminated field soil having a Cd concentration of 1.60 mg/kg (Zhang *et al.*, 2005). In the presence of mycorrhizal fungi, the Cd concentration in rice shoots decreased (7.0 mg/kg to 14.2 mg/kg) relative to the control (11.7 to 22.8 mg/kg), regardless of whether root Cd concentrations increased or decreased. The effect was consistent across two different cultivars of rice, and there was no difference in P nutrition between plants. However, metal accumulation may have been reduced by tissue dilution due to increased yield, and translocation may have been reduced by the fungi immobilizing Cd in the roots.

1.6.19 Cultivar

Cadmium uptake and accumulation in grains has been shown to differ for different rice cultivars. He *et al*. (2006) found that Cd concentration in paddy rice grain varied from 0.06 to 0.99 mg/kg between 38 genotypes in a field study. Arao and Ae (2003) also found differences in Cd uptake in two soil types in a comparison of forty-nine cultivars in pot experiments conducted under drained conditions over a period of two years. Soil A (grey lowland soil) was characterized as ‘slightly polluted’ with a Cd concentration of 0.9 mg/kg and soil B (Andosols) as ‘heavily polluted’ with a Cd concentration of 7.4 mg/kg. Over the two years of the experiments the Cd grain concentrations varied between cultivars from 0.19 to 4.41 mg/kg in Soil A and from 0.59 to 7.71 mg/kg in soil B (Arao and Ae, 2003). This variability between cultivars was also observed in rice grown in a submerged field trial in China across two seasons at two soil Cd concentrations (added as $CdNO_3$); the variation in grain Cd concentrations between cultivars was up to an order of magnitude (Yu *et al.*, 2006). Supporting this, variability in grain Cd concentrations between 138 japonica genotypes was found to be up to one order of magnitude in a field trial using highly contaminated, moderately contaminated and slightly contaminated soils. Cadmium concentrations in the grain ranged from 0.13 to 1.09 mg/kg, 0.05 to 0.65 mg/kg and from 0.04 to 0.39 mg/kg in each soil respectively (Zeng *et al.*, 2008). Liu *et al*. (2007d) found similar results in a pot experiment with two cultivars and soil Cd concentrations of 0, 10 and 50 mg/kg; different cultivars accumulated Cd to a different extent, and secreted organic acids of differing composition.

1.6.20 Cadmium translocation within the rice plant

Cadmium taken up by rice plants is known to be translocated throughout the plant to different tissues (e.g. shoots, leaves and grain) (Ito and Iimura, 1976a; Kashem and Singh, 2001; Kitagishi and Obata, 1979; Kukier and Chaney, 2002; Saito and Takahashi, 1978a).

Cadmium is mostly accumulated in rice roots, but is also translocated to leaves, and a lesser extent grains. Ito and Iimura (1976a) found a root:shoot:grain (brown rice) concentration ratio of 100:10:1 for Cd in rice grown in pot experiments where soil was spiked with between 0.3 and 2,000 mg/kg Cd. Liu *et al*. (Liu *et al.*, 2003)found an even higher root:shoot:grain (brown rice) concentration ratio of 617:12:1 for Cd in rice grown in soil spiked with 100 mg/kg Cd. Zinc was found to follow the same pattern of accumulation in a Chinese field study (Yang *et al.*, 2008).

Saito and Takahashi (1978a) found the same order of accumulation in solution culture experiments using a solution Cd concentration of 0.3 mg/L. At harvest Cd was mostly accumulated in the rice roots, then leaves, then ear. Other workers (Dabin *et al.*, 1978) found the same order of accumulation above 0.010 mg Cd/L in solution culture, however at 0.005 mg Cd/L the shoots accumulated the most Cd followed by roots, then grains. Kitagishi and Obata (1979) found that ^{115}Cd (used as tracer for Cd) is mostly accumulated in rice roots and to a lesser extent in the rice leaves. Kukier and Chaney (2002) found that roots accumulated approximately 30 times more Cd than shoots in solution-culture experiments (0.09 to 0.5 mg Cd/L) run for 89 days in a growth chamber. The work of Kashem and Singh (2001) found that Cd was translocated to rice grain in pot experiments using contaminated soil ranging from

0.11 to 2.5 mg Cd/kg, with Cd in the rice grain approximately 10 times lower than Cd in the shoots. Rascio *et al*. (2008) found that Cd accumulation in rice shoots was less than 10 % of that accumulated in roots of 10-day old plants grown in solution culture exposed to up to 250 μM Cd nitrate.

In contrast, Reddy and Patrick (1977) found that Cd accumulation in rice does not follow the order roots > leaves > grains. In this study, rice plants were grown for 10 days in soil suspensions with 99% of Cd taken up by the plants and translocated to the shoots. The soil used was a silt loam unamended with Cd (natural Cd concentration not stated). This was observed from pH 5 to 8 and for redox potentials between -200 and +400. Cadmium and Mn were taken up by the shoots similarly, and were thought to move through the plant by similar mechanisms. Reddy and Patrick (1977) may have observed higher Cd concentrations in rice shoots than roots due to the use of lower Cd concentration in the natural soil used, compared to other studies using solution culture and contaminated soils.

Within the rice grain, the bran to endosperm ratio for Cd has been found to be significantly lower compared to that for As or Pb (Williams *et al.*, 2009). This suggests for As and Pb the bran layer may ‘shield’ the endosperm somewhat, resulting in polished rice which is much less contaminated than the corresponding brown rice. Cadmium behaves differently, with milling of brown rice resulting in only a marginal decrease in grain Cd concentration. These findings came from a grain survey of mine-impacted paddy rice in Hunan, China. However, other workers found that polishing rice can significantly decreased the Cd concentration of rice grains from 142 to 122 ng/g (Zhang *et al.*, 1997).

The extent to which Cd is translocated to rice grain is influenced by the growth stage at which Cd-exposure occurs. For example, when plants were exposed to Cd for a two-week period during different growth stages in solution culture and pot experiments under flooded cultivation (Ito and Iimura, 1976c), the growth stage most sensitive to Cd uptake was between ear primordia and ear sprouting; 6% of the total Cd taken up by the plant was translocated to grain during this time. Only 2% of total Cd absorbed by the plant was translocated to grain when Cd exposure occurred at other times. Minagawa *et al.* (1974) found that the Cd concentration in brown rice grain was higher when paddies were drained before heading (as compared to drainage after heading) in a field study over two consecutive years. These findings indicate that although rice plants are exposed to soil Cd throughout the growth cycle, it is during the grain development stage of growth that rice plants are able to translocate the greatest amount of soil Cd is to the grains. Because grain development typically occurs concurrently with pre-harvest drainage which renders the soil Cd more bioavailable, the grain-filling stage of rice growth is therefore the most favorable for Cd accumulation in paddy rice grain.

Cadmium is likely translocated within rice plants as an organo-metal complex. The form of Cd in rice roots was elucidated using radioisotopes in solution culture with a solution Cd concentration of 100 μg/L (Chino and Baba, 1981). The formation of stable organo-metal complexes with insoluble proteins in the root was suspected by the authors, as metals of greater electronegativity were translocated the least. Other workers found supporting evidence for this from plants grown in solution culture (Dabin *et al.*, 1978). Solutions were spiked with 0.005, 0.010 and 0.100 mg/kg Cd spiked with ^{109}Cd, and 0.700 mg/kg Zn spiked with ^{65}Zn. After one month the roots were harvested, homogenized and chromatography used to examine metal complexes; ^{109}Cd and ^{35}S-cysteine were detected in the same fractions; indicating that Cd (but not Zn) binding by metallothionein in the root may be important in plant metabolism. Other workers (Hart *et al.*, 2006) found that all soluble Cd in durum wheat roots (*Triticum turgidum* var. *durum*) was stored as a low molecular weight phytochelatin complex (approx. 1305 g/mol), as determined by capillary electrophoresis. The form of Cd in rice shoots has also been shown to be an organo-metal complex with citric acid (or similar), as elucidated by electrophoresis experiments which investigated the form of Cd in the xylem of an excised stem (Chino and Baba, 1981). It is believed that this type of complex may be involved in the mechanism of Cd translocation within rice plants, as it was found in the stem.

Cadmium translocation by rice plants increases with exposure time. Fujimoto and Uchida (1979) found with Cd concentration of 0.01 mg/L in solution culture that the percentage translocation (leaf+stem/total x 100) of ^{115}Cd in 25-day old rice seedlings increased markedly with time. Cadmium translocation increased from ~ 2% after 30 minutes to 31% after 24 hours. However, in reality the rate of Cd-translocation from root to shoot is limited by the rate of Cd transport across the root membrane (Fujimoto and Uchida, 1979). The cell walls of plants are negatively charged and the cytoplasm has a high pH, resulting in a difficult environment for uptake and transport of Cd(II). Fujimoto and Uchida (1979) found in a separate experiment that most of the ^{115}Cd absorbed by roots from solution culture (solution Cd concentration of 10 mg/L) was taken up in the first 20 minutes, with far less absorbed between 20 and 40 minutes. The rate of Cd absorption continued to decrease for the next 6 hours, but increased from 6 to 24 hours. The increase in absorption after 6 hours coincided with an increase in Cd translocation to shoots, indicating that once Cd is removed from the roots by translocation there are surface sites available for more to be absorbed. Although some work has been carried out on the kinetics of Cd translocation within the rice plant, environmentally unrealistic concentrations of Cd have generally been utilized in solution culture experiments without Zn present.

Cadmium may be translocated within rice plants through mass flow of water and inorganic ions through the phloem from root to shoot, or by transport through sieve elements of the phloem from photosynthetically active leaves to photosynthate-consuming sink regions, i.e. developing grains (Kitagishi and Obata, 1981). It is believed that the accumulation of metals in rice grains is a result of both the root to grain and leaf to grain pathways (i.e. leaf metals are root-derived and stored in leaf). It was found in flooded pot trials that Cd translocated to rice grains is 0.73% (average of 6 cultivars ranging from 0.26 to 1.14%) of total Cd absorbed by the plants (Liu *et al.*, 2007c). Cadmium distribution ratios indicated that Cd concentration in rice grain is governed somewhat by root to shoot translocation, but mostly by shoot to grain translocation. However, this study did not utilize standard pre-harvest drainage of the pots, and the soil was spiked with an unrealistically high Cd concentration of 100 mg/kg. In another study comparing root and foliar application of ^{115m}Cd to rice at grain filling stage following foliar-application, it was found that after 10 days Cd was mostly retained in the leaves and only a small amount passes through to the grains (Chino, 1981). With root application up to 10% was translocated to both leaves and grains (Chino, 1981). This raises the question of whether Cd taken up from soil is stored in leaves before being translocated to grain, and if so whether foliar application of competing cations could limit grain Cd concentrations.

Ishikawa *et al*. (2009b) showed that different rice cultivars translocate Cd from root to shoot in a different manner, giving rise to the genotypic differences in shoot Cd accumulations. A positron-emitting tracer system utilizing ^{107}Cd was used to show Cd accumulation in shoots with time for 6 rice cultivars. Cadmium first appeared in the basal portion of the shoot after only 2 hours of exposure, with accumulation in the upper shoots increasing with time for *indica* cultivars but decreasing with time for *japonica* cultivars. The same type of experiment carried out on soybean plants (Ito *et al.*, 2009) showed that Cd was also translocated from roots to shoots within 2 hours, but by 2 days of exposure Cd was detected in seeds but not leaves, indicating that Cd was translocated directly from roots to grains during a 2-hour exposure timeframe.

A hydroponic study on wheat translocation in two cultivars (*Triticum turgidum* L. var. *durum*) has shown that the shoot to grain pathway is more important than the root to grain pathway for the accumulation of Cd in grain. Plants were grown to maturity and neither variety transferred ^{106}Cd from the root to the grain during the ripening stage of development (Chan and Hale, 2004). Rather, grain-Cd was derived predominantly via remobilization from the stem, as demonstrated in another experiment by the application of a foliar-spray once per day for 3 days (22.5 mL in total of 500 or 5000 μg/L $CdNO_3$ solution). Additionally, it was discovered that the plants also translocate foliarly-applied Cd from shoot to root. Also in wheat, Cakmak *et al*. (2000) found that the root to grain and shoot to grain pathways interact, when Cd (spiked with ^{109}Cd) applied to leaves was translocated less to roots/grains over 11 days when Zn supply in solution culture was increased from 0.1 to 1.0 to 5.0 μM. Curiously at 0.1 μM Zn exposure, translocation was higher than at nil-Zn. The percentage of translocation from the leaf was small, varying from 1.3 to 6.5%, however translocation was mostly to other shoot parts rather than down to the root. The authors suggested an inhibitory action of increased Zn concentration in shoot tissues on phloem-mediated Cd transport.

A few authors have studied Cd translocation to rice grains, and despite the different watering regimes and greatly varying Cd concentrations (some of which were environmentally unrealistic) Cd has been shown to be translocated through both root to grain and leaf to grain pathways as an organo-metal complex. Studies on wheat plants have supported this.

1.6.21 Interactions with zinc

Zinc is an essential micronutrient in plants and well known to be a competing ion for Cd uptake by rice (Chino and Baba, 1981; Dabin *et al.*, 1978). Zinc can have an antagonistic or synergistic role in Cd uptake by rice plants, and the Zn:Cd ratio in soil and tissues has been shown to influences the uptake, translocation and accumulation of Cd in rice (Chino and Baba, 1981; Hassan *et al.*, 2005b; Honma and Hirata, 1978; Saito and Takahashi, 1978a; Saito and Takahashi, 1978b). Additionally, the Zn:Cd ratio in the edible plant parts influences the ultimate toxicity of ingested Cd to humans; it is desirable that Zn:Cd ratios are high in both soils and plant parts. Zinc is present naturally in soils and soil solutions at concentrations of approximately 50 mg/kg and between 4 and 270µg/L respectively (Bohn *et al.*, 2001). At low pH (< 4.0) however, Zn concentrations in contaminated soil solutions can be as high as 7,137 µg/L, or even 17,000 µg/L (Bohn *et al.*, 2001). In comparison, Cd is present naturally in soils at an average concentration of 0.2 to 0.3 mg/kg (Garrett, 1995), and in soil solutions typical Cd concentrations range between 0.2 and 6 µg/L (Bohn *et al.*, 2001). In the soil solution of contaminated soils, the Cd concentration can range from 300 to 400 µg/L (Kabata-Pendias and Pendias, 1984).

Uptake of Cd and Zn by the rice plant is controlled by the concentration in soil as well as other factors such as electronegativity, temperature and depth in soil layer (Chino and Baba, 1981). When both metals were present in equimolar amounts, Cd uptake by rice seedling roots over 24 hours was slightly higher than Zn uptake for concentrations below 0.445 µmol each (0.051 mg Cd/L). However, from 0.445 to the 8.900 µmol, Zn uptake exceeded that of Cd. In spinach, shoot uptake of Cd has been shown to be greater than that of Zn, from nutrient solution, however the Cd and Zn concentrations in solutions were not reported (Smolders *et al.*, 1997).

Not only uptake, but translocation within the rice plant is different for Cd and Zn. Chino and Baba (1981) used radioisotopes to track Cd and Zn within rice plants grown in solution culture, finding that metal translocation from root to shoot was higher for Zn than Cd when both were applied at a rate of 100 µg/L, an observation which is in agreement with other work

(Homma and Hirata, 1984). Presumably Cd was translocated less due to the increased formation of organo-metal complexes with insoluble proteins in the root. However, Cd and Zn are rarely found at equimolar concentrations in nature so the results cannot be reliably extrapolated to a field environment. Other workers found that the translocation factor (grain/shoot and shoot/root) was consistently higher for Zn than Cd in a range of aerobically cultivated field crops (corn, wheat, oats, barley, soybeans, bush beans) in the U.S.A (Kubota *et al.*, 1992). Dabin *et al.* (1978) also found that Zn was more readily translocated to the aerial plant-parts than Cd in solution culture experiments on rice taken to harvest. In their work, Cd concentrations in solution were between 0.005 and 0.100 mg/L, and Zn was 0.100 mg/L. Conversely, Cd has also been shown to accumulate in brown rice in preference to Cu, Pb and Zn in flooded pot experiments which were drained at panicle-initiation (Iimura and Ito, 1978). The Zn:Cd ratio was 100:1, with irrigation water concentrations of 0.22 μg/L Cd and 22 μg/L Zn.

Addition of Zn to soils can increase Cd accumulation in plant parts (Hassan *et al.*, 2005b; Honma and Hirata, 1978; Saito and Takahashi, 1978a; Saito and Takahashi, 1978b). Increasing the Zn concentration in solution culture from 13 to 65 μg/L (Zn-sufficient concentrations for the plants) enhanced Cd translocation when rice seedlings were grown for 24 days at Cd concentration of 0, 112 or 562 μg/L (Hassan *et al.*, 2005b). The result is perhaps irrelevant to natural systems however, as Zn is rarely found at lower concentrations in soil solution than Cd (Chaney *et al.*, 1996). It was hypothesised that Zn facilitated Cd translocation by competing for phytochelatin ligands that can bind metals in the vacuole of root cells. Saito and Takahashi (1978a) also found that Cd concentrations in rice shoots grown in solution culture increased as the Zn:Cd ratio increased from 10 to 20 to 100. However, the result is confounded by the fact that Cd and Zn concentrations were not varied consistently to increase the Zn:Cd ratio; at Zn:Cd ratios of 10 and 100, Cd concentration in solution was

0.04 mg/L Cd, whereas at Zn:Cd ratio 20, Cd concentration in solution was 0.3 mg/L. Contrary to observations in the shoots, Cd concentrations in the roots always decreased in the presence of Zn, indicating that the Zn was accelerating Cd translocation rather than total uptake by the rice plant. These findings are supported by the results of Liu *et al*. (2007b), where Zn addition to solution culture decreased the Cd concentration in rice roots while increasing it in rice shoots. Zhou *et al*. (2003) also found that the presence of Zn enhanced Cd uptake for rice plants grown in a greenhouse pot experiment. When plants were exposed to 200 mg Zn/kg, Cd accumulation in the roots, shoots and grains was higher than the control containing 0.135 mg Cd/kg and 56.93 mg Zn/kg.

Cadmium and Zn can compete with each other for uptake by rice plants. In solution culture experiments with rice (Saito and Takahashi, 1978a), Cd concentrations in plant shoots increased as solution Zn:Cd ratios increased from 10 to 20 at solution Cd concentrations of 0.04 and 0.3 mg Cd/L respectively. When the solution Zn:Cd ratio was 100 (solution Cd concentration of 0.04 mg/L), Cd concentrations in the shoots were decreased for most of the growth period but increased by harvest time. Other workers found that at higher Zn:Cd ratios, Cd translocation was enhanced for solution Cd concentrations 0, 0.05, 0.5 and 5 mg/L and Zn concentrations 0, 0.05, 0.5 and 5 mg/L (Saito and Takahashi, 1978b). However, these concentrations are not realistic when compared with typical solution Cd concentrations for contaminated soils of 300 to 400 µg/L (Kabata-Pendias and Pendias, 1984). Other workers found that Cd translocation was enhanced by increasing Zn:Cd ratios from 1:1 to 10:1 using ten-day old rice seedlings exposed to 0.05 mg Cd/L spiked with ^{109}Cd and 0.05, 0.5 or 5 mg Zn/L in solution culture. This effect diminished with further increasing the Zn:Cd ratio from 10:1 to 100:1 (Honma and Hirata, 1977). The results from a greenhouse pot experiment (soil containing 6.79 mg/kg Cd and 71.1 mg/kg Zn) showed that Zn application at rates of 25 (Zn:Cd ratio 14:1) and 76 mg/kg (Zn:Cd ratio 22:1) were able to reduce Cd accumulation in rice grain from 2.39 (control) to 2.31 and 2.26 mg/kg respectively (Li *et al.*, 2008). However, available Cd in soil (0.01 M $CaCl_2$ extraction) and Cd taken up by rice roots were not significantly different between Zn treatments and the control (Li *et al.*, 2009), indicating that the effect was due more to competition between Zn and Cd during translocation within the plant.

The importance of the Zn:Cd ratio in soils is further illustrated by a transect study of Cd/Zn contaminated soil and paddy rice with distance from a Zn-contamination source. The contamination source was a road, with Zn derived from the corrosion of galvanized crash barriers and lamp pylons, as well as from tire wear (ZnO added during tire processing as a vulcanizing agent). From 0 to 25 m from the source, Zn concentration in soil decreased from 1.54 to 1.18 mg/kg (DTPA extractable), while Cd rose slightly from 0.97 to 1.11 mg/kg. In the rice grains, increasing source-distance from 5 m to 25 m increased Cd concentrations in the rice grain from 0.01 to 0.83 mg/kg, which was presumably due to the lower Zn concentrations in the soil to compete for plant uptake (Supaphol *et al.*, 2007). Similar results have been found in other plant species, e.g. in a field study on hyperaccumulator *Thlaspi caerulescens* and metal tolerant *Silene vulgaris* grown on sludge-amended soils Brown *et al.* (1995) found that increasing the soil Zn:Cd ratio from 33 (Cd 3.0 mg/kg, Zn 119 mg/kg) to 146 (Cd 0.33 mg/kg, Zn 48 mg/kg) and 144 (Cd 1.0 mg/kg, Zn 144 mg/kg) resulted in decreased uptake of Cd but little change to uptake of Zn. The result is confounded however, by the fact that the Cd concentration changed along with the Zn:Cd ratio, so the two effects cannot be isolated. Other workers found that in durum wheat (*Triticum turgidum* L. var *durum*), Zn decreased Cd accumulation in the grain of plants grown to maturity in solution culture containing 0.5 μM Cd (Hart *et al.*, 2005; Jiang *et al.*, 2007a). This was true for two near-isogenic lines differing in grain Cd accumulation; low and high. Increasing the Zn activities in the nutrient solution from deficient (1 μM Zn, 2:1 Zn:Cd ratio) to sufficient (10 μM Zn, 20:1 Zn:Cd ratio) levels at the time of flowering, reduced the concentration of Cd in grains and shoots of both isolines relative to the controls (kept deficient after flowering). Conversely, decreasing the Zn activities from sufficient to deficient at the time of flowering raised the concentration of Cd in grains and shoots relative to controls which were kept sufficient for the entire growth period. The presence of sufficient Zn in the first half of the growth cycle was more effective in reducing Cd accumulation in grains than the presence of sufficient Zn during flowering and grain filling. Increasing the Zn:Cd ratio also decreased shoot Cd concentrations in lettuce (*Lactuca sativa L.* var. *longifolia*) in pot experiments (Chaney *et al.*, 1996). This effect was relative to Zn-deficient plants, and it was hypothesized that the Zn-deficiency was in fact a result of the high soil Cd concentrations interfering with Zn uptake.

In summary, the literature suggests that around the usual Zn:Cd ratio of > 100:1 as in geogenic, biosolids-amended and Zn-mine contaminated soils, Zn will not enhance Cd translocation to grain but rather should suppress it.

1.6.22 Differential translocation of cadmium and zinc

Cadmium and Zn have been shown to be translocated to different parts of the rice plant to a different extent. The Cd:Zn ratio in the different plant parts is important to consider as well as the Cd concentration in grain, because nutrient concentrations (e.g. Fe and Zn) in foods are known to decrease the intestinal absorption (decreasing the health risk) of ingested Cd (Chaney *et al.*, 1996).

The Cd:Zn ratio in the aerial parts of rice (shoots and grains) has been found to be lower than in roots (Silva *et al.*, 2007). Shoot to grain translocation was not described, but the result indicates that Zn is translocated from roots to a greater extent than Cd. Seven sampling sites were tested with soil Cd concentrations ranging from 20 to 28 mg Cd/kg and soil Zn concentrations from 102 to 554 mg Zn/kg. It was demonstrated in another field study that the Cd:Zn ratio was higher in rice grains than in stems or leaves (Simmons *et al.*, 2003). Surface rice plant samples were collected at physiological maturity from an agricultural area of Thailand which had received irrigation from a mineralized area containing extensive Zn silicate and Zn sulfate deposits containing Cd.

There is evidence to suggest that Cd and Zn accumulate in rice grains via different translocation pathways (Jiang *et al.*, 2007b; Kashiwagi *et al.*, 2009). Haslett *et al*. (2001) demonstrated that Zn transport in wheat plants occurs by phloem transport; finding that ^{65}Zn applied to leaves was translocated to shoots above and below the point of application as well as to the roots, and that ^{65}Zn applied to roots was conversely translocated to shoots. Jiang *et al*. (2007b) showed that grain-Zn in aerobic, solution-cultured rice originates mostly from root-uptake after flowering than Zn remobilization from the leaves. This was tracked using ^{65}Zn applied to roots, flag leaves or lower senescent leaves after flowering. At maturity up to 50 % of absorbed ^{65}Zn was translocated from the treated leaves, but was transported to roots, other green leaves and sheaths rather than to panicle parts or grains. Up to 23 % of ^{65}Zn applied to roots however, was translocated to grains by maturity.

Cadmium however, has been shown in field trials to be translocated to rice grains (*Oryza sativa* subsp. *Japonica* cv. *Nipponbare*) after heading from Cd already accumulated in leaf blades, sheaths, and in the culm (Kashiwagi *et al.*, 2009). During early growth stages the lower leaves had the highest Cd contents, but by harvest they contained almost no Cd, suggesting that lower leaves are the primary storage organs during the vegetative stage. After heading, Cd concentrations decreased in upper and lower leaves and increased in culms and ears. The final Cd concentration in grain depended upon translocation after heading from Cd already accumulated in leaf blades and sheaths, and in the culm. It appears that Zn follows a root to grain pathway whereas Cd follows a root to leaf storage to grain pathway, however this has not been demonstrated in rice plants exposed to both metals.

Where the translocation of both Cd and Zn has been investigated in the one study, it has been verified for wheat plants that the two metals are translocated differently (Hart *et al.*, 2006). Wheat (*Triticum turgidum* var. *durum*) plants were grown for 20 days in solution culture, and two near-isogenic lines which differed in Cd accumulation were used; high and low. Plants were exposed to 0.5 μM Cd in the solution culture and either 1 (deficient) or 10 (sufficient) μM Zn concentrations. Throughout the 20 days, the low Cd accumulator had higher root Cd concentrations, whereas the high Cd accumulator had higher shoot Cd concentrations. This is suggestive of lower Cd translocation in the low Cd accumulator, which may have been caused by reduced Cd loading into the xylem, or cellular sequestration of Cd in root cells. There was no difference between the two lines in Zn root and shoot concentrations. This indicates that Cd and Zn translocation occurs by different mechanisms. Phytochelatin was investigated as a possible explanation for this since it is known to have a higher binding affinity for Cd than Zn. All of the soluble Cd in plant roots was associated with phytochelatin in both isolines. Since Cd translocation was different between isolines but phytochelatin binding in the root wasn't, this cannot explain differences in metal partitioning. The divergence must instead occur during movement of Cd through the root and into the transpiration stream.

1.6.23 Interactions with iron

Iron is an essential nutrient to rice but can be toxic at elevated concentrations. Plants suffer Fe-deficiency at DTPA-extractable soil-Fe concentrations between 0.2 and 4.5 mg/kg (Kabata-Pendias and Pendias, 1984). This can be corrected by foliar application of Fe (Singh *et al.*, 2003). Iron becomes toxic to rice at soil concentrations above 800 mg/kg (Medhi *et al.*, 1999), and above soil solution concentration of 250 mg/L Fe (Becker and Asch, 2005; De Dorlodot *et al.*, 2005). Rice crops can be susceptible to Fe-toxicity (Kabata-Pendias and Pendias, 1984), as in paddy fields Fe(III) is reduced to other Fe species, including the more soluble Fe(II) species (Singh *et al.*, 2003). Natural concentrations of Fe in soil solution range from 30 to 550 μg/L but at low pH (< 4.0) can reach as high as 2,000 μg/L (Bohn *et al.*, 2001).

Cadmium and Fe are well known to compete with each other for uptake by rice plants. Liu *et al.* (2003) showed that in the presence of Cd, Fe uptake was significantly lower in rice leaves at maturity in pot experiments. Other workers have shown that as Cd uptake by rice roots increased, Fe uptake decreased for plants grown for 10 days in soil suspensions (Reddy and Patrick, 1977). This was true over the pH range 5 to 8 and for redox potentials between -200 and +400 mV.

Conversely, the presence of Fe has been shown to limit the uptake and translocation of Cd in plant parts. In solution culture experiments in a growth chamber, Adhikari *et al.* (2006), observed that translocation of Cd to rice shoots was reduced when Fe(II) activity was higher, between pFe (-log Fe(II) activity) 17.0 and 17.8, and for pCd (-log Cd(II) activity) 0, 7.9 (9.072 mg/L), 8.2 (4.480 mg/L) and 8.5 (2.240 mg/L). Preliminary experiments confirmed that both pFe levels used were Fe-sufficient, ruling out deficiency effects at low Fe activities. The presence of Fe was also found to decrease ^{109}Cd uptake (8 hours exposure) by 20-day old rice plants (grown to be Fe-deficient or Fe-sufficient) in solution culture (Nakanishi *et al.*, 2006). In contrast to these results, the addition of Fe(II) sulfate was found to have no influence on Cd uptake in continuously flooded pot experiments (Koshino, 1973). However, this may simply be due to the fact that under flooded conditions, Cd may be unavailable for plant uptake due to CdS precipitation, no matter what the Fe concentration is. Additionally, Fe(II) sulfate would not compete for plant uptake due to transformation to insoluble FeS.

The presence of Fe may reduce Cd uptake in rice due to the establishment of Fe-deficiency and stimulation of the Fe-acquisition system. This may increase absorption of Cd as it could be bound by the same transport proteins. This could be a particular problem for plant uptake in flooded paddy soils when pre-harvest drainage occurs. In this situation the soil becomes aerobic and Cd is solubilised by CdS dissolution, whereas Fe becomes less soluble upon drainage as Fe oxyhydroxides precipitate. This removes Fe(II) from solution causing Fe-deficiency, which in turn causes increased Cd uptake especially as drainage has increased Cd concentration in solution. Furthermore, the high nutrient demands of the new grains could help induce Fe-deficiency. Indeed, it has been shown that if Fe-deficient plants were subsequently supplied with Fe in the nutrient solution (8.4 mg/L), then translocation of Cd to the shoots was enhanced (Nakanishi *et al.*, 2006). This suggests that rice plants store Fe/Cd in the roots when faced with deficiency, but utilize the root-stored metal when nourishing conditions are subsequently encountered.

The effects of pre-harvest drainage on Cd and Fe uptake in paddy fields are confirmed by the results of field experiments. The Cd:Fe ratio was higher in rice grains than stems or leaves, which is thought to be due to oxidation of Fe(II) to Fe(III) during the pre-harvest drained period, resulting in a lower availability of Fe during the grain filling stage (Simmons *et al.*, 2003).

1.6.24 Foliar application of zinc

The presence of Zn in soil has the potential to either enhance or suppress the uptake of Cd from soil into rice plants. However, other methods of Zn delivery to the plant have not been fully investigated for rice plants in flooded conditions. This is important to investigate, as foliar Zn-application would eliminate complex soil-level Cd/Zn interactions and possibly decrease Cd accumulation in rice grains.

It has been shown that the Zn concentration of rice grains can be enhanced by foliar application of Zn (Fang *et al.*, 2008). In a field trial with rice, the foliar application of 0.90 kg Zn/ha at the heading stage enhanced rice grain accumulation of Zn by 37 % with no effect on yield and protein content. However, there is no literature addressing the specific possibility that foliar application of Zn to rice plants would be an effective way to reduce Cd accumulation in rice grain.

1.6.25 Foliar application of EDTA

Foliar application of EDTA is also a potential method for limiting the risk from Cd accumulation in paddy rice grain. The Cd:Fe molar ratio in brown rice was found to be reduced by 25 % with foliar application of Fe(II)-EDTA to rice plants in a pot experiment (Shao *et al.*, 2008). Concentrations of both Cd and Fe in the grain were found to increase following foliar Fe(II)-EDTA application, which suggested that enhanced translocation of metal-EDTA complexes may have occurred in the plants. This phenomenon requires further investigation, particularly as other workers have shown that Cu, Fe, Mn, and Zn concentrations in wheat grains were unaffected by foliar application of EDTA (Modaihsh, 1997).

1.7 Conclusions and knowledge gaps

The literature on Cd phytoavailability in soil and translocation to rice grains contains knowledge gaps in the areas of the role of redox (especially sulfide reduction and oxidation) and effects of the competitive ions Fe and Zn (through soil and foliar applications).

With regard to the role of redox and S cycling, one of the major knowledge gaps identified was the influence of sulfide composition and crystallinity on the form and relative amounts of Cd, Fe and Zn released to soil solution during oxidation. Cadmium is thought to be taken up and accumulated by rice preferentially to Zn in rice grains due to CdS being more readily oxidized than ZnS during pre-harvest drainage of the paddy, however this has not been directly confirmed for environmentally realistic sulfide compositions/mixtures. Solid-solution work on oxidative dissolution rates of Cd and Zn from a mixed Cd/Zn sulfide (McBride, Barrett *et al.* 2007) has shown that when the Zn:Cd ratio is high enough (above 20), Zn can be preferentially released during oxidation, helping to limit Cd accumulation in the rice grains. It is yet to be discovered whether this phenomenon holds true for soil-sulfide mixtures, or for solid-solution sulfides including Fe. Additionally, it has been suggested that metals are released in nanoparticulate form (Barrett and McBride, 2007), which would render them unavailable for plant uptake. Furthermore, the relative importance of the various processes controlling Cd and Zn availability in paddy soils has not been evaluated i.e. reductive dissolution and oxidative precipitation of oxides which sorb Cd, pH-dependent sorption, and sulfide precipitation and oxidative dissolution.

The competitive effect of Zn with Cd for relative solubility and plant uptake has been studied extensively in flooded environments (Bingham *et al.*, 1976; Honma and Hirata, 1974; Iimura and Ito, 1978; Khaokaew *et al.*, 2007; Koshino, 1973). However, environmentally relevant Zn:Cd ratios of >100:1 have generally not been employed, and no previous research has examined Zn:Cd interactions under flooded cultivation with standard pre-harvest drainage practices. Another unexplored area is the effects of foliar Zn-application on Cd uptake. To combat the problem of Cd translocation from shoots to grain after drainage of paddy fields, a Zn foliar-spray could be applied during this phase. This has only been tried with Okra plants where application compositions, quantities and frequencies were unclear (Salim *et al.*, 1995).

The competitive relationship between Fe and Cd requires further study. The effects of Fe have been studied mostly under solution culture (Adhikari *et al.*, 2006; Nakanishi *et al.*, 2006; Reddy and Patrick, 1977), with few results from pot (Koshino, 1973) or field experiments (Simmons *et al.*, 2003). The general consensus from solution culture is that Fe decreases Cd uptake and translocation to the grain (Adhikari *et al.*, 2006; Nakanishi *et al.*, 2006). However, field experiments have found higher Cd:Fe ratio in grains than other plant parts, presumably due to pre-harvest drainage facilitating oxidation of Fe(II) to precipitate as Fe(III) minerals, leaving relatively more Cd to be taken up by the plant (Simmons *et al.*, 2003). One pot study found that under continuous flooding, Fe (added as sulfate) had no effect on Cd uptake (Koshino, 1973). These conflicting reports should be clarified by examining the Cd concentration in grain at different soil Fe concentrations.

2 Aims and Objectives

The soil factors of redox, Fe and Zn are the focus of this thesis as they are particularly important for minimizing Cd transfer to rice grain. The outcomes of this research may eventually allow us to devise treatments so that these soils can still be used to grow rice and other crops without concentrating potentially harmful concentrations of Cd from the soil.

2.1 Thesis aims and objectives

Cadmium accumulates in paddy rice grain and becomes enriched relative to Fe and Zn during pre-harvest drainage, but the mechanism by which this occurs remains unclear. The risk to human health posed by Cd enrichment in paddy rice grain cannot be mitigated without an adequate understanding of this phenomenon.

There were two main objectives for this thesis:

1. To examine soil processes and mechanisms responsible for Cd accumulation and enrichment relative to Fe and Zn in paddy rice grain, and;
2. To determine whether soil amendments to paddy fields or foliar sprays to rice plants could be used to limit Cd accumulation and enrichment in paddy rice grain.

2.2 Release of dissolved cadmium and sulfur nanoparticles from oxidizing sulfide minerals (study 1)

Cadmium enrichment (relative to Fe and Zn) in paddy rice grain occurs during the pre-harvest drainage of flooded soil, which causes oxidative dissolution of sulfide minerals present in reduced soil. This process was investigated over a range of environmentally realistic Cd-containing sulfide minerals/mixtures, comparing Cd and Fe Co-precipitated sphalerites (0.6 to 1.2 mol % Cd, 24 mol % Fe, 74.8 to 75.4 mol % Zn) with mixtures of the pure sulfides at concentration ratios equal to co-precipitated sphalerites, as well as comparing freeze- and oven-dried sulfide minerals. Solutions were analyzed for dissolved and nanoparticulate elements to determine potential phytoavailability.

The specific aims of this study were to investigate the effects of co-precipitated (Cd, Fe, Zn) sulfide composition (at environmentally realistic metal ratios) and crystallinity on Cd release (relative to Fe and Zn) into solution during oxidation, as well as to determine whether Cd release is affected by galvanic interactions in physical mixtures of pure sulfides compared to co-precipitated sulfides of similar bulk chemical composition. A further aim of this research was to identify whether Cd and S are released in nanoparticulate form.

2.3 Cadmium solubility in paddy soils: effects of soil oxidation, metal sulfides and competitive ions (study 2)

Cadmium is a non-essential element for human nutrition and is an agricultural soil contaminant. Cadmium solubility in paddy soils affects Cd accumulation in the grain of rice. This is a human health risk, exacerbated by the fact that rice grains are deficient in iron (Fe) and zinc (Zn) for human nutrition. To find ways of limiting this potential risk, I investigated factors influencing Cd solubility relative to Fe and Zn during pre-harvest drainage of paddy soils, in which soil oxidation is accompanied by the grain-filling stage of rice growth. This was simulated in temperature-controlled “reaction cell” experiments by first excluding oxygen to incubate soil suspensions anaerobically, then inducing aerobic conditions.

The aim of this study was to determine the dominant processes controlling Cd solubility (relative to Fe and Zn) in flooded soils subjected to oxidizing conditions by simulating pre-harvest drainage of soils.

2.4 Limiting cadmium in paddy rice grain relative to iron and zinc (study 3)

In this study, foliar applications and soil amendments were examined to limit the accumulation of Cd in rice grain relative to Fe and Zn. Rice plants (*Oryza Sativa* L.) were grown in Cd-spiked soil in a laboratory pot experiment under flooded conditions incorporating pre-harvest drainage. Soil treatments were combinations of Fe, Mn, Zn and/or EDTA. Foliar treatments were Zn or EDTA applied during grain-filling.

The aims of this study were to examine the influence of competitive ions (Fe, Mn and Zn) in soils, foliar applied Zn, foliar applied EDTA and soil applied EDTA on the relative accumulation of Cd, Fe and Zn in brown rice from a rice-growing soil collected in Griffith, New South Wales (NSW), Australia.

3 Release of dissolved cadmium and sulfur nanoparticles from oxidizing sulfide minerals

3.1 Contribution Statement

The journal article titled 'Release of dissolved cadmium and sulfur nanoparticles from oxidizing sulfide minerals' has been accepted for publication in the Soil Science Society of America Journal on the 10th of January 2011, and was authored by Jennifer de Livera, Mike J. McLaughlin, Douglas Beak, Ganga M. Hettiarachchi and Jason K. Kirby.

The conceptualisation of this work was carried out by Jennifer de Livera, Mike J. McLaughlin, Ganga M. Hettiarachchi and Jason K. Kirby. Experimentation and documentation was carried out by Jennifer de Livera in consultation with Mike J. McLaughlin, Ganga M. Hettiarachchi, Douglas Beak and Jason K. Kirby. The conclusions drawn from this research were developed by Jennifer de Livera in consultation with Mike J. McLaughlin, Douglas Beak, Ganga M. Hettiarachchi and Jason K. Kirby. Comments on the draft document were given by Mike J. McLaughlin, Ganga M. Hettiarachchi and Douglas Beak, and incorporated in the final manuscript by Jennifer de Livera.

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Jennifer de Livera
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I verify that the above statement of contribution is accurate and give my consent for this document to be included in the PhD thesis of Jennifer de Livera.

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Jason Kirby
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de Livera, J., McLaughlin, M., Beak, D., Hettiarachchi, G. & Kirby, J. (2011) Release of dissolved cadmium and sulfur nanoparticles from oxidizing sulfide minerals.
Soil Science Society of America Journal, v. 75 (3), pp. 842 -854

4 Cadmium solubility in paddy soils: effects of soil oxidation, metal sulfides and competitive ions.

4.1 Contribution Statement

The journal article titled 'Cadmium solubility in paddy soils: effects of soil oxidation, sulfide equilibria and competitive ions' was accepted for publication in Science of the Total Environment on the 17th of December 2010, and was jointly authored by Jennifer de Livera, Mike J. McLaughlin, Ganga M. Hettiarachchi and Jason K. Kirby and Douglas G. Beak.

The conceptualisation of this work was carried out by Jennifer de Livera, Mike J. McLaughlin, Ganga M. Hettiarachchi and Jason Kirby. Experimentation and documentation was then carried out by Jennifer de Livera in consultation with Mike J. McLaughlin, Ganga M. Hettiarachchi, Douglas Beak and Jason K. Kirby. The conclusions drawn from this research were developed by Jennifer de Livera in consultation with Mike J. McLaughlin, Douglas Beak, Ganga M. Hettiarachchi and Jason K. Kirby. Comments on the draft document were given by Mike J. McLaughlin, Ganga M. Hettiarachchi, Douglas Beak and Jason K. Kirby, and incorporated into the finished manuscript by Jennifer de Livera.

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4.2 Abstract

Cadmium (Cd) is a non-essential element for human nutrition and is an agricultural soil contaminant. Cadmium solubility in paddy soils affects Cd accumulation in the grain of rice. This is a human health risk, exacerbated by the fact that rice grains are deficient in iron (Fe) and zinc (Zn) for human nutrition. To find ways of limiting this potential risk, we investigated factors influencing Cd solubility relative to Fe and Zn during pre-harvest drainage of paddy soils, in which soil oxidation is accompanied by the grain-filling stage of rice growth. This was simulated in temperature-controlled "reaction cell" experiments by first excluding oxygen to incubate soil suspensions anaerobically, then inducing aerobic conditions. In treatments without sulfur addition, the ratios of Cd:Fe and Cd:Zn in solution increased during the aerobic phase while Cd concentrations were unaffected and the Fe and Zn concentrations decreased. However, in treatments with added sulfur (as sulfate), up to 34 % of sulfur (S) was precipitated as sulfide minerals during the anaerobic phase and the Cd:Fe and Cd:Zn ratios in solution during the aerobic phase were lower than for treatments without S addition. When S was added, Cd solubility decreased whereas Fe and Zn were unaffected. When soil was spiked with Zn the Cd:Zn ratio was lower in solution during the aerobic phase, due to higher Zn concentrations. Decreased Cd:Fe and Cd:Zn ratios during the grain filling stage could potentially limit Cd enrichment in paddy rice grain due to competitive ion effects for root uptake.

Abbreviations: CEC (cation exchange capacity), Cd (cadmium), Cl^- (chloride), DIC (dissolved inorganic carbon), DOC (dissolved organic carbon), EC (electrical conductivity), Eh (redox potential), Fe (iron), IC (ion chromatography), ICP-MS (inductively coupled plasma mass spectrometry), ICP-OES (inductively coupled plasma optical emission spectroscopy), Mn (manganese), NO_3^- (nitrate), PO_4^{3-} (phosphate), PZC (point of zero charge), SO_4^{2-} (sulfate), S (sulfur), SRB (sulfate-reducing bacteria), Zn (zinc).

4.3 Introduction

Cadmium (Cd) accumulates in soil through both natural (e.g. weathering) and anthropogenic (e.g. industrial) processes. Anthropogenic sources of Cd in soil include fertilizers, biosolids, mine, smelter and other industrial wastes (Friberg *et al.*, 1974; Nordberg, 1995). The main risk of soil Cd to animals and humans is through exposure from contaminated agricultural soils through the food chain (Chaney *et al.*, 1996). Cadmium is accumulated in plants more readily than most other metals, and can be translocated into the edible parts before any signs of phytotoxicity (Li *et al.*, 1994; Tudoreanu and Phillips, 2004). The health effects caused by Cd exposure to humans are well documented. The consumption of rice with elevated Cd concentrations has been shown to be responsible for 'Itai-Itai' disease which arose in Japan in the 1960s (Nordberg, 2004). Even today, rice is the leading source of Cd burden for the Japanese population (Tsukahara *et al.*, 2003). Rice is one of the most important crops grown for human consumption in the world, and Cd accumulation in rice grains poses a potential health risk (McLaughlin and Singh, 1999). It is often not economically feasible to remediate Cd-contaminated soils, so subsistence farmers continue to use them for paddy rice production. Paddy rice grains accumulate more Cd than the nutrients iron (Fe) and zinc (Zn), based on their relative soil concentrations (Chaney *et al.*, 1996). This combination of high Cd and low Fe and Zn in rice grain can contribute to enhanced Cd bioavailability to people who subsist on a rice diet (McLaughlin *et al.*, 1999b). It is therefore important to investigate the mechanism(s) by which the soil solution contributes to the enrichment of Cd in paddy rice grain relative to Fe and Zn.

The enrichment of Cd in paddy rice grain occurs during soil oxidation, which accompanies pre-harvest drainage of the flooded paddy (Iimura, 1981b; Inahara *et al.*, 2007). Pre-harvest drainage is typically initiated two weeks before plant maturity, coinciding with the grain-filling phase of rice growth (Figure 4-1). Therefore, it is during this time that Cd becomes more available to the plant relative to Fe and Zn (Honma and Hirata, 1977; Saito and Takahashi, 1978a). This may be due to higher Cd:Fe and Cd:Zn ratios in the soil solution, and therefore less competition from Fe and Zn with Cd at the root surface for uptake into the rice plant (Smolders *et al.*, 1997). It has been suggested that the increased Cd:Fe and Cd:Zn ratios are due to higher concentrations of Cd in soil solution as a result of differential oxidation of sulfide minerals (Chaney *et al.*, 1996). However, it has been shown that this is unlikely to give rise to higher Cd:Zn ratios in soil solution for Cd-Zn solid solution sulfide minerals (Barrett and McBride, 2007). Differential oxidation of sulfide minerals are also unlikely to give rise to higher Cd:Fe and Cd:Zn ratios when all three metals are present in sulfide minerals at molar ratios that are environmentally realistic in soils (de Livera *et al.*, 2008).

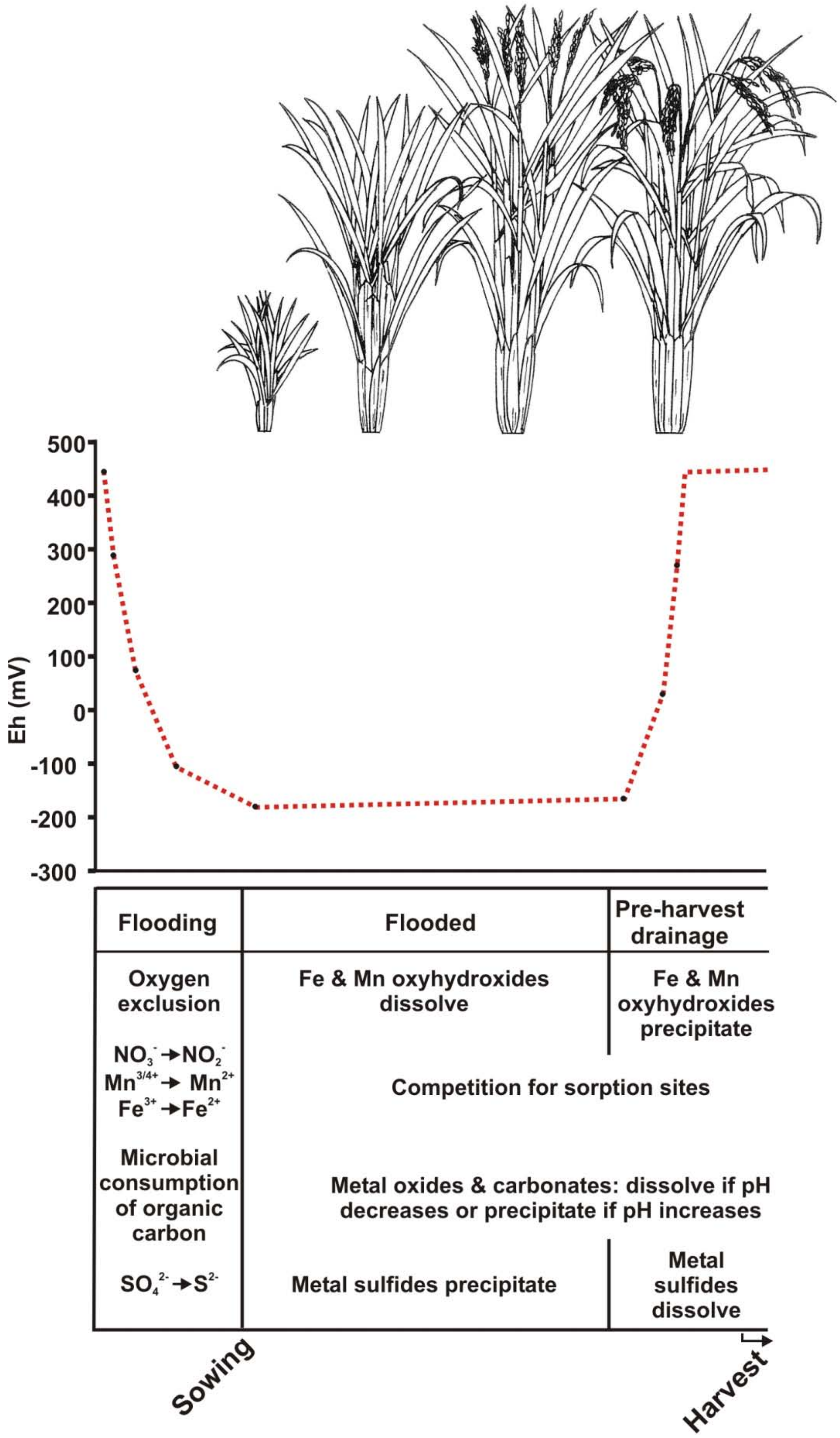

Figure 4-1. Potential redox driven changes in soil chemistry occurring during rice cultivation.

Alternatively, Cd:Fe and Cd:Zn ratios in soil solution may become higher by competitive sorption of Zn over Cd (Davranche and Bollinger, 2000). Iron and manganese (Mn) oxides and oxyhydroxides precipitate during soil oxidation, decreasing the Fe concentration in soil solution and providing adsorbent surfaces which are known to control Cd, Fe and Zn solubilities (Atkinson *et al.*, 2007; Charlatchka and Cambier, 2000; Chuan *et al.*, 1996). Different affinities of adsorbent soil constituents for each metal may result in higher Cd:Zn ratios in soil solution during oxidation. Therefore, addition of Zn to soil may be a feasible method of lowering the Cd:Zn ratio in soil solution during oxidation by providing a higher concentration of Zn in soil solution. This would give rise to lower Cd:Zn ratios in soil solution and reduce the risk to human health from Cd accumulation in paddy rice grain.

The aim of this study was to determine the dominant processes controlling Cd solubility (relative to Fe and Zn) in flooded soils subjected to oxidizing conditions by simulating pre-harvest drainage of soils. Field contaminated and laboratory spiked paddy soils were compared to ascertain the effect of soil aging on Cd solubility (relative to Fe and Zn) under variable redox conditions. A laboratory spiked soil was used to test the effects of soil redox processes and the presence of added SO_4^{2-} or Zn on the Cd:Fe and Cd:Zn ratios in soil solution. The effects of soil redox processes and the presence of added SO_4^{2-} on the Cd:Fe and Cd:Zn ratios in soil solution were also tested using a field contaminated paddy soil.

4.4 Materials and methods

4.4.1 Preparation and characterization of materials

Uncontaminated Ferrous Hydragric Anthrosol (Soil Survey Staff, 1992) (Loam) paddy soil was collected from Griffith, New South Wales, Australia (0-20 cm) and a Ferrous Hydragric Anthrosol (Soil Survey Staff, 1992) field contaminated (Zn mine waste) paddy soil was obtained from ChangSha, Hunan Province, China (0-20 cm) (Table 4-1). These two soils were chosen in order to compare metal solubilities between laboratory spiked and field-contaminated soils. It is recognized that field aging influences speciation (solid and solution phase) of metals including Cd, which in turn has a significant influence on their fate and behavior in soils (Cui *et al.*, 2008; Kirkham, 2006).

Table 4-1. Selected soil properties of uncontaminated and contaminated paddy soils used in reaction cell experiments. Cation exchange capacity (CEC) is the sum of exchangeable calcium (Ca), potassium (K), sodium (Na) and magnesium (Mg).

Soil property	**Units**	**Uncontaminated**	**Contaminated**
pH	$-\log_{10}[H^+]$	6.4	8.0
Electrical conductivity	µS cm^{-1}	141	430
Cation exchange capacity	$cmol_{(+)}\ kg^{-1}$	21.1	19.0
Soil less than 2 µm in particle size	wt %	42.4	16.1
Soil greater than 2 µm in particle size	wt %	56.7	83.0
Quartz, SiO_2	wt %	40-50	50-70
Kaolinite, $Al_2Si_2O_5(OH)_4$	wt %	10-20	5-10
Illite, $K_{0.65}Al_2(Al_{0.65}Si_{3.35}O_{10})(OH)_2$	wt %	10-20	-
Interstratified illite-smectite	wt %	10-20	-
Muscovite, $KAl_2(OH_2)AlSi_3O_{10}$	wt %	-	20-40
Chlorite $(Mg,Fe)_3(Si,Al)_4O_{10}(OH)_2{\cdot}(Mg,Fe)_3(OH)_6$	wt %	-	1-5
K- and Na- feldspar, $K(AlSi_3O_8)$, $Na(AlSi_3O_8)$	wt %	5-10 for each	1-5 for each
Anatase, TiO_2	wt % < 2 µm	1-5	-
Hematite, Fe_2O_3	wt % < 2 µm	1-5	1-5
Clay content	% < 2 µm	40	13
Silt content	% 2-20 µm	9	38
Sand content	% 20-2000 µm	51	47
Total carbon	wt %	1.2	1.55
Extractable iron	wt %	1.1	1.30
Cadmium	mg kg^{-1}	0.18	4.5
Zinc	mg kg^{-1}	42	1,625

Soils were ground and sieved to < 2 mm diameter. The pH and electrical conductivity (EC) were determined in 1:5 (m/v) soil:water suspensions (Houba *et al.*, 2000) using a model 420 pH electrode (Thermo Orion, Waltham, MA, U.S.A) and a model CDC641T conductivity electrode (Radiometer Analytical, Lyon, France). Extractable sulfur (S), as a proxy for 'plant-available' S, was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Spectro Analytical Instruments, Kleve, Germany) following extraction with 0.25 M potassium chloride (KCl) at 40 °C (Blair *et al.*, 1991). Extractable Fe was determined by ICP-OES following extraction of 1.0 g soil with 50 mL 0.74 M sodium citrate and 5.7 mmol sodium dithionate on an end over end shaker for 16 h at 25 ºC (Rayment and Higginson, 1992). Mineralogical composition was determined by powder x-ray diffraction (XRD); patterns were collected using an X'Pert Pro (PANalytical, Almelo, the Netherlands) multi-purpose diffractometer using Fe-filtered Co K_α radiation, a 1/2° divergence slit, a 1° anti-scatter slit and a fast silicon strip detector. The diffraction pattern was recorded from 0 to 80 in steps of 0.017 °2θ with a 0.5 second counting time per step, and logged to data files for analysis. X-ray diffraction was performed on both the bulk soil and the < 2 µm sized fraction.

The uncontaminated soil was spiked with varying concentrations of Cd and Zn chloride salt solutions by evenly spraying the soil with a fine mist of these solutions. Solutions were prepared using reagent-grade chemicals. Chloride salts were used rather than nitrate or sulfate salts due to the potentially oxidizing effect of nitrate (Ponnamperuma, 1972) and interference of sulfate with the sulfate treatment. The final concentrations of metals spiked (mol kg^{-1}) were such that Cd and Zn would be at concentrations relevant to anthropogenically contaminated soils (Table 4-2). For example, the average concentration of Cd in soils typically ranges from 1.7 to 2.7 µmol kg^{-1} (Garrett, 1995), but can occur geogenically at up to 62 µmol kg^{-1} (Bohn *et al.*, 2001). Zinc concentrations in polluted rice producing soil have been found at between 0.21 to 3.8 mmol kg^{-1} in Zhejiang Province (specifically the Hangzhou-Jiazing-Huzhou Plain) in South-East China (Liu *et al.*, 2006). After spiking the soil, it was mixed by hand and air-dried overnight.

Table 4-2. Soil treatments used in controlled-atmosphere reaction cell experiments. Metal concentrations shown for the uncontaminated soil (first 4 treatments) are spiked nominal additions (i.e. excluding geogenic concentrations). Where no spike was added geogenic concentrations of iron (Fe) and zinc (Zn) are shown in parentheses. The geogenic concentration of cadmium (Cd) was 0.0016 mmol kg^{-1}. Sulfur (S) concentrations shown in parentheses are extractable S. Geogenic Fe concentrations shown are from citrate/dithionate extractions. Molar ratios are based on the sum of geogenic and spiked metals. Soil treatments were Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn), Cd and low Zn-spike with added SO_4^{2-} (Cd + LZn + SO_4^{2-}), contaminated soil (contaminated) and contaminated soil with added SO_4^{2-} (contaminated soil + SO_4^{2-}).

Treatment name	Cd (μmol kg^{-1})	Fe (mmol kg^{-1})	Zn (mmol kg^{-1})	S (mmolkg^{-1})	Cd/Fe molar ratio	Cd/Zn molar ratio
Cd	24	(197)	(0.65)	(0.41)	0.00012	0.0372
Cd + LZn	24	(197)	1.49	(0.41)	0.00012	0.0112
Cd + HZn	24	(197)	3.05	(0.41)	0.00012	0.0065
Cd + LZn + SO_4^{2-}	24	(197)	1.49	40	0.00012	0.0112
Contaminated soil	40	233	25	3.44	0.00017	0.0016
Contaminated soil + SO_4^{2-}	40	233	25	92	0.00017	0.0016

The EC of the spiked soils was determined (Rayment and Higginson, 1992) and the soils were leached to remove excess soluble salts that may have resulted from the spiking of the soil. Leaching was carried out 6 times with artificial rainwater (Stevens *et al.*, 2003) until the EC stabilized at a value < 500 µS cm^{-1}. Spiked soils were aged for 4 weeks by keeping the soil at field capacity to allow aging reactions to occur (McLaughlin *et al.*, 2010). The field capacity of the soils were determined by recording the gravimetric water content of the soils after equilibration on a tension plate with a 100 cm hanging water column (Marshall and Holmes, 1979).

After leaching and aging, total metal concentrations in the soils were determined using open vessel strong acid extraction with *aqua regia* 1:3 (v/v) mixture of analytical grade concentrated nitric and hydrochloric acids (Fisher Scientific) (Zarcinas *et al.*, 1996) and analysis by ICP-OES (Table 4-1).

To provide a carbon source for microbially-mediated reactions in the soil, straw was harvested from rice plants (*var.* Japonica, *cv.* Quest) grown in the laboratory in flooded, uncontaminated paddy soil. Before use, the straw was dried, ground and analyzed for total C by infrared detection of CO_2 (LECO CNS-2000, St. Joseph, Michigan, U.S.A) following high temperature combustion in an oxygen atmosphere. Straw was also digested in analytical-grade concentrated nitric acid (Fisher Scientific) and analyzed for Cd, Fe and Zn concentrations by ICP-OES (Zarcinas and Cartwright, 1983).

4.4.2 Redox adjustment experiments

Redox adjustment of soil suspensions was carried out using a temperature-controlled "reaction cell" apparatus (Figure 4-2). Soil suspensions were kept under 'reduced conditions' through air exclusion using nitrogen, then oxidation was induced by exposure to the atmosphere. Experiments were run in triplicate and the soil treatments (Table 4-2) were: Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn), Cd and low Zn-spike with added SO_4^{2-} (Cd + LZn + SO_4^{2-}), contaminated soil (contaminated) and contaminated soil with added SO_4^{2-} (contaminated soil + SO_4^{2-}). Into each reaction cell 5.0 g soil, 2.0 g rice straw and sulfate (when necessary) were added. Sulfate, as sodium sulfate (Na_2SO_4), was added equimolar to the sum of the maximum solution concentrations of Fe, Mn and Zn observed in control experiments. Finally, 400 mL of deionised water was added to the reaction cell. The lid, which had a sampling port, nitrogen port, stirring wand (spHinner, Gerard Scientific), silver (Ag)/AgCl ORP electrode (Model S500C, Sensorex, Garden Grove, CA, U.S.A) and stoppered hole, was then screwed on. The sampling tube was installed vertically into each reaction cell near the bottom of the suspension, with the end of the tubing outside of the cell and fitted with a gas tight valve keeping the reaction cell airtight. After sealing the reaction cells, they were submerged in a 25 ± 2 °C water bath so that the reaction kinetics were controlled during the course of the experiment.

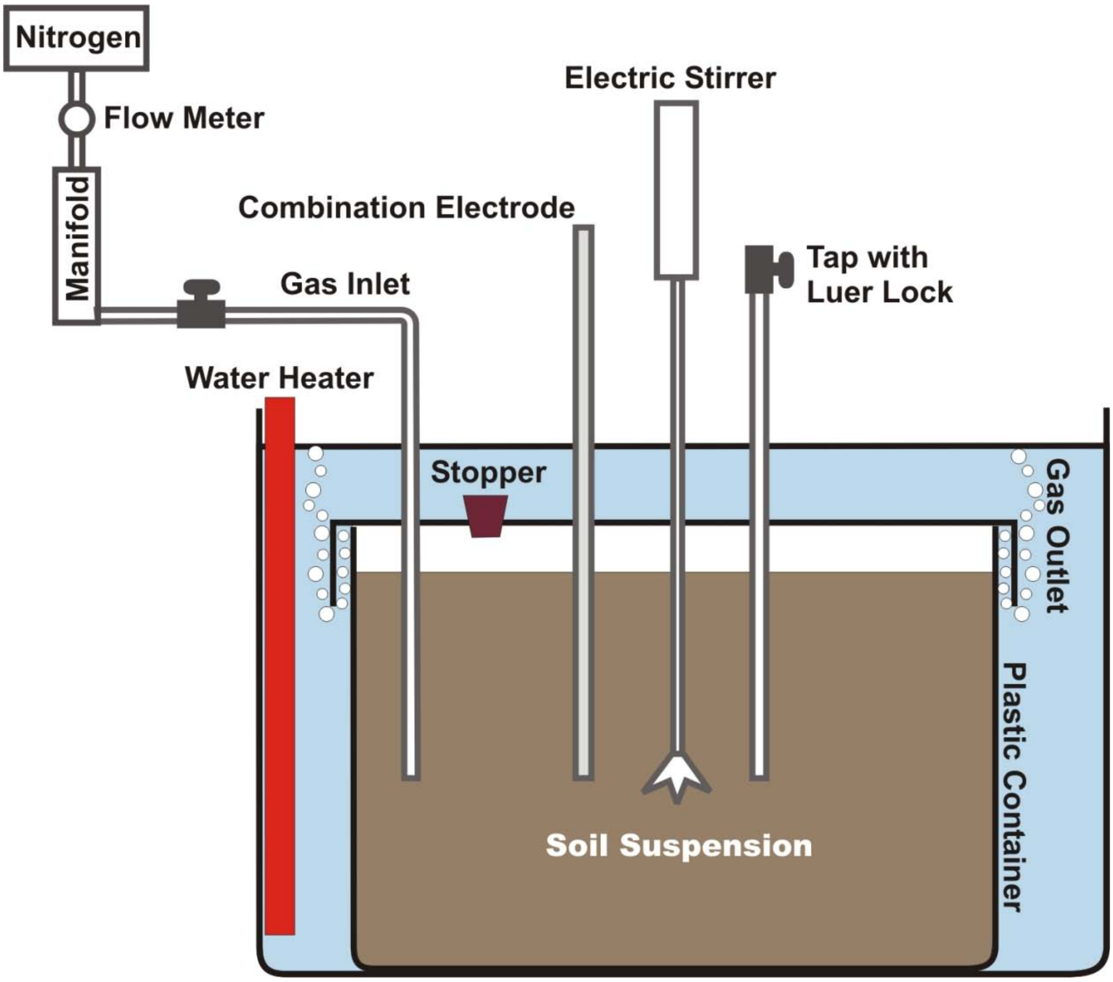

Figure 4-2. Temperature controlled reaction cell apparatus used to conduct redox adjustment experiments through an oxygen-exclusion followed by exposure to the atmosphere.

After submergence, nitrogen gas (N_2) was bubbled for 42 days in the contaminated soil and 30 days in the uncontaminated soil, and samples collected at 0, 10, 20, 30 and 42 days (in the contaminated soil). This comprised the anaerobic phase, the time period of which was determined in preliminary experiments and based on the length of time required for Fe concentrations to become constant in solution. Oxidation was initiated on day 42 for the contaminated soil and contaminated soil + SO_4^{2-} treatments and on day 30 for the other treatments, by stopping N_2 bubbling, moving flasks onto a raised platform in the water bath, and opening the stoppered hole to allow exposure to air. Samples were collected at 1, 3, 7 and 14 days after initiation of oxidation.

Samples were collected by drawing 20 mL soil slurry through the sampling port after discarding the first 2 mL, then disconnecting the syringe and transferring it directly into an anaerobic chamber. Inside the anaerobic chamber, the samples were passed through a 2.5 µm filter to remove larger particles, and then passed through a 0.2 µm filter. The syringes and filters were flushed with nitrogen in the anaerobic chamber before use. The pH of the filtered solution was measured and an aliquot of filtrate was acidified with 100 µL 6 M hydrochloric acid (HCl) for ICP-OES analysis of calcium (Ca), Fe, magnesium (Mg), Mn, sodium (Na), phosphorus (P), S and Zn concentrations, and inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500ce, Santa Clara, CA, U.S.A) determination of copper (Cu), Cd and nickel (Ni) concentrations. Another aliquot of the filtered sample was transferred to a glass vial and frozen for analysis of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), nitrate (NO_3^-) and SO_4^{2-}. Dissolved organic carbon was determined by the difference between total carbon and DIC. Total carbon (C) was determined by high temperature combustion (Skalar Formacs HT TOC Analyzer, Breda, Netherlands) of C to carbon dioxide (CO_2) and measurement by absorbance at 2349 cm^{-1} as determined by a non-dispersive infrared detector. Inorganic C was similarly determined by infrared following CO_2 generation by sample acidification. Nitrate and SO_4^{2-} were determined by ion chromatography (IC) (Dionex ICS-2500, Sunnyvale, CA, U.S.A). Finally, an aliquot of sample was used for colorimetric determination of soluble Fe(II) and S^{2-} (Greenberg *et al.*, 1992; Sparks, 1996) using a UV-visible spectrophotometer (Shimadzu UV-1601, Kyoto, Japan), with color developed in the anaerobic chamber.

The redox potential (Eh) was recorded at sampling times by converting ORP measurements from the *in situ* electrode (Figure 4-2) to Eh values by adding 199 mV

(Rabenhorst *et al.*, 2009). The redox status of the soil suspension was determined by Eh in conjunction with the absence or appearance of reduced and oxidized species, i.e. under anaerobic conditions and upon reduction we observed a decrease in concentrations of NO_3^-, increases in Mn (presumably Mn(II) as other species are only sparingly soluble), Fe(II) and DOC, and a decrease in SO_4^{2-}. Upon the addition of air, aerobic conditions and oxidation we observed decreases in concentrations of Mn (presumably Mn(II)), Fe(II) and DOC as well as an increase in SO_4^{2-}. The pH was not controlled in these experiments and was allowed to change with the Eh, as this represented a closer approximation to the chemical changes occurring in a paddy field.

The minimum amount of Fe associated with the solid phase during reduction was estimated by the difference between maximum solution Fe concentrations and extractable soil Fe determined prior to experiments. The calculation was based on the Cd treatment, as Fe concentrations in solution were not significantly different between Cd, Cd + LZn, Cd + HZn, and Cd + LZn + SO_4^{2-} treatments. The amount of Cd and Zn associated with the solid phase was estimated for the reduced and oxidized phases separately, by calculating the difference between the mean solution concentration and the total soil metal initially present.

To test for the presence of sulfide minerals during the reduced phase, samples from Cd + LZn + SO_4^{2-} and contaminated soil + SO_4^{2-} were flash-frozen in liquid nitrogen and analyzed using the chromium-reducible sulfide (CRS) method (Ahern *et al.*, 2004).

4.4.3 Data analysis and interpretation

Analysis of variance (ANOVA) was carried out using Genstat statistical software package (10th edition, VSN International, Hempstead, UK) with treatments and time as factors. Before ANOVA, data were divided into two groups; a ‘reduced’ group containing all data points from day 10 to the day of oxidation, and an ‘oxidized’ group containing all data points from the day of oxidation onwards. Data were transformed before statistical analysis when necessary to normalize variances: In the reduced group, Ca, Cd, Cd:Zn and Zn were square root transformed, and Cd:Fe, Fe, Mn, Ni, and NO_3^- were $\log_{10}$ transformed. In the oxidized group, DOC, Mn, NO_3^-, S, and SO_4^{2-} were square root transformed, Cd:Fe, Cd:Zn, Fe, Fe(II), Ni, and Zn were $\log_{10}$ transformed and Cd was $\log_e$ transformed. Analysis of variance was then carried out with significant effects of soil treatments determined by the least significant differences of means (l.s.d) at the 5 % confidence level, and are indicated on graphs and tables by the letters of the alphabet such that different letters indicate significant differences, and treatments sharing a letter are not significantly different.

Geochemical modeling was carried out using Visual MINTEQ version 2.53 (Jon Peter Gustafsson, KTH, Dept. of Land and Water Resources Engineering, Stockholm, Sweden) to assist in identifying which minerals were potentially involved in precipitation/dissolution reactions during experiments. Solutions of all treatments were modeled at the last day of the reduced phase (day 30 for spiked soils and day 42 for contaminated soils) and the last day of the oxidized phase (day 44 for spiked soils and day 56 for contaminated soils). Experimentally-determined Eh values and concentrations of Ca, Cd, Cu, Fe(II), K, Mg, Mn(II), Na, NO_3^{2-}, Ni, SO_4^{2-}, and Zn were specified. Experimentally-derived DOC was specified and defined as Gaussian, DIC as carbonate and P as phosphate (PO_4^{3-}). Iron(III) was calculated by subtracting Fe(II) from total Fe. Manganese(III), although likely precipitated had to be included in the model in order to correctly calculate Mn speciation between redox states. Manganese(III) was estimated at oxidized time points by subtracting Mn in solution (assumed to be soluble Mn(II)) on the last day of oxidation from Mn in solution on the last day of the reduced phase. Manganese(VI) was not specified as it is not in the MINTEQ database. Chloride (Cl^-) concentrations were adjusted in the model to obtain charge balance, after confirmation in a subset of treatments that this was the ion accounting for the charge imbalance. Iron(II/III), Mn(II/III) and HS^-/SO_4^{-2} were specified as redox couples and ionic strength was calculated by MINTEQ. Carbon dioxide gas was specified at atmospheric pressure at oxidized time points but not specified for the reduced time points, to accurately reflect the experimental conditions; i.e. the system was closed to the atmosphere during reduction (with any evolved gases flushed out of the system by N_2 bubbling) but open to the atmosphere during oxidation. Over-saturated solids were not allowed to precipitate, i.e. mineral precipitation was not allowed to reach equilibrium in the modeling calculations. Saturation indices were determined for bixbyite ($(Mn,Fe)_2O_3$), cadmium phosphate ($Cd_3(PO_4)_2$), ferrihydrite ($FeOOH.0.4H_2O$), FeS, goethite (α-FeO(OH), cadmium sulfide (CdS), greigite (Fe_3S_4), hausmannite (Mn_3O_4), hematite (Fe_2O_3), lepidocrocite (γ-FeO(OH), mackinawite (FeS), maghemite (γ-Fe_2O_3), magnesioferrite ($MgFe_2O_4$), magnetite (Fe_3O_4), manganite (MnO(OH)), pyrite (FeS_2), rhodocrosite ($MnCO_3$) and sphalerite (ZnS).

4.5 Results and discussion

4.5.1 Soil oxidation

Metal concentrations in the soil solution were likely derived mainly from the soil rather than decomposition of the rice straw. The added rice straw contained < 8 µmol kg^{-1} Cd, 2.3 mmol kg^{-1} Fe, and 0.95 mmol kg^{-1} Zn, much lower than the contribution of these metals from the spiked soils.

In all soil treatments the Eh decreased upon the exclusion of air and remained negative for the duration of the reduced phase. In the absence of oxygen, soil microbes respire by using oxidized soil components in the order of NO_3^-, Mn(III/VI) (present in oxide phases), Fe(III)(present in oxide phases), SO_4^{2-} and dissimilation products of organic matter. These species accept electrons in the process, causing a decrease in the redox potential as NO_2^-, Mn(II), Fe(II), low molecular weight organic acids and S^{2-} are generated (Ponnamperuma, 1964). In contrast, Alewell *et al.* (1996) found in an investigation of the redox status of three different forested wetlands that the sequential reduction chain did not seem applicable at the micro- or mesoscale because of (1) high small-scale heterogeneity and (2) an absence of clear relationships between redox indicative parameters. The authors suggested the second point may be due to redox processes occurring simultaneously at the investigated spatial and temporal scales.

When air was introduced into the reaction cells, oxidation occurred and the Eh increased to positive values within 24 hours (Figure 4-3) as the reduced species were oxidized. In all treatments without SO_4^{2-} added, soil oxidation caused Cd:Fe and Cd:Zn molar ratios in solution to increase with time (Figure 4-4 and Figure 4-5). This occurred as a result of significant decreases in Fe (equivalent to Fe(II) in all solution samples, data not shown) and Zn concentrations whereas Cd concentrations were not affected by oxidation. This result is supported by the work of Miao *et al*. (2006), where Cd solubility was unaffected by oxidation of a reduced sediment suspension.

The pH increase during oxidation (Figure 4-3), was likely due to aerobically-driven microbial consumption of DOC and H^+ to form CO_2 and H_2O (Barekzai and Mengel, 1993; Dupraz *et al.*, 2009). This explanation is supported by observations of a concurrent decrease in DOC and increase in DIC (CO_2) (data not shown) as was also observed by Yan *et al*. (1996).

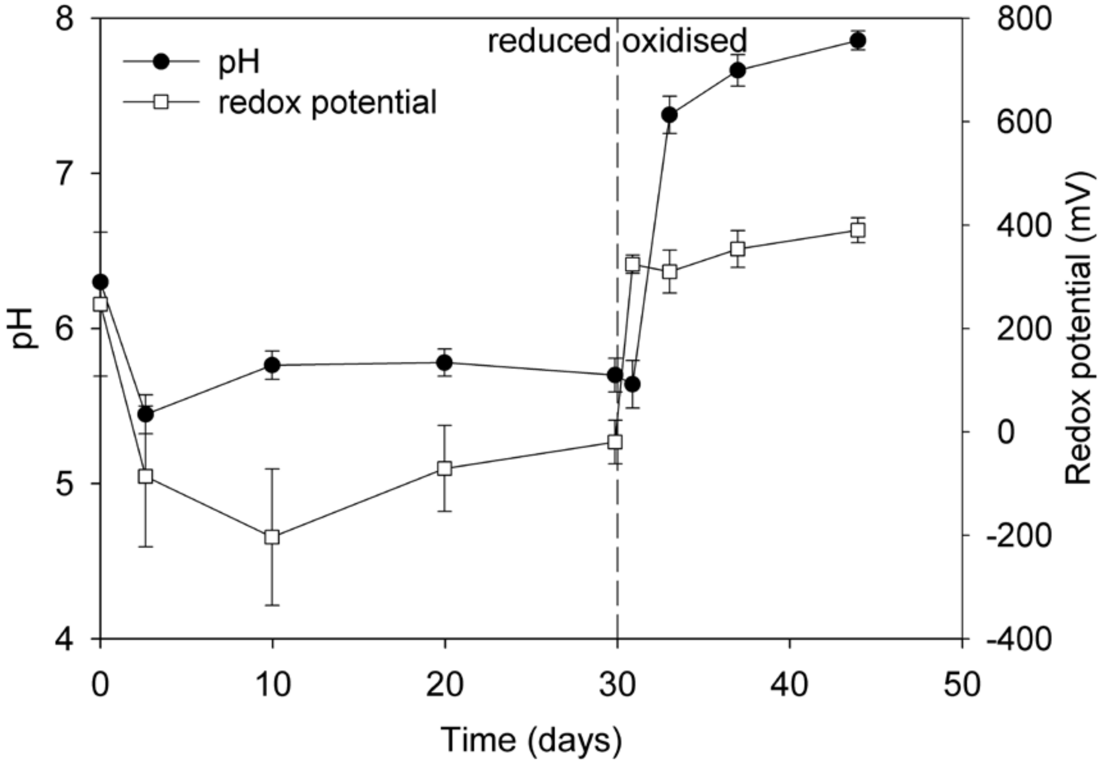

Figure 4-3. pH and redox changes with time during reduction and oxidation. Only Cd-spiked soil is shown as it is representative of all treatments. Error bars are standard error (n = 3).

In treatments without SO_4^{2-} added, the increase in Cd:Fe and Cd:Zn ratios in solution during oxidation was not likely controlled by redox-driven transformations of S, as the time trends in Fe and Zn concentrations were not correlated with concentrations of SO_4^{2-}. The S in solution was present entirely as SO_4^{2}, which significantly decreased during the reduced phase in all treatments (Figures 1 and 2, supporting information), and significantly increased during oxidation only in the Cd + LZn + SO_4^{2-} treatment (Figure 1, supporting information), whereas concentrations of Fe and Zn only decreased significantly during oxidation and Cd was unchanged (Figure 4-3; Figure 4-4; Panel B, Figure 4-5; and Figure 6, supporting information).

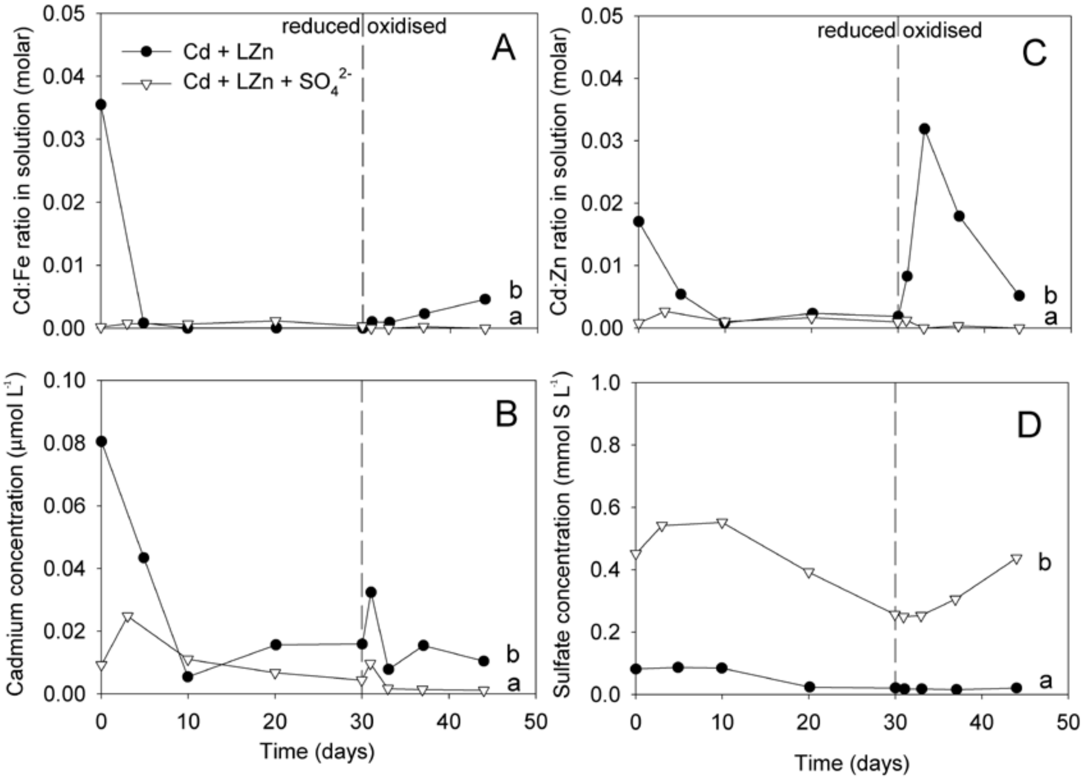

Figure 4-4. Effect of sulfur (S) addition on the cadmium:zinc (Cd:Zn) ratio in solution during reduction and oxidation. All panels share the same legend and x axis. Significance letters are for the oxidized phase. Panel A: Cd:iron(Fe) molar ratio in soil solution. Panel B: Cadmium concentration. Panel C: Cd:Zn molar ratio. Panel D: Sulfate concentration. Iron and Zn concentrations are shown in supporting information. Soil treatments are Cd and low Zn-spiked (Cd + LZn), and Cd and low Zn-spike with added SO_4^{2-} (Cd + LZn + SO_4^{2-}).

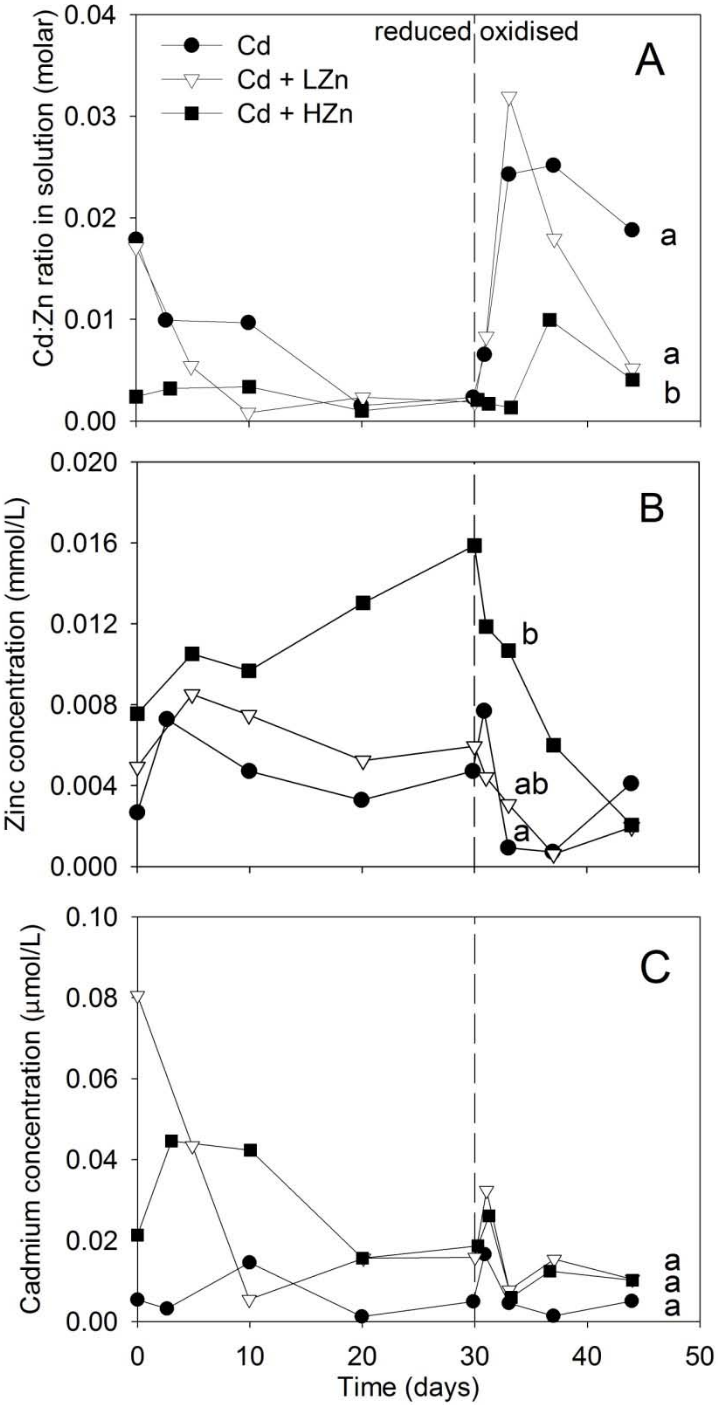

Figure 4-5. Effect of zinc (Zn) spiking on cadmium(Cd):Zn ratio in solution during reduction and oxidation. All panels share the same legend and x axis. Significance letters are for the oxidized phase. Panel A: Cd:Zn ratio. Panel B: Zinc concentration. Panel C: Cadmium concentration. Sulfate concentration is shown in supporting information. Soil treatments are Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn spiked (Cd + HZn).

The decrease in Fe and Zn concentrations during oxidation is likely due to the increased formation of Fe/Mn oxides and oxyhydroxides (Ponnamperuma, 1972), and subsequent increased adsorption of Zn to the newly precipitated Fe/Mn solid phase and other soil constituents as a result of the pH increase. This is supported by the modeling results, which indicated that Fe/Mn oxides and oxyhydroxides were more dissolved during flooding and more precipitated during oxidation (Table 2, supporting information). Additionally, our calculations (based on solution concentrations and Fe content of the starting material) showed that the proportion of soil Fe that was precipitated increased from 80 % during the reduced phase to 100 % during the oxidized phase. Soil Fe was likely present in the soil initially as minerals such as hematite, which was detected by XRD analysis of the soil at 1 to 5 wt % in the < 2 µm fraction (Table 4-1) (Li *et al.*, 2006; Music and Ristić, 1992), and other x-ray amorphous Fe oxides such as ferrihydrite. Soil Fe that was reprecipitated following the reduction and oxidation cycle was more likely dominated by highly adsorbent amorphous Fe(III) oxhydroxides.

In contrast to the changes in Zn solubility with oxidation, Cd concentrations did not change with oxidation, and were consistently low throughout the experiment. This trend is likely due to a stronger association of Cd (relative to Zn) with soil Fe minerals. This is supported by calculations that the proportion of Cd associated with the solid phase was greater than that of Zn, at > 92 % in all treatments during the reduced and oxidized phases, compared to between 36 to 97 % for Zn (Table 3, supporting information). Both Cd and Zn are expected to be adsorbed by Fe oxides and oxyhydroxides (Harvey *et al.*, 1983; Li *et al.*, 2006; Music and Ristić, 1992). Cadmium and Zn may also have been associated with other soil constituents such as organic matter and clay minerals (Atanassova, 1999; Gao *et al.*, 2004b; Huang and Fuerstenau, 2001; Kashem and Singh, 2004; Lackovic *et al.*, 2004; MacNaughton, 1977).

Table 4-3. Change in sulfate, cadmium and zinc concentrations in solution during the reduced phase. Positive values indicate a decrease in concentration. Soil treatments were Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn), Cd and low Zn-spike with added SO_4^{2-} (Cd + LZn + SO_4^{2-}), contaminated soil (contaminated) and contaminated soil with added SO_4^{2-} (contaminated soil + SO_4^{2-}).

Treatment name	**Sulfate (mmol L^{-1})**	**Cadmium (µmol L^{-1})**	**Zinc (mmol L^{-1})**
Cd	0.093	0.036	0.0026
Cd + LZn	0.065	0.028	0.0026
Cd + HZn	0.10	0.026	0.0056
Cd + LZn + SO_4^{2-}	0.29	0.021	0.0054
Contaminated soil	0.13	0.074	0.020
Contaminated soil + SO_4^{2-}	1.2	10.0	0.039

4.5.2 Metal sulfide precipitation and dissolution

The Cd + LZn + SO_4^{2-} treatment had significantly lower solution ratios of Cd:Fe and Cd:Zn than the Cd + LZn treatment during oxidation (Panels A and C, Figure 4-4). This was due to lower concentrations of Cd (Panel B, Figure 4-4) in the Cd + LZn + SO_4^{2-} treatment, while Fe and Zn were unaffected by the treatment (Figure 3 and Figure 4, supporting information). The effect of SO_4^{2-} addition was similar in the contaminated soil, where Cd:Fe and Cd:Zn ratios in solution were lower in the contaminated soil + SO_4^{2-} treatment during oxidation (Panels A and C, Figure 5, supporting information), as compared to the contaminated soil (without SO_4^{2-}) treatment. Cadmium and Fe concentrations were both decreased by the treatment but to a greater extent for Cd (Panels B and D, Figure 5, supporting information), while the concentration of Zn was unaffected (Figure 6, supporting information). These lower Cd:Fe and Cd:Zn ratios in the soil solutions of the SO_4^{2-} added treatments could potentially result in lower Cd:Fe and Cd:Zn ratios in paddy rice grain, and should be investigated further in plant-based studies.

The lower Cd:Fe and Cd:Zn ratios observed in soil solutions of the SO_4^{2-} added treatments may be due to greater precipitation of sulfide minerals during the reduced phase. In all treatments, SO_4^{2-} was removed from solution during the reduced phase (Panel D, Figure 4-4; Figures 1 and 2, supporting information). It is unlikely that SO_4^{2-} was adsorbed by soil Fe minerals, as the pH did not change and would be expected to increase as a result of SO_4^{2-} adsorption (Ishiguro *et al.*, 2006). It is more probable that SO_4^{2-} was removed from solution by reduction to S^{2-} and precipitation to form metal sulfides (Ponnamperuma, 1972). Dissolved sulfide (S^{2-}) was not detected in soil solutions (data not shown). The amount of SO_4^{2-} removed from solution between days 0 and 30 of the reduced phase was calculated to be sufficient to precipitate with all of the Cd and Zn lost from solution (Table 4-3). The reduction of SO_4^{2-} to S^{2-} and subsequent precipitation of Fe(II) to form FeS_x(s) is also expected, although the continuous dissolution of Fe oxyhydroxides masks the effect of this on Fe concentrations in solution.

Metal sulfides likely precipitated to a greater extent in the Cd + LZn + SO_4^{2-} and contaminated + SO_4^{2-} soil treatments as compared to the Cd + LZn and contaminated soil treatments. The Eh and pH conditions encountered were more favorable for sulfide formation in the SO_4^{2-}-containing treatments (Brookins, 1988). Additionally, CRS analyses showed that 31 ± 5 % and 34 ± 1 % of added SO_4^{2-} was precipitated as sulfide in the Cd + LZn + SO_4^{2-} and in the contaminated soil + SO_4^{2-} treatments (Table 4-4). Although one can argue that the CRS determination might have been inflated by the presence of organic S in the added rice straw, this was unlikely as CRS was not detected in the oxidized phase control samples which contained the same quantity of straw (Table 4-4).

Table 4-4. Percentage of total S present as chromium-reducible sulfide (CRS) in selected soil suspensions. Soil treatments tested were contaminated soil with added SO_4^{2-} (contaminated soil + SO_4^{2-}) and, Cd and low Zn-spike with added SO_4^{2-} (Cd + LZn + SO_4^{2-}).

Treatment	Soil condition	CRS (% of total S)
Contaminated soil + SO_4^{2-}	pre-reduction	3.28 ± 0.00
	reduced	34.43 ± 1.16
	oxidized	0.35 ± 0.04
Cd + LZn + SO_4^{2-}	pre-reduction	0.00 ± 0.00
	reduced	31.01 ± 5.48
	oxidized	0.00 ± 0.00

Modeling results supported that cadmium sulfide, greigite, pyrite and sphalerite were precipitated under the solution conditions in the contaminated soil + SO_4^{2-} treatment (Table 2, supporting information). It must be noted that pyrite may not actually have formed, as this requires microbial mediation (Bostick *et al.*, 2000; Framson and Leckie, 1978; Schenau *et al.*, 2002) which may not have been available under the experiment conditions. Modeling results did not predict metal sulfide precipitation under the solution conditions in the Cd + LZn + SO_4^{2-} treatment, however we believe this may be due to the uncertainty in the measurements of experimental Eh measurements used in the model. A comprehensive discussion on the uncertainties in Eh measurements and the effect on modeling can be found in Stefànsson *et al*. (2005).

During oxidation, Cd solubilisation was limited relative to Fe and Zn, when greater metal sulfide precipitation occurred during reduction (i.e. when SO_4^{2-} was added to the system). This explanation is supported by findings from the oxidative dissolution of synthetic sulfide minerals with environmentally realistic compositions of Cd, Fe and Zn where Fe and Zn were released to a greater extent than Cd from co-precipitated sulfide minerals, as well as from mixtures of pure CdS, FeS and ZnS (de Livera *et al.*, 2008). The idea that Cd solubility could be limited during oxidation as a result of enhanced sulfide precipitation during reduction is relatively novel, and is the opposite of the currently accepted paradigm for the mechanism behind Cd enrichment in paddy rice grain. This idea is supported by the results of Cantwell *et al*. (2008), who found that oxidative release of both Cd and Zn was limited when higher amounts of sulfide were present in reduced sediments. The results of the present study indicate that there may be a higher risk of Cd enrichment in paddy rice grain when metal sulfide precipitation is limited during the flooded (reduced) phase of rice growth.

4.5.3 Effect of soil

The effect of SO_4^{2-} addition on metal solubilities was different between the field contaminated and laboratory spiked soils. In the Cd + LZn + SO_4^{2-} treatment, Cd concentrations during the reduced phase were significantly lower (Panel B, Figure 4-4) relative to the control (Cd + LZn), whereas Fe (Figure 3, supporting information) and Zn (Figure 4, supporting information) concentrations were similar with or without SO_4^{2-} addition. In the contaminated soil, concentrations of both Cd and Fe were significantly lower during the reduced phase with SO_4^{2-} addition (Figure 5, supporting information), indicating that both of these metals were removed from solution, likely by precipitation as metal sulfides.

Cadmium was the only metal with lower concentrations during oxidation when SO_4^{2-} was added to the Cd + LZn soil. This was likely because Cd sulfide precipitates out of solution more readily than ZnS for a system at equilibrium (Brennan and Lindsay, 1996; Weast, 1988), and Fe sulfides precipitate only after Cd and Zn sulfides, due to the relative thermodynamic stabilities and formation kinetics of these species (Weber *et al.*, 2009). It is also possible that Cd was removed from solution by co-precipitation or adsorption to pyrite (Bostick *et al.*, 2000), which may be readily formed by sulfate-reducing bacteria (SRB) (Fortin and Beveridge, 1997).

Iron concentrations were lower during oxidation when SO_4^{2-} was added to the contaminated soil, but not when SO_4^{2-} was added to the Cd + LZn treatment. This is likely due to greater precipitation of Fe as metal sulfide in the contaminated soil with SO_4^{2-} added, as compared to the Cd + LZn spiked soil with SO_4^{2-} added, during the reduced phase. This may be an indication of the relatively greater health of the SRB community in the field contaminated soil as compared to laboratory spiked soil. It is plausible that in the Cd + LZn + SO_4^{2-} treatment, SRB suffered from toxic effects from the Cd and/or Fe(II) in the system and hence were less active in converting SO_4^{2-} to S^{2-} during their respiration (Vig *et al.*, 2003). Cadmium may be toxic to bacteria at concentrations > 2.0 mg Cd kg^{-1} soil, depending upon the soil type (Vig *et al.*, 2003), and Fe may cause a 50 % inhibition of the growth of heavy-metal tolerant sulfate-reducing bacteria (in pure cultures) at Fe concentrations of 0.7 mmol L^{-1} (Azabou *et al.*, 2007). Heavy metal toxicity to microbes may not have occurred in the contaminated soil + SO_4^{2-} treatment due to lower bioavailability of metals as a result of field aging reactions (Lock and Janssen, 2003) and/or a developed tolerance of soil microbes to the metals present (Vig *et al.*, 2003).

4.5.4 Zinc-spiked soil

The effect of spiking soil with Cd and Zn was tested under an S-limited scenario. Soil spiked with Zn at the high rate (Cd + HZn) had a lower Cd:Zn ratio in soil solution during oxidation (Panel A, Figure 4-5) relative to the control (Cd spike). This lower ratio was due to higher concentrations of Zn in solution (Panel B, Figure 4-5), while Cd concentration remained constant (Panel C, Figure 4-5). The lower Cd:Zn ratio at the high Zn rate could potentially result in a lower Cd:Zn ratio in paddy rice grain, the possibility of which should be investigated in plant-based studies. Zinc-spiking at the higher rate also gave higher solution concentrations of exchangeable cations (Ca, K, Mg, Na) and Mn(II) during oxidation (data not shown), likely due to the additional Zn displacing these cations from exchange/sorption sites (Diatta *et al.*, 2004). Zinc concentrations in soil solution were at least an order of magnitude lower than exchangeable cation concentrations (results not shown). The exchangeable cations were not previously removed from the soil by leaching, as this was carried out with artificial rainwater containing Ca, K, Mg and Na to replace these cations in the soil. The fact that Cd solubility during oxidation was unaffected by Zn-spiking indicates that Cd may be specifically sorbed to soil constituents and is therefore non-exchangeable by Zn (Diatta *et al.*, 2004). The Zn spike may have been expected to increase Cd concentrations in soil solution through competitive displacement of exchangeable Cd from solid phases due to the higher concentrations of Zn in soil solutions (Lambert *et al.*, 2007).

4.5.5 Implications

It is important to consider the potential effects of changes in Cd:Fe and Cd:Zn ratios in oxidizing soil solutions on Cd enrichment in paddy rice grain. The greatest significant effect we observed on the mean Cd:Zn ratio in soil solution during oxidation was a 7-fold decrease from 0.019 to 0.0028 (0.023 to 0.014 μmol L^{-1} Cd and 0.0034 to 0.0049 mmol L^{-1} Zn). This effect was observed with Zn-spiking at the high rate, and as mentioned previously, the Cd concentration was not significantly affected whereas Zn was significantly higher. Although our metal concentrations were low due to the high solution to soil ratio in our experiments, a ratio change of this magnitude could potentially reduce the risk posed by Cd accumulation in paddy rice grain based on results of Cd uptake by plants from solution culture and field observations in the literature (Hart *et al.*, 2005; Supaphol *et al.*, 2007). For example, in wheat plants, Hart *et al*. (2005) found a 10-fold decrease in the Cd:Zn molar ratio in the solution culture from 0.29 to 0.029 (0.5 μmol L^{-1} Cd, 0.001 to 0.01 mmol L^{-1} Zn) resulted in a 12-fold decrease in the Cd:Zn molar ratio in the grain from 0.019 to 0.0015 (0.4 to 0.1 μg kg^{-1} Cd, 12 to 38 μg kg^{-1} Zn). In addition, Supaphol *et al*. (2007) found that a decrease in the Cd:Zn molar ratio in soil from 0.55 to 0.36 (0.01 to 0.009 mmol kg^{-1} Cd and 0.018 to 0.024 mmol kg^{-1} Zn), brought about by increased distance from a Zn polluted roadside (Zn predominantly derived from tyre wear), resulted in a 70-fold decrease in the Cd:Zn molar ratio (Cd:Zn ratios calculated from diethylene triamine pentaacetic acid (DTPA)-extractable metal concentrations reported in Supaphol *et al*., 2007) in paddy rice grain from 0.02 to 0.0003 (0.0074 to 0.00009 mmol kg^{-1} Cd and 0.37 to 0.32 mmol kg^{-1} Zn).

The impact of changes in the Cd:Fe ratio in soil solution on the Cd:Fe ratio in rice grain is likely to only be significant if changes in the Cd concentration in soil solution (rather than Fe) are driving the Cd:Fe ratio change. It may be expected that when the Cd:Fe ratio in soil solution decreases as a result of changes in the Fe concentration, there will be no impact on the Cd:Fe ratio in paddy rice grain. This is because in rice plants transport of Fe to the grain is highly regulated. Simmons *et al*. (2003) reported there was no relationship between DTPA-extractable Fe (a proxy for ‘plant-available’ Fe) in paddy soil and the concentration of Fe in paddy rice grain, whereas there was a significant relationship for Cd ($P < 0.001$). Therefore, it is only when Cd concentrations in solution change that we may expect to see an effect on the Cd:Fe ratio in paddy rice grain. The only treatment in which we observed a decrease in the Cd concentration was in the SO_4^{2-} spiked treatments, where the mean Cd concentration during the oxidized phase was 0.0035 μmol L^{-1} relative to 0.053 μmol L^{-1} in the control. (2003)

4.6 Conclusions

It is well-known that drainage of paddy fields in the period leading up to rice harvesting results in a transformation from reducing to oxidizing conditions in the soil. This laboratory study has shown that such a transformation led to oxidative precipitation of Fe oxyhydroxide minerals and dissolution of metal sulfides; resulting in removal of Fe from soil solution, release of SO_4^{2-} and Zn into the soil solution, and no change in Cd. Higher concentrations of SO_4^{2-} and/or Zn in the soil solution corresponded to lower Cd:Fe and Cd:Zn ratios in soil solution; in a plant-based system this would likely limit the accumulation of Cd in paddy rice grain. Laboratory experiments also showed that oxidation of a reduced paddy soil suspension caused an increase in the Cd:Fe and Cd:Zn ratios in solution when SO_4^{2-} was not added. We conclude that this was due to differential adsorption of metals to soil constituents during the oxidized phase. When additional SO_4^{2-} was introduced at the beginning of an experiment, the ensuing reduction and precipitation reactions produced larger quantities of insoluble metal sulfides. In the subsequent oxidizing phase of these experiments the resultant solution phase Cd:Fe and Cd:Zn ratios were lower. This laboratory study also showed that SO_4^{2-} reduction occurred more readily when the metals were more aged in a field contaminated soil as compared to a laboratory spiked soil.

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5 Limiting cadmium in paddy rice grain relative to iron and zinc

5.1 Abstract

Cadmium (Cd) accumulation in rice grain can pose a significant threat to human health. Rice is one of the most important human dietary crops, and can absorb Cd from soil contaminated by fertilizers, biosolids or industrial wastes. This study aims to examine the influence of co-contaminant metals, iron (Fe), manganese (Mn) and zinc (Zn) in soils, foliar applied Zn, foliar-applied ethylenediamine tetraacetic acid (EDTA) and soil-applied EDTA on the relative accumulation of Cd in brown rice from a rice-growing soil collected in Griffith, New South Wales (NSW), Australia. The information gained will be used to develop management strategies to limit Cd accumulation in rice grain relative to Fe and Zn and hence reduce the risk to human health. Rice plants (*Oryza Sativa* L.) were grown in Cd-spiked soil in a laboratory pot experiment under flooded conditions incorporating pre-harvest drainage. Soil treatments were combinations of Fe, Mn, Zn and/or EDTA. Foliar treatments were Zn or EDTA applied during grain-filling. Cadmium accumulation in rice grain was not significantly reduced compared to the control by any treatment. Spiking soil with EDTA significantly decreased grain Zn concentrations and the Cd:Fe ratio in grain due to higher Fe concentrations in grain, and spiking of soil with Mn increased grain Zn concentrations. Cadmium accumulation was significantly increased compared to the control by spiking soil with Fe and/or Zn, without affecting Fe or Zn concentrations in grain. Foliar treatments had no significant effect on Cd, Fe or Zn concentrations in grain. Addition of EDTA to soil may limit the risk to human health posed by Cd accumulation in paddy rice grain by virtue of a lowered Cd:Fe ratio in grain.

5.2 Introduction

Cadmium accumulation in rice (*Oryza Sativa* L.) grain is considered a human health risk, with the maximum allowable limit for unpolished rice soon to be set at 0.4 mg/kg by the Japanese government (Ishikawa *et al.*, 2009a), based upon the Codex Alimentarius standard for polished rice of 0.4 mg/kg (FAO/WHO, 2009). Rice is one of the most important human dietary crops, and can absorb Cd from soil contaminated by fertilizers, biosolids or industrial wastes (Chaney *et al.*, 1996; McLaughlin *et al.*, 1999b). Because rice plants show no physical signs of toxicity when contaminated with Cd, they can pose a significant risk to human and animal health (McLaughlin and Singh, 1999).

Research into Cd uptake and translocation in rice plants was sparked by the ‘Itai-Itai’ disease that struck subsistence rice farmers in Japan in the 1960s who were also deficient in iron (Fe) and zinc (Zn) (Nordberg, 2004). There have been no similar reports of health problems like this in western society due to better micronutrient nutrition (McLaughlin *et al.*, 1999b). Therefore, it is subsistence rice farmers with poor micronutrient nutrition who are most at risk from Cd accumulation in paddy rice grain, as they are often using agricultural soils which have been polluted with Cd through the continued fertilization of agricultural soils with phosphate rock, the land application of Cd-rich biosolids and contamination with mine waste (Hutton, 1982).

Cadmium accumulates in rice grains to a greater extent than the nutrients Fe and Zn, based on relative soil concentrations (Iimura, 1981b), and subsistence on rice can result in human nutrient deficiency. Nutrient deficiency can enhance Cd bioavailability (Reeves and Chaney, 2008); the mechanism is poorly understood but is believed to involve changes in intestinal absorption and/or increased uptake by the kidney (Spivey Fox, 1988). In order to limit the risk to human health, soil management strategies are required that can reduce Cd accumulation relative to Fe and Zn in rice (i.e. reduce Cd concentrations and also reduce the Cd:Fe and Cd:Zn ratios).

It has been found that Cd becomes enriched relative to Fe and Zn in paddy rice grain during the soil oxic phase that accompanies pre-harvest drainage of the paddy (Minagawa *et al.*, 1974; Römkens *et al.*, 2009b). Paddy rice is typically cultivated by growing plants in flooded soil which is drained to facilitate harvest approximately two weeks before the grains are mature and harvested. The increased accumulation of Cd by rice during soil drainage (oxic conditions) has been suggested to occur due to the differential oxidation of mixed Cd sulfide phases formed during soil flooding (anoxic conditions) (Chaney *et al.*, 1996), such that the Cd:Fe and Cd:Zn ratios in soil solution become higher during pre-harvest drainage. However, this mechanism is not likely as the oxidation of sulfide minerals with environmentally realistic Cd, Fe and Zn compositions does not give rise to higher concentrations of Cd relative to Fe and Zn in soil solutions (Chapter 3). Furthermore, it has been shown that when sulfide precipitation occurs in a soil environment under anoxic conditions, the Cd:Fe and Cd:Zn ratios in soil solution during subsequent oxic conditions are lower than when sulfide precipitation does not occur (Chapter 4). Therefore, it is more likely that the Cd:Fe and Cd:Zn ratios in paddy rice grain increase during pre-harvest drainage as a result of competition between Cd, Fe and Zn for solid phase sorption, which in turn influences the metal concentrations in paddy soil solutions which are available for root uptake and/or competition between Cd, Fe and Zn within the plant.

Cadmium accumulation may be decreased in soils that contain higher amounts of Zn (for a given level of Cd contamination), due to a decrease in the Cd:Zn ratio that the plant is exposed to. A decrease in Cd uptake in the presence of higher Zn concentrations in solutions or soils has been suggested to occur due to competition between Cd and Zn for translocation within the plant (Li *et al.*, 2009). Honma and Hirata (1977) showed that Cd accumulation in paddy rice grain decreased when the Zn:Cd ratio was increased from 10:1 to 100:1 in solution culture experiments. However, further investigations are required as plants were not grown to maturity, the effect of soil oxidation was not considered, and results may be different in a soil environment where minerals, organic carbon and biota are present. In greenhouse pot experiments (Li *et al.*, 2008) found Cd concentrations in rice grain were significantly reduced, by 20% and 23 %, when Zn was applied to a paddy soil at 25 (Zn:Cd ratio 14:1) and 76 mg/kg (Zn:Cd ratio 22:1), respectively relative to the control soil containing 2.4 mg Cd/kg. However, it is unclear whether pre-harvest drainage was carried out in this study.

Cadmium accumulation in paddy rice grain may be limited relative to Fe and Zn in soils high in Fe and/or Mn. During pre-harvest drainage, Fe and Mn oxyhydroxides precipitate in paddy soil (Ponnamperuma, 1972) and can remove metals from solution by adsorption (Tiller and Wassermann, 1973). Iron and Mn oxyhydroxides are the primary sinks for Cd and Zn under aerobic soil conditions (Du Laing *et al.*, 2009), and even when metals bind with dissolved organic carbon (DOC), DOC can readily adsorb to Fe and Mn phases (Kothawala *et al.*, 2009). Iron and Mn oxyhydroxide minerals preferentially adsorb Zn over Cd (Bohn *et al.*, 2001; Davis *et al.*, 1994), so greater relative bioavailability of Cd may occur during grain-filling as Cd is outcompeted by Zn for sorption sites on solid phases. Chaney *et al*. (2006) found that addition of Fe and Mn oxides to soil had no effect on the Zn:Cd ratio in lettuce leaves. However the authors noted that results may be different for species such as rice which use phytosiderophores (PSs) to solubilise soil Fe. Increasing the availability of Fe through soil addition may reduce PS secretion, and as PSs may also transport Cd, this may reduce Cd uptake (Chaney *et al.*, 2006). Cadmium accumulation in paddy rice grain may also be able to be limited by cation competition for plant uptake with additional Fe in soil solution. To the authors' knowledge, no research has been conducted on the effect of addition of Fe and Mn to soil on Cd accumulation in paddy rice grain.

The addition of EDTA to soil has been previously shown to enhance the uptake of Zn (Karak *et al.*, 2006) and to decrease the uptake of Cd (Shao *et al.*, 2008) into rice. However, it is unclear whether decreased uptake of Cd in the study by Shao *et al*. (2008) was due to the addition of EDTA or the addition of Fe, as soil was spiked with Fe(II)-EDTA. Conversely, EDTA has been shown to enhance shoot uptake of Cd, Fe (January *et al.*, 2008) and Zn in hyperaccumulator plants (Lai and Chen, 2006), likely due to increased uptake of the chelated metal from soil solutions and the greater translocation of chelated metals within plants. The influence of EDTA on Cd uptake and accumulation in rice grain has not previously been examined under variable redox conditions.

Foliar application of Fe(II)-EDTA may also be a potential method for limiting Cd accumulation into paddy rice grain. The Cd:Fe molar ratio in brown rice was found to be reduced by 25 % with foliar application of Fe(II)-EDTA to rice plants in a pot experiment (Shao *et al.*, 2008). Concentrations of both Cd and Fe in the grain were found to increase following foliar Fe(II)-EDTA application, which suggested that enhanced translocation of metal-EDTA complexes may have occurred in the plants. This phenomenon requires further investigation, particularly as other workers have shown that Cu, Fe, Mn, and Zn concentrations in wheat grains were unaffected by foliar application of EDTA (Modaihsh, 1997).

Foliar application of Zn may be an effective method of limiting Cd accumulation in paddy rice grain. It has been suggested that Cd and Zn accumulate in rice grains via different translocation pathways (Jiang *et al.*, 2007b; Kashiwagi *et al.*, 2009). Grain Zn is believed to be derived from root-uptake after flowering rather than Zn remobilization from the leaves (Jiang *et al.*, 2007b), whereas Cd is thought to be translocated to grains after heading from Cd already accumulated in leaf blades, sheaths, and in the culm (Kashiwagi *et al.*, 2009). However, this has not been demonstrated in a situation where plants are exposed to both Cd and Zn in the same experiment. Foliar-applied Zn is known to be absorbed by rice plants and translocated to grains (Fang *et al.*, 2008), so foliar application of Zn during grain filling (when Cd is present in leaves) may be a viable method of limiting Cd accumulation in rice grain through competition for transport from leaves to grain..

This study therefore aimed to examine the influence of competitive ions (Fe, Mn and Zn) in soils, foliar-applied Zn, foliar-applied EDTA and soil-applied EDTA on the relative accumulation of Cd, Fe and Zn in brown rice from a rice-growing soil collected in Griffith, New South Wales (NSW), Australia. The information gained will be used to develop management strategies to limit Cd accumulation in rice grain relative to Fe and Zn and hence reduce the potential risk to human health.

5.3 Materials and Methods

5.3.1 Soil characterization

An uncontaminated soil used for paddy rice production was collected from Griffith, NSW (Hanwood Loam) (Table 5-1). The soil was air dried, homogenized and sieved to < 2 mm. The pH and EC were determined in 1:5 (m/v) soil: water using a model 420 pH electrode (Thermo Orion, Waltham, MA, U.S.A) and a model CDC641T conductivity electrode (Radiometer Analytical, Lyon, France). Soil moisture content at field capacity was determined by the tension plates method at 100 cm (Marshall and Holmes, 1979). Total metal concentrations in soils were determined by strong acid extraction using *aqua regia* (Zarcinas *et al.*, 1996) and analysis by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Spectro Analytical Instruments, Kleve, Germany).

Table 5-1. Selected soil properties and constituents of paddy soil used for rice cultivation. The pH and electrical conductivity (EC) were determined in 1:5 soil:water. Cation exchange capacity (CEC) is sum of exchangeable Ca, K, Na and Mg. Extractable iron (Fe) was determined by citrate/dithionate extraction. Weight percent (wt %) values were determined by x-ray diffraction (XRD) and are for the bulk soil unless a size fraction is stated. Because anatase and hematite wt % values were below the detection limit of the bulk soil, values given are for the less than 2 micrometre (< 2 µm) soil fraction

Soil property	**Units**	**Soil content**
pH	-	6.4
Electrical conductivity	µS/cm	141
Cation exchange capacity	cmol (+)/kg	21.1
Moisture content (field capacity)	wt%	37
Quartz, SiO_2	wt%	40-50
Kaolinite, $Al_2Si_2O_5(OH)_4$	wt%	10-20
Illite, $K_{0.65}Al_2(Al_{0.65}Si_{3.35}O_{10})(OH)_2$	wt%	10-20
Interstratified illite-smectite	wt%	10-20
Muscovite, $KAl_2(OH_2)AlSi_3O_{10}$	wt%	-
Chlorite $(Mg,Fe)_3(Si,Al)_4O_{10}(OH)_2\cdot(Mg,Fe)_3(OH)_6$	wt%	-
K- and Na- feldspar, $K(AlSi_3O_8)$, $Na(AlSi_3O_8)$	wt%	5-10 for each
Anatase, TiO_2	wt% <2 µm	1 to 5
Hematite, Fe_2O_3	wt% <2 µm	1 to 5
Clay content by XRD	% <2 µm	56.7
Clay content	% <2 µm	40
Silt content	% 2-20 µm	9
Sand content	%20-200 µm	51
Total carbon	% 200 - 2000µm	1.2
Extractable iron	wt%	1.1
Cadmium	mg/kg	0.18
Zinc	mg/kg	42

5.3.2 Soil preparation and spiking

Soil was spiked with varying concentrations of Cd, Fe, Mn and Zn as chloride salt solutions (Sigma) to avoid interactions of sulphate or nitrate salts with soil redox processes, and EDTA as a sodium salt solution (Sigma). Spiking rates for soils were chosen such that Cd, Fe, Mn and Zn would be at environmentally realistic molar ratios (Bohn *et al.*, 2001; Hettiarachchi *et al.*, 2000) (Table 5-2). The soil spiking rate of EDTA was equimolar with the maximum concentrations of Cd, Fe and Zn detected in soil solutions of the reaction cell experiments described in Chapter 4 (0.45 µmol Cd/L, 1.15 mmol Fe/L, 0.03 mmol Zn/L). Control treatments were included to support the assumptions made in interpreting results, i.e. that cultivation under flooding and drainage gives rise to more Cd in plant parts relative to cultivation at field capacity “upland” conditions, and that Cd detected in plant parts and soil solutions was derived from the Cd spiked into the soil. Control treatments were unspiked control at field capacity (FC control), unspiked control (control) undergoing flooding and drainage, and Cd-spiked control (Cd) undergoing flooding and drainage. The following soil treatments all underwent flooding and drainage. Soil treatments to examine the influence of competition of added soil Zn on the accumulation of Cd in rice grain were: low Zn-spike (Cd + LZn) and high Zn-spike (Cd + HZn). Soil treatments to examine the influence of competition of added soil Fe and Mn on the accumulation of Cd in rice grain were: Cd+Fe-spike (Cd + Fe) and Cd+Mn-spike (Cd + Mn). Soil treatments compared against Cd + LZn to test the effect of added soil Fe or Mn concentrations on Cd accumulation in rice were Cd and low Zn with Fe spike (Cd + LZn + Fe) and Cd and low Zn with Mn spike (Cd + LZn + Mn). The soil treatment compared against Cd + LZn + Fe to test the effect of added soil EDTA in a soil with multiple metals added was Cd and low Zn with Fe and EDTA (Cd + LZn + Fe + EDTA). Soil treatments were spiked, homogenized and air-dried overnight.

Table 5-2. Nominal and actual spiking rates and metal ratios in soil treatments. Nominal soil metal concentrations shown in brackets are based on the pre-spiking soil concentrations, and indicates that metal was not added to that treatment. Other expected soil metal concentrations are calculated from the addition of the spiked amount of metal to the concentration of metal in the unspiked (control) soil.

Treatment name	Description	Nominal soil metal concentration (mg/kg)				Nominal soil metal ratio (x 10 000)		Metal concentration by acid digestion (mg/kg)				Actual soil metal ratio (x 10 000)	
		Cd	Fe	Mn	Zn	Cd:Fe	Cd:Zn	Cd	Fe	Mn	Zn	Cd:Fe	Cd:Zn
FC control	Field capacity control	(0.18)	(25873)	(378)	(43)	0.07	42	0.11	25036	320	53	0.04	21
control	Unspiked control	(0.18)	(25873)	(378)	(43)	0.07	42	0.12	24216	335	50	0.05	24
Cd	Soil spiked with Cd	2.88	(25873)	(378)	(43)	1.11	670	2.93	24862	313	52	1.18	563
Cd + LZn	Soil spiked with Cd and Zn	2.88	(25873)	(378)	140	1.11	206	2.60	23553	316	128	1.10	203
Cd + HZn	Soil spiked with Cd and Zn	2.88	(25873)	(378)	248	1.11	116	3.03	24249	318	243	1.25	125
Cd + Fe	Soil spiked with Cd and Fe	2.88	32575	(378)	(43)	0.88	670	0.86	29733	186	48	0.29	179
Cd + LZn + Fe	Soil spiked with Cd, Fe and Zn	2.88	32575	(378)	140	0.88	206	0.62	29609	191	107	0.21	58
Cd + LZn + Fe + EDTA	Soil spiked with Cd, Fe, Zn and EDTA	2.88	32575	(378)	140	0.88	206	0.90	30478	178	113	0.30	80
Cd + Mn	Soil spiked with Cd and Mn	2.88	(25873)	4158	(43)	1.11	670	2.11	25061	3088	58	0.84	364
Cd + LZn + Mn	Soil spiked with Cd, Mn and Zn	2.88	(25873)	4158	140	1.11	206	2.09	24017	3125	153	0.87	137
Cd + foliar Zn	Foliar spray of Zn during drainage	2.88	(25873)	(378)	(43)	1.11	670	2.97	26602	365	56	1.12	530
Cd + foliar EDTA	Foliar spray of EDTA during drainage	2.88	(25873)	(378)	(43)	1.11	670	2.93	25782	330	55	1.14	533

The EC of the spiked soils was determined (Rayment and Higginson, 1992) and the soils were leached to remove excess soluble salts. The soils were leached using artificial rainwater (pH 5.9) containing calcium chloride, calcium nitrate and magnesium chloride at 0.5 mmol/L, as well as sodium sulfate and potassium chloride at 0.1 mmol/L (Stevens *et al.*, 2003). Leaching events were carried out by saturating soils from beneath (to avoid entrapment of air, transport of fine particles and blockage of pores), equilibrating overnight, and draining the following day. Soils were exposed to approximately 2 pore volumes of artificial rainwater in each leaching event. The soils were leached 6 times until the EC decreased to < 500 µS/cm (from >7500 µS/cm). The concentration of chloride in soil solution during drainage was significantly ($P<0.001$) higher in the Cd + Fe (max 790 mg/L), Cd + LZn + Fe (max 573 mg/L), and Cd + HZn (max 512 mg/L) treatments compared to the Cd (max 267 mg/L) and Cd + LZn (max 358 mg/L) treatments. Spiked soils were aged for 4 weeks at field capacity in a growth chamber under a 12 hour day/night cycle at 30º/24º to allow aging reactions to occur (McLaughlin *et al.*, 2010).

After leaching and aging, total metal concentrations in soils were determined by strong acid extraction using *aqua regia* (Zarcinas *et al.*, 1996) and analysis by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Spectro Analytical Instruments, Kleve, Germany) (Table 5-1). Expected soil metal concentrations were calculated from the addition of the amount of spiked metal to the amount of metal that was present in the soil prior to spiking.

Nutrients required for healthy rice growth were added to soils, dissolved in water and mixed into the soil by hand (Doran *et al.*, 2006; Gunawardena *et al.*, 2003). Nutrients were nitrogen as urea (326 kg N/ha), phosphorus as trisodium phosphate (25 kg P/ha), potassium chloride (192 kg K/ha), copper sulfate (0.3 kg Cu/ha), boric acid (0.1 kg B/ha) and ammonium molybdate (0.01 kg Mo/ha)(Gunawardena *et al.*, 2003).

Plastic pots were lined with a plastic bag and filled with a 2 cm layer of quartz sand topped with 2 kg of the soil treatments.

5.3.3 Water management

Plastic pots were flooded from two weeks before the planting of rice seedlings until 21 days after flowering, by keeping the water between 2 and 5 cm above soil surface. Pots were drained of water at 21 days after flowering, by piercing the plastic lining to allow water to drain freely. After drainage, pots were kept at field capacity for 14 days until harvest (Fageria, 2001). Field capacity was maintained by watering pots to the weight recorded at 1 day after drainage.

5.3.4 Rice cultivation

Rice plants (*var.* Japonica, *cv.* Amaroo) were grown from seeds in a growth chamber under a 12 hour day/night cycle at 30º/24º. Seeds were pre-germinated for 48 hours between layers of moistened paper towel. Sprouted seeds were then planted approximately 0.5 cm deep into a shallow tray filled with moist quartz sand. Seedlings were grown in the tray for approximately three weeks until the shoots were between 5 and 10 cm tall. Two seedlings were transplanted into each pot. Pots were randomized in the growth chamber and moved to a different position once every week. Plants were checked weekly for signs of N deficiency and urea applied when required to all pots consistently across treatments at a rate of 65 kg/ha (Doran *et al.*, 2006) when yellowing of the leaves was observed.

Foliar treatments were applied to the appropriate treatments once per day during the drainage period (grain-filling), amounting to a total of 100 mL per pot. Plants grown in Cd-spiked soil received a Zn foliar spray (Cd + foliar Zn) or an EDTA foliar spray (Cd + foliar EDTA) (Table 5-1). The rice grains and soil surface were shielded from the foliar spray using plastic sheeting. The Zn spray was prepared at a concentration of 20 mg Zn/L by dissolving zinc sulfate ($ZnSO_4.H_2O$) in 1% Spreadwet 1000 (non-ionic wetting agent, SST Australia, Bayswater, Victoria, Australia). The Cd + Zn foliar treatment received a total application of ~ 2.0 mg Zn/pot similar to the recommended foliar-fertilization rate which is equivalent to 1.7 mg Zn/pot (Nutri-Tech-Solutions, 2007). The EDTA spray was prepared at a concentration of 50 g EDTA/L by dissolving sodium EDTA in 1% Spreadwet 1000. The Cd + EDTA foliar treatment received a total application of 5 g EDTA per pot. The foliar application rate of EDTA was equivalent to the EDTA per plant exposure rate of the Cd + LZn + Fe + EDTA treatment.

5.3.5 Soil sampling

Soil from each treatment replicate was sampled on the last day of flooding (96 days) for chromium-reducible sulfides (CRS) (Ahern *et al.*, 2004). Soil was sampled from pots using a 20 mm stainless steel corer. Soil was immediately dislodged from the corer directly into a nitrogen-filled centrifuge tube. Tubes were then snap-frozen in liquid nitrogen for storage prior to CRS analysis.

5.3.6 In situ redox measurements

Platinum electrodes were connected to a datalogger with reference electrodes (Ionode, Tennyson, Australia) and installed at 10 cm depth in duplicate pots of selected treatments: FC control, control, Cd, Cd + LZn, Cd +HZn, Cd + LZn + Fe + EDTA and Cd + LZn + Mn. Redox potential measurements shown are a mean of the two duplicates, as there was good agreement between the duplicate electrodes with no significant effect of replicates found. The redox potential (Eh) was recorded at sampling times (see under heading ‘soil solution sampling’) and corrected as per datalogger specifications by dividing by two and adding + 199 mV.

5.3.7 Soil solution sampling and analysis

Soil solution samples were collected 2 days before flooding, on the day of flooding, on the day of planting, at 42 and 82 days after flooding, on the day of drainage, and at 1, 3, 7 and 14 days after drainage from each treatment replicate. Soil solutions were collected using Rhizon samplers (Eijkelkamp, Giesbeek, the Netherlands) installed vertically and approximately 10 cm deep into the soil of each pot. Samplers consisted of a 10 cm hydrophilic porous polymer (0.1 µm filter) probe reinforced with a stainless steel wire connected to a flexible PVC tube and Luer-lock connector.

Immediately before soil solution 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 oxygenation of the sample with 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. The vacuum induced the soil solution to drip slowly into the tube (approximately 8 mL in an hour).

Samples were transferred directly to an anaerobic chamber (modular atmosphere controlled system, DW Scientific, West Yorkshire, U.K.) for processing. Inside the chamber, the pH was measured and an aliquot was acidified with 100 µL 12 M hydrochloric acid (HCl) for ICP-OES analysis for Ca, Cu, Fe, Mg, Mn, Na, Ni, P and S concentrations and ICP-MS

(Agilent 7500ce, Santa Clara, CA, U.S.A) determination of Cd and Zn concentrations. Another aliquot was transferred to a glass vial and preserving by freezing prior to analysis of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), nitrate (NO_3^-) and sulfate (SO_4^{2-}) concentrations. Dissolved organic carbon was determined by the difference between total carbon and inorganic carbon concentrations in samples. Total carbon (C) concentrations were determined by high temperature combustion (Skalar Formacs HT TOC Analyzer) through conversion to carbon dioxide (CO_2) and measurement by a non-dispersive infrared detector. Inorganic C was similarly determined by CO_2 generated following sample acidification. Concentrations of NO_3^{2-} and SO_4^{2-} were determined by ion chromatography (IC) (Dionex ICS-2500, Sunnyvale, CA, U.S.A).

Another aliquot of sample was used for colorimetric determination of Fe(II) and S(-II) (Greenberg *et al.*, 1992; Sparks, 1996) using a UV-visible spectrophotometer (Shimadzu UV-1601, Kyoto, Japan), with color developed inside the nitrogen filled anaerobic glovebox. Dissolved sulfide S(-II) concentrations were not detected in any soil solutions in this study.

5.3.8 Rice plant harvest and analysis

Plants were harvested at 35 days after flowering, which was equivalent to 14 days after soil drainage (aerobic conditions), between 110 and 144 days after flooding depending upon the development of plants in individual pots. Shoots and grains were separated from individual plants rinsed with deionised water, oven dried at 55° for 5 days and weighed. The roots of plants were not examined in this study because of potential contamination with soil (despite washing in sodium lauryl sulfate), as indicated by concentrations of aluminum >

4000 mg/kg. The plant parts were ground in a stainless steel mill which was cleaned with compressed air between samples.

Plant parts were open vessel digested by weighing 0.5 g of plant material into a 75 mL digestion tube with 5 mL of concentrated nitric acid (Mallinckrodt) (HNO_3). The samples were digested at 140 °C for 8 hours. After this time the acid in the tubes was boiled down to ~ 1 mL and allowed to cool to room temperature. The samples were diluted 1:20 with 0.1 % HNO_3,vortex mixed and 0.45 μm (Sartorius) filtered prior to ICP-OES analysis of Ca, Fe, Mg, Mn, Na, S and Zn concentrations (Kashem and Singh, 2001). Blank samples and certified reference plant materials (National Institute for Environmental Studies Certified Reference Material No. 10-a, No. 10-b and No. 10-c) (CRMs) were included in the digestion and analysis procedure as quality control samples for the determination of metal concentrations. Blank concentrations were subtracted from all certified reference materials and samples prior to calculations, statistical analyses and data presentation. Recoveries for CRMs 10-a, 10-b and 10-c were as follows: 113 ± 1 %, 108 ± 3 % and 106 ± 3 % for Ca; 137 ± 6 %, 118 ± 10 % and 116 ± 4 % for Fe; 87 ±1 %, 86 ± 0 % and 86 ± 0 % for Mn; and 105 ± 1 %, 113 ± 1 % and 111 ± 0 % for Zn.

Total Cd concentrations in rice plant tissues were determined using a closed vessel microwave strong acid digestion procedure (Horwitz, 2000). This was carried out by weighing ~ 0.5 g of sample into a Teflon microwave digest vessel with 5 mL of concentrated HNO_3 (Mallinckrodt) and 3 mL of hydrogen peroxide (30 % v/v). The samples were digested at room temperature for 15 min before vessels were sealed. Digestion vessels were then placed in a microwave oven (Ethos E touch control, Milestone, Bergamo, Italy) and digested at 600 W for 20 min. Vessels were then removed from the microwave oven and cooled in a water bath for 15 min before opening. The digested samples were diluted 1:20 with 0.1 % HNO_3, vortex mixed and 0.45 μm (Sartorius) filtered prior to ICP-MS analysis of Cd concentrations. Blanks and certified reference plant material for Cd (Community Bureau of Reference BCR no. 191, lyophilized brown bread) were included in the acid digestion for quality control of metal concentrations and recovery of Cd was 99 ± 5 %.

5.3.9 Data analysis and interpretation

Analysis of variance (ANOVA) was carried out on solution variables Ca, Cd, Cd:Fe ratio, Cd:Zn ratio, Cl^-, Cu, DIC, DOC, Eh, Fe, Fe(II), K, Mg, Mn, Na, Ni, NO_3^-, pH, S, SO_4^{2-}, Zn and element concentrations in plant tissues (e.g. shoots and grain) using Genstat statistical software package (10th edition, VSN International, Hempstead, UK). Solution variables were analyzed in two groups; a ‘reduced’ group containing all data points from day 10 to the day of soil drying (oxidation), and an ‘oxidized’ group containing all data points from the day of soil drying onwards. Before ANOVA analysis, data were transformed when necessary to correct for non-normal distribution of residuals. Analysis of variance was then carried out with significant effects of soil treatments determined by the least significant differences of means (l.s.d) at the 5 % confidence level.

5.4 Results & Discussion

Soil treatments spiked with Fe contained lower than expected concentrations of Cd, Fe and Zn, while soils spiked with Mn contained lower than expected concentrations of Cd. It is likely that the added Fe and Mn in soils displaced or prevented the sorption of Cd onto soil phases (Lambert *et al.*, 2007) facilitating the removal of Cd during leaching events. It is also likely that the chloride associated with the added Fe and Mn in soils assisted the partitioning of Cd to the solution phase through chloro-complexation (Ohtani *et al.*, 2007; Smolders *et al.*, 1998), thereby facilitating the removal of Cd during leaching events.

5.4.1 Soil redox potential and pH during rice growth

In all of the submerged treatments, the soil Eh decreased from between 446 and 637 mV on the day of flooding (day 0) to between -16 and 75 mV at 96 days after flooding (Panel B, Figure 5-1). During drainage, the soil Eh and soil solution pH of all flooded treatments increased significantly due to oxidation reactions following soil drainage (Chapter 2 and 3). The Eh was significantly higher in the field capacity control compared to the submerged treatments (Figure 5-1). Differences in Eh were not significant among the flooded treatments during either flooding or drainage phases.

The onset of flooding resulted in an initial decrease then increase in soil pH, then during drainage the pH did not change significantly with time (Figure 5-1). The initial decrease (e.g. acidification) in pH is likely due to the production of H^+ in soil solutions during flooding by the hydrolysis of Fe(III) (Stefànsson and Seward, 2008) and other metals (Antoniadis and Tsadilas, 2007; Ponnamperuma, 1972). The increase in pH towards neutral that occurred during flooding is typical of soils with a pH < 7, and occurs due to the consumption of H^+ during reduction of NO_3^-, Mn(III)/Mn(IV), Fe(III), SO_4^{2-} and dissimilation products of organic matter (Ponnamperuma, 1972). The pH was significantly lower in the soil solutions of Cd treatments spiked with Fe or Mn (Figure 5-1) throughout rice growth. This is likely a result of hydrolysis of the added metals, and resultant generation of relatively more H^+ in solutions (Speir *et al.*, 1999). The more acidic pH of the Fe and Mn-spiked treatments indicates that Fe and Mn were present more in their reduced forms in these treatments (Brookins, 1988) and as a consequence less oxyhydroxide minerals may have been precipitated. Thus, rather than the addition of Fe or Mn decreasing Cd solubility through adsorption as hypothesised, Fe or Mn addition may potentially have worked to enhance Cd solubility through acidification (Degryse *et al.*, 2004) and as a consequence relatively fewer adsorptive surfaces being available compared to other treatments (Brookins, 1988).

The more acidic pH of the Fe and Mn spiked treatments was most pronounced at time 0, and the pH became more similar to the controls over the time course of the rice growth. This is likely the result of ageing of the added metals in soil during rice growth, i.e. ageing decreases metal solubility (Lock and Janssen, 2003) and therefore decreases the extent of hydrolysis and H^+ generation (Antoniadis and Tsadilas, 2007). Therefore, the effects of more acidic pH in these treatments on Cd solubility can be expected to be less marked towards the end of rice growth, i.e. during soil oxidation.

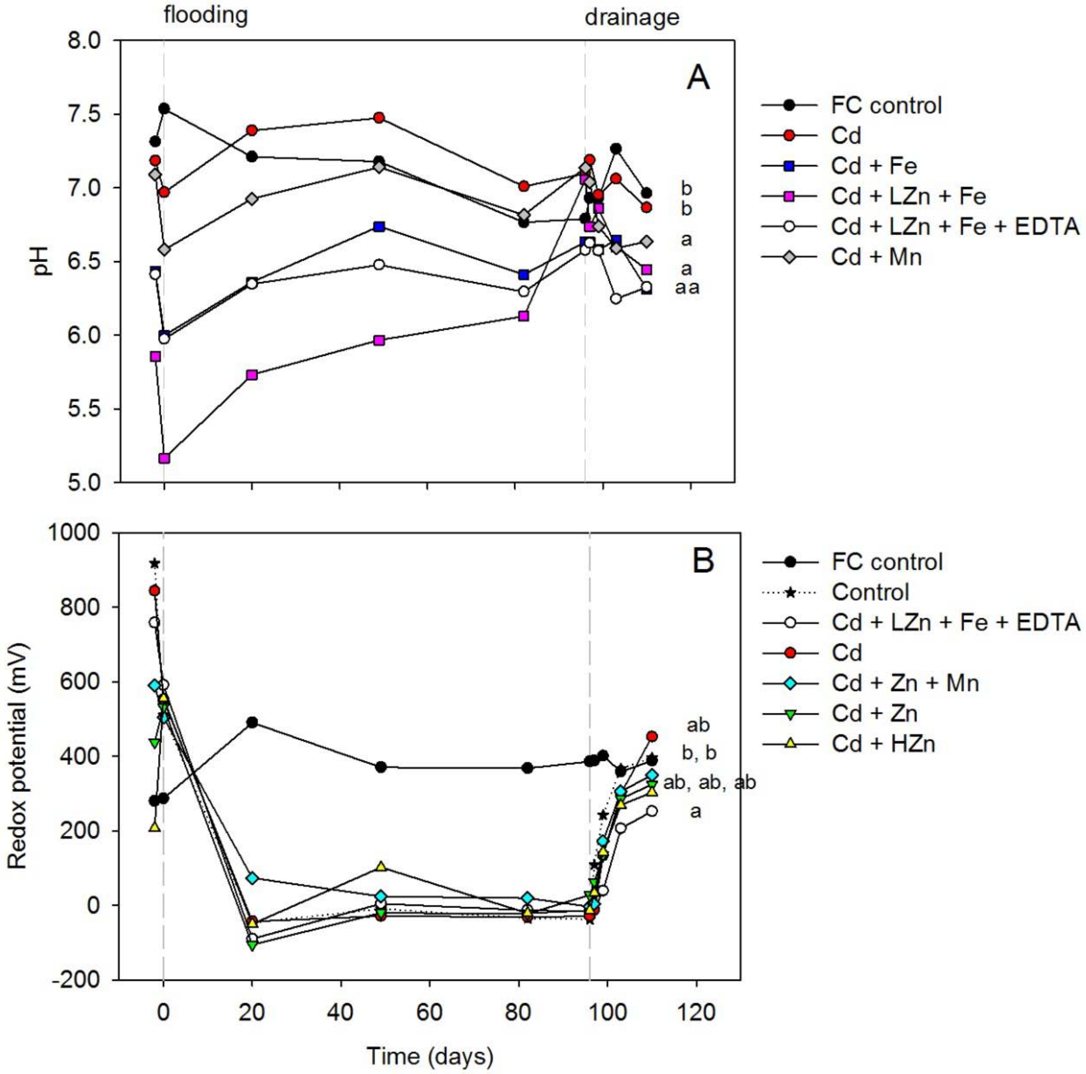

Figure 5-1. Changes in pH and redox potential with time in different treatments. Soil treatments were: Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA). Analysis of variance letters are such that where treatments share a letter they are not significantly different. Letters were derived from day 103 data. Panel A: pH in all treatments, mean of 3 replicates, Cd + LZn, Cd + HZn and Cd + foliar Zn treatments are not shown as the pH was not significantly different to Cd treatment; Cd + LZn + Mn is not shown as the pH was not significantly different to Cd + Mn; unspiked control is not shown as the pH was not significantly different to FC control. Panel B: Redox potential of selected treatments. Significant effects of soil treatments are indicated by different letters of the alphabet such that a is significantly different from b.

5.4.2 Metal Concentrations in Soil Solutions - Flooded Phase

During the flooded phase (days 20 to 96, coincident with rice cultivation), concentrations of Cd (Panel A, Figure 5-2), Ca, Cl, K, Mg, Mn, Na (results not shown; Appendix C) and Zn (Panel C, Figure 5-2) all decreased in the soil solutions of all submerged treatments. The decrease in these elemental concentrations in soil solutions may be due to uptake by rice plants and/or their continuing partitioning to solid phases. All of these elements with the exception of Cd are nutrients for higher plants (Marschner, 1995), so it is likely that loss of these elements from solution during rice cultivation was due to plant uptake. During soil flooding reduction of Mn(III)/Mn(IV) solid phases to soluble Mn(II) can occur (Brookins, 1988; Gotoh and Patrick Jr, 1972; Ponnamperuma, 1964). In the soil solutions of submerged treatments from this study, Mn concentrations were not observed to increase, suggesting that Mn was either partitioned to the solid phase following release as Mn(II), and/or removed from soil solution by plant uptake. Similarly, the decrease in exchangeable cations (Ca, K, Mg and Na) in soil solutions was likely due to continued plant uptake.

In all submerged treatments, except the Cd + LZn + Fe + EDTA treatment (Panel B, Figure 5-2), the concentration of Fe in soil solutions increased from day 20 to day 96 during the flooded phase. The increase in Fe concentrations in soil solutions may be due to the reductive dissolution of Fe phases such as hematite and ferric hydroxide and accompanying release of soluble Fe into solution (Brookins, 1988; Ponnamperuma, 1964). Hematite was the only Fe oxyhydroxide mineral detected by XRD analysis of the soil, and was present at 1 to 5 wt % in the < 2 µm fraction (Table 5-2). Although ferric hydroxide was not detected in soil treatments by x-ray diffraction (Table 5-2), it is a common Fe phase in soils and may be present in amorphous form and therefore undetectable by XRD analysis (Schwertmann and Cornell, 1991). In the Cd + LZn + Fe + EDTA treatment, concentrations of Fe (Panel B, Fig.

2) in soil solutions decreased between day 20 and day 96 during the flooded phase. This trend was not observed in the other soil treatments and is likely due to loss of Fe-EDTA complexes from solution due to the slow microbial degradation of EDTA (Bucheli-Witschel and Egli, 2001) over the time course of the experiment and the uptake and/or partitioning of soluble Fe(II) to solid phases (Jeon *et al.*, 2003).

The concentration of total S and SO_4^{2-} in soil solutions was found to decrease during flooding in all treatments and was coincident with the decrease in Eh (Appendix C). Total S determined by ICP-OES was slightly less than SO_4^{2-} determined by IC, likely due to systematic underestimation of total S due to loss as H_2S gas during sample acidification (which did not occur during IC sample preparation). Therefore, the trend in total S concentrations may be considered to be reliable, however the magnitude of total S is not quantitative. It is unlikely that SO_4^{2-} was removed from soil solutions during flooding by sulfide mineral precipitation, as the Eh and pH conditions encountered were unfavorable (Brookins, 1988). The decrease in SO_4^{2-} concentration in soil solutions during the flooded phase may be due to adsorption under the pH conditions encountered (Ishiguro *et al.*, 2006) and/or the uptake of SO_4^{2-} by rice plants (Marschner, 1995; Samosir and Blair, 1983).

The flooding of soils resulted in a decrease in Cd and Zn soil solution concentrations from time 0 to time 96 days in Cd and Zn spiked soil treatments (Figure 5-2). These decreases in Cd and Zn concentrations in soil solutions may have been due to plant uptake (Marschner, 1995) or sorption to solid phases (Li *et al.*, 2006; Musić and Ristić, 1992). Precipitation of Cd or Zn as metal sulfides was unlikely, as analysis for Cr-reducible sulfide at 96 days did not indicate the presence of sulfide minerals in soils, and the soil conditions (e.g. pH and Eh) were not favorable for precipitation of sulfide minerals (Brookins, 1988). However, it is feasible that small quantities of undetectable sulfide mineral may have formed in low redox microenvironments in the soil (e.g. microsites).

Cadmium soil treatments spiked with Zn (e.g. Cd + LZn and Cd + HZn) had significantly higher concentrations of Zn in soil solution during the flooded phase, and there was no treatment effect found on Cd or Fe concentrations in soil solutions (Table 5-3).

Table 5-3 - Mean and standard error (n=3) solution concentrations of cadmium, iron and zinc during flooded and drainage phases. Statistical significance letters are not shown due to interactions between the treatments and time. Statistical significance is instead considered between individual treatments and is discussed in the text. Soil treatments were: Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA). Detection limits (DLs) were 0.1 µg Cd/L, 0.1 mg Fe/L and 0.001 mg Zn/L. Values below the DL are indicated by ‘ nd’ for not-detected.

	Zinc (mg/L)				Iron (mg/L)				Cadmium (µg/L)			
Phase:	Flooded	error	Drainage	error	Flooded	error	Drainage	error	Flooded	error	Drainage	error
FC control	0.06	0.01	0.05	0.00	1.12	0.32	nd	0.00	0.47	0.21	0.47	0.23
control	0.03	0.00	0.07	0.01	3.33	0.85	1.57	0.79	nd	0.02	nd	0.00
Cd	0.02	0.00	0.05	0.00	2.28	0.54	1.12	0.38	0.85	0.18	nd	0.01
Cd + LZn	0.05	0.01	0.05	0.00	2.02	0.20	1.18	0.37	0.41	0.10	nd	0.00
Cd + HZn	0.07	0.00	0.06	0.00	1.18	0.23	0.96	0.28	1.21	0.17	0.49	0.06
Cd + Fe	0.03	0.00	0.06	0.00	12.65	3.15	11.79	4.21	1.76	0.59	nd	0.00
Cd + LZn + Fe	0.09	0.01	0.10	0.01	14.18	4.85	17.96	5.47	1.92	0.49	nd	0.00
Cd + LZn + Fe + EDTA	1.47	0.19	0.23	0.04	26.41	1.16	13.16	1.32	12.64	2.13	0.11	0.02
Cd + LZn + Mn	0.08	0.01	0.08	0.01	1.63	0.40	2.62	0.76	2.62	0.65	nd	0.00
Cd + Mn	0.02	0.00	0.04	0.01	1.72	0.19	0.45	0.22	1.06	0.25	nd	0.00
Cd + Zn spray	0.04	0.01	0.08	0.02	2.66	0.56	1.03	0.51	0.87	0.15	nd	0.00
Cd + EDTA spray	0.02	0.00	0.44	0.10	2.56	0.50	1.34	0.38	0.45	0.09	31.14	4.12

Cadmium soil treatments spiked with Fe (e.g. Cd + Fe and Cd + LZn + Fe) had significantly higher concentrations of total Fe and Fe(II) in soil solutions compared to the Cd and Cd + LZn treatments (Table 5-3). The Fe spike was found not to significantly affect Cd concentrations in soil solutions during flooding (Table 5-3). Soil treatments spiked with Fe in the presence of Zn (Cd + LZn + Fe) had significantly higher concentrations of Zn in soil solution compared to the Cd + LZn treatment even though the total Zn concentration in soil was lower (Table 5-3). The Fe-spiked soil treatments were found to have a significantly lower soil pH compared to other Cd-spiked treatments (e.g. Cd + LZn) (Figure 5-1). The addition of Fe to soils may have increased the concentration of Zn in soil solutions through cation exchange mechanism(s) and/or the lower pH resulting in lesser adsorption to solid phases. It must be noted the Cd concentrations in Cd + Fe and Cd + LZn + Fe treatments were lower than the Cd and Cd + LZn treatments so a direct comparison on Cd solubility due to the presence of Fe cannot be undertaken. Because the Fe treatment had significantly less total soil Cd but did not show significantly lower Cd concentrations in soil solution, the data indicate that the Fe treatment may have actually enhanced Cd solubility.

Soil treatments spiked with Mn (e.g. Cd + Mn and Cd + LZn + Mn) had significantly higher concentrations of Mn in soil solution during flooding compared to the Cd and Cd + LZn treatments (Table 5-3). The addition of Mn to soils did not affect Cd concentrations in soil solution during flooding (Table 5-3). The Cd soil spiked with Mn in the presence of Zn (Cd + LZn + Mn) had significantly higher Zn concentrations in soil solutions compared to the Cd + LZn treatment (Table 5-3). Soil spiking with Mn had no effect on the pH of soil solutions (Figure 5-1). Soil spiking with Mn may increase Zn in solution through cation exchange with the increased concentrations of Mn in solution, while Cd may be more strongly associated with the solid phase, as noted above.

The soil treatment spiked with EDTA (Cd + LZn + Fe + EDTA) had significantly higher concentrations of Cd, total Fe and Zn in soil solutions during flooding compared to the Cd + LZn + Fe treatment (Table 5-3). Iron(II) was unaffected by the treatment but it must be noted that the results of colorimetry were determined to be insensitive to Fe(II) in the presence of EDTA due to interference by competition between EDTA and reagents for Fe complexation. The presence of EDTA likely enhanced Cd, total Fe and Zn solubility through chelation. The stability constants (log K) for Cd-EDTA and Zn-EDTA are 16.5 (log molar units), compared to 14.3 (log molar units) for Fe(II)-EDTA (Martell and Smith, 1974). Iron(III) is most strongly complexed, with a log K of 25.1 (Hutcheson *et al.*, 2005), indicating that Fe(III) was likely solubilised by the EDTA spike.

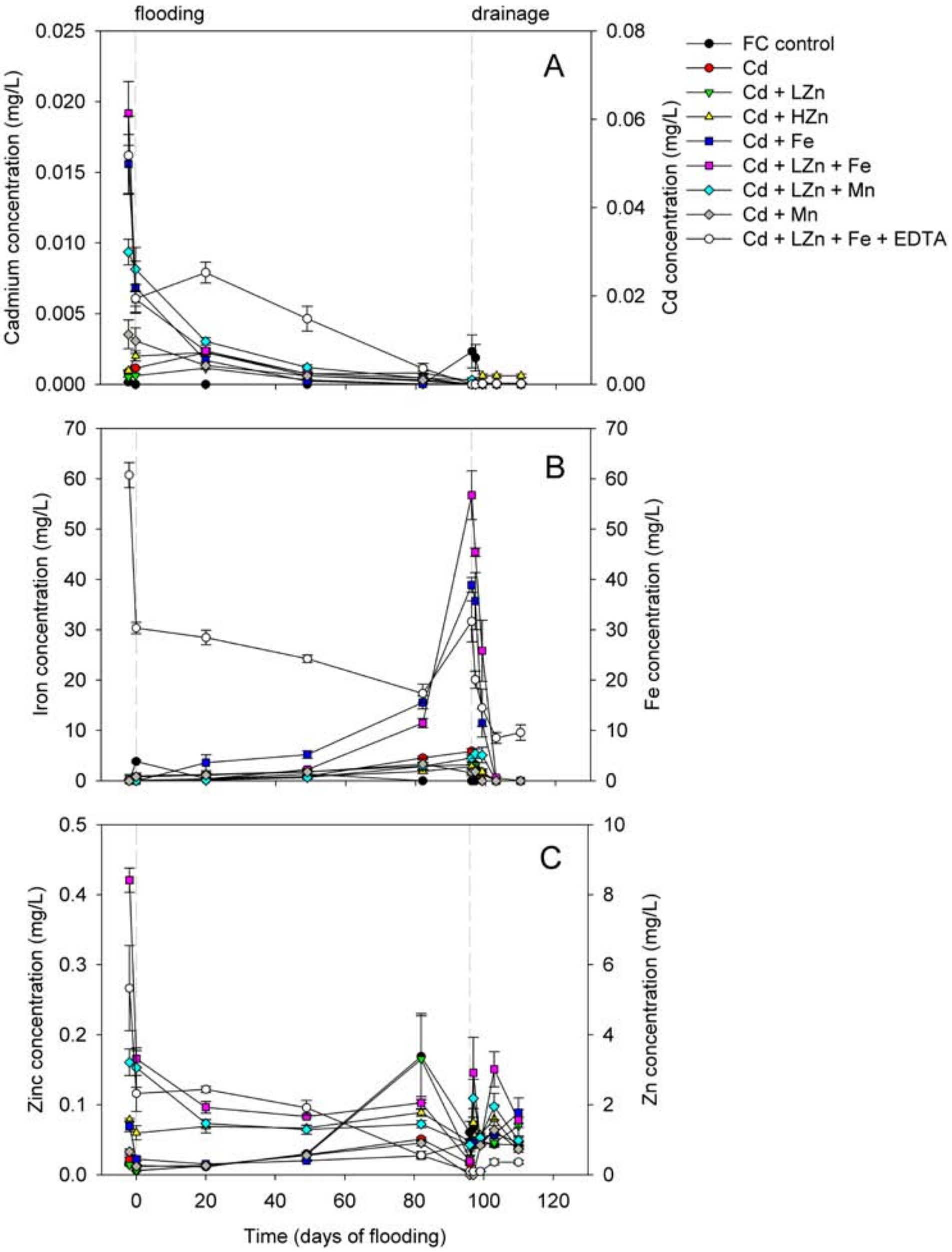

Figure 5-2. Cadmium (Cd), iron (Fe) and zinc (Zn) concentrations in soil solution during rice cultivation. The Y2 axis refers only to the Cd + LZn + Fe + EDTA treatment. Soil treatments were: Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA). Unspiked control and Cd + foliar Zn treatments not shown as Cd, Fe and Zn concentrations were not significantly different to Cd treatment. Panel A: Cadmium concentration (mg per litre); Panel B: Zinc concentration (mg per litre); Panel C: iron concentration (mg/L). Flooding was commenced at day 0 and drainage was initiated at day 96. Detection limits were 0.0001 mg/L for Cd, 0.001 mg/L for Zn and 0.1 mg/L for Fe. Blanks were at the detection limit.

5.4.3 Metal Concentrations in the Soil Solution - Drainage Phase

Upon drainage of all submerged treatments, the soil solution concentrations of Cl, K and Na (Appendix C) remained constant whereas Ca (data not shown), total Fe (Panel B, Figure 5-2), Mg and Mn (Appendix C) significantly ($P < 0.001$) decreased and Cd, Zn (Panels A and C, Figure 5-2) and SO_4^{2-} (Appendix C) significantly ($P <0.001$) increased. All of these elements with the exception of Cd are nutrients for higher plants (Marschner, 1995), so it is likely that some loss of these elements from solutions after drainage was due to plant uptake.

Manganese and Fe may be removed from soil solutions during drainage through the additional mechanisms of oxidation of soluble Mn(II) to insoluble Mn(III/IV) and Fe(II) to Fe(III) and precipitation as oxyhydroxide phases (Brookins, 1988; Gotoh and Patrick Jr, 1972; Ponnamperuma, 1964; Ponnamperuma, 1972), and/or adsorption of Mn(II) and Fe(II) to freshly precipitated solid phases (Thompson *et al.*, 2006). The decrease in divalent exchangeable cations (Ca and Mg) during drainage (Appendix C) may be due to plant uptake and adsorption to precipitated Fe and Mn oxyhydroxide phases. Monovalent cations (K and Na) were unaffected by drainage (Appendix C) as these elements are less readily sorbed to solid phases than divalent cations (Bohn *et al.*, 2001). The increase in the SO_4^{2-}concentration in soil solutions during drainage may be due to SO_4^{2-} desorption. Sulfate may also increase during drainage as a result of the oxidative dissolution of small quantities of sulfide minerals that were formed (and undetectable) in the soil during the flooded phase (Chapters 2 and 3).

The Cd soil treatment spiked with the high rate of Zn (Cd + HZn) had significantly ($P < 0.001$) higher concentrations of Cd and Zn in soil solutions during the soil drainage phase (Table 5-3) compared to the Cd-only spike treatment. The Zn spike may have increased Cd concentrations in soil solution due to competitive displacement of Cd from solid phases due to the higher concentrations of Zn in soil solutions (Lambert *et al.*, 2007). The pH was not significantly different between the Cd-only soil treatment and Cd soil treatments spiked with Zn, so it would appear that in this instance the concentration effect has overcome the fact that Cd is more strongly held than Zn as previously discussed.

The Cd soil treatment spiked with Fe (e.g. Cd + Fe) had significantly higher concentrations of Cl, Mn and Fe(II) in soil solution during drainage, significantly lower pH (Figure 5-1), and unchanged Cd and Zn concentrations compared to the flooded phase (Figure 5-2 and Table 5-3). In addition, soil spiked with Fe in the presence of added Zn (Cd + LZn + Fe) had significantly higher Zn concentrations in soil solutions compared to the Cd + LZn treatment (Figure 5-2). Cadmium concentrations in soil solution were unaffected by Fe-spiked treatments, despite the presence of less total soil Cd in these treatments. The soil Cd in Fe-spiked treatments therefore underwent greater solubilisation to the soil solution than in non-Fe spiked treatments, possibly a result of the more acidic pH with Fe spiking (Degryse *et al.*, 2004). The Zn concentration in soil solution was likely unaffected by the Cd + Fe treatment due to the geogenic nature of the soil Zn present. Geogenic metals are generally more aged and therefore less available for exchange reactions (Lock and Janssen, 2003). The significantly higher Zn concentrations in soil solutions in the Cd + LZn + Fe treatment may be due to the lower pH solubilising the added Zn to a greater extent than Zn removal from solution through adsorption to additional oxyhydroxide mineral surfaces. For example, over the pH range encountered in the experiment, adsorption to the common soil constituent hematite is expected to be greater for Cd than Zn, based on experimentally-derived adsorption isotherms (Li *et al.*, 2006; Musić and Ristić, 1992).

The soil treatments spiked with Mn in the absence or presence of added Zn did not significantly affect Cd, Fe or Zn concentrations in soil solution during drainage (Figure 5-2; Table 5-3).

The soil treatment spiked with EDTA (Cd + LZn + Fe + EDTA) had significantly higher soil solution concentrations of total Fe and Zn (Panels B and C, Figure 5-2, EDTA is on Y2 axis); and unaffected Cd (Panel A, Figure 5-2, EDTA is on Y2 axis) and Fe(II) (Appendix C) concentrations during drainage compared to the Cd + LZn + Fe treatment (Figure 5-2; Table 3). This suggests that with EDTA addition, during soil drainage Cd is more strongly retained by the soil solid phases than Zn and is not readily partitioned to the solution phase and hence is less potentially available to rice plants.

5.4.4 Metal concentrations in rice shoots

Elemental concentrations in rice shoots at maturity indicated plants were not suffering from deficiencies of Ca, K, Mg, Mn, P, S or Zn (Reuter and Robinson, 1997). However, plants were marginal with respect to Cu nutrition and all treatments which were not Fe-spiked may have been Fe deficient as shoot concentrations were less than 37 mg Fe/kg (Pal *et al.*, 2008) (Figure 5-3). It is known that rice can secrete PSs from their roots to increase the availability of Fe in submerged soils (Singh *et al.*, 2003). These compounds are known to chelate and solubilise Fe(III) for transport across the root membrane (Singh *et al.*, 2003), but have been shown to also increase plant uptake of Cd and Zn (Shenker *et al.*, 2001; Zhang *et al.*, 1998).

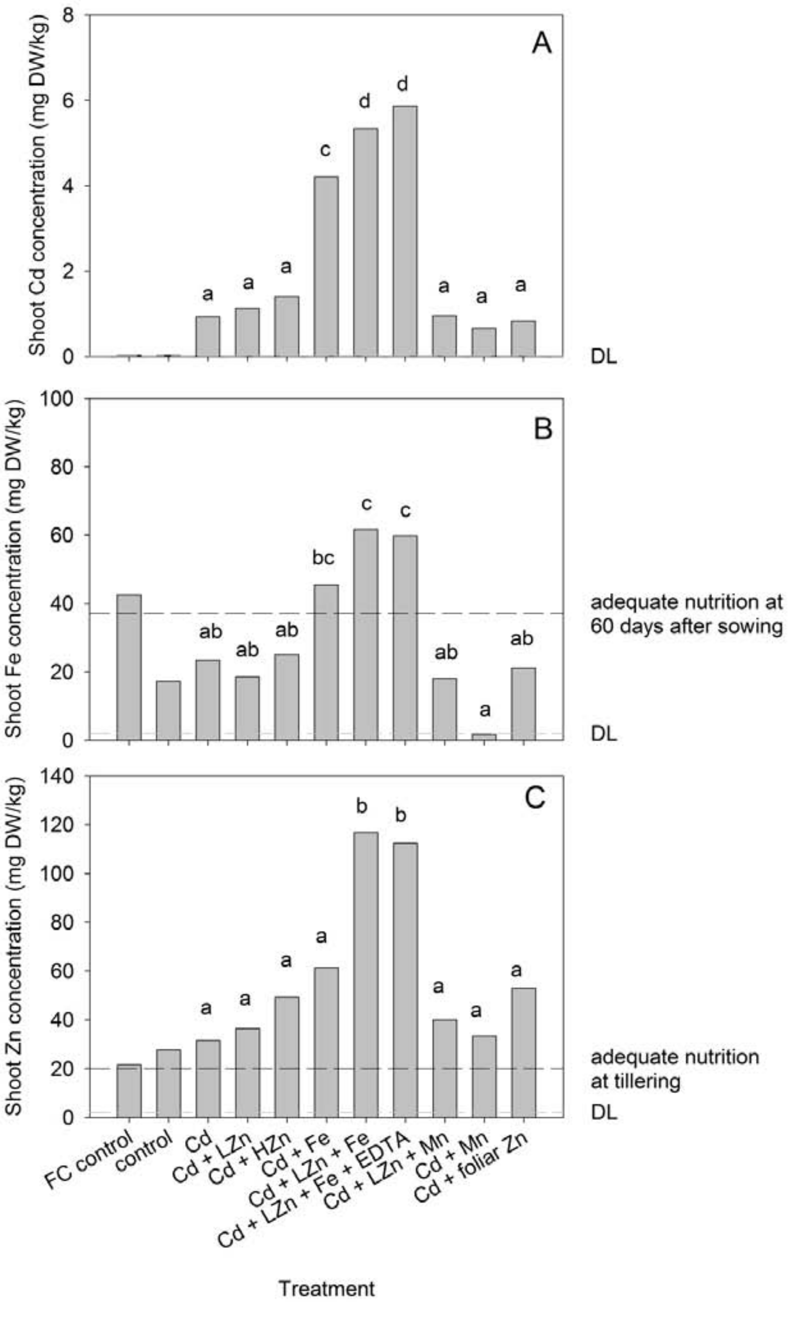

Figure 5-3. Cadmium, iron and zinc concentrations in rice shoots on a dry weight basis. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA). Analysis of variance letters are such that where treatments share a letter they are not significantly different. DL denotes detection limit. Panel A: cadmium; Panel B: iron; Panel C: zinc. Significant effects of soil treatments are indicated by different letters of the alphabet such that a is significantly different from b.

Spiking soils with Zn (i.e. Cd + LZn and Cd + HZn) had no significant influence on the Cd concentrations in the shoots of rice compared to the Cd-only treatment (Figure 5-3 and Figure 5-4). In contrast, soil treatments spiked with Fe (i.e. Cd + Fe and Cd + LZn + Fe) resulted in significantly higher concentrations of Cd in rice shoots compared to the Cd only treatment (Figure 5-3). The accumulation of Cd in Fe-spiked treatments is significantly higher even though there was a lower initial Cd concentration in these soils. Cadmium uptake by plants in the Fe-spiked treatments may be enhanced by the higher concentrations of Cl in Fe-spiked soil solutions (Ohtani *et al.*, 2007), and therefore greater speciation of Cd as ($CdCl_n^{2-n}$) , despite similar total Cd concentrations in solution (Smolders *et al.*, 1998). Other workers have demonstrated that soil solution chloride concentrations of 700 mg/L significantly enhanced Cd accumulation in rice shoots relative to control treatments (< 20 mg Cl/L) (Ohtani *et al.*, 2007). This occurs due to greater chloro-complexation of the Cd cation (Smolders *et al.*, 1998).

Soil spiked with Fe in the presence of Zn (Cd + LZn + Fe) significantly increased the Cd, Fe, and Zn concentrations in shoots (Figure 5-3). This is in agreement with the results of Yoshihara *et al*. (2010) who found that Fe addition enhanced rice shoot uptake of Zn; it was suggested that the excess Fe resulted in an overall decrease in the accumulation of Zn by the plant, and that ‘induced metal deficiency’ caused a redistribution of Zn from roots to shoots. The pH of the Fe-spiked treatments was significantly lower than the Cd spike treatment, and Cl concentrations were higher. Accordingly, the Fe, Mn and Zn concentrations in soil solution were higher and therefore these metals were more available for plant uptake. The Cd concentration in soil solution was no different to the control, so greater plant uptake of Cd cannot be explained by any difference in soil solution concentration.

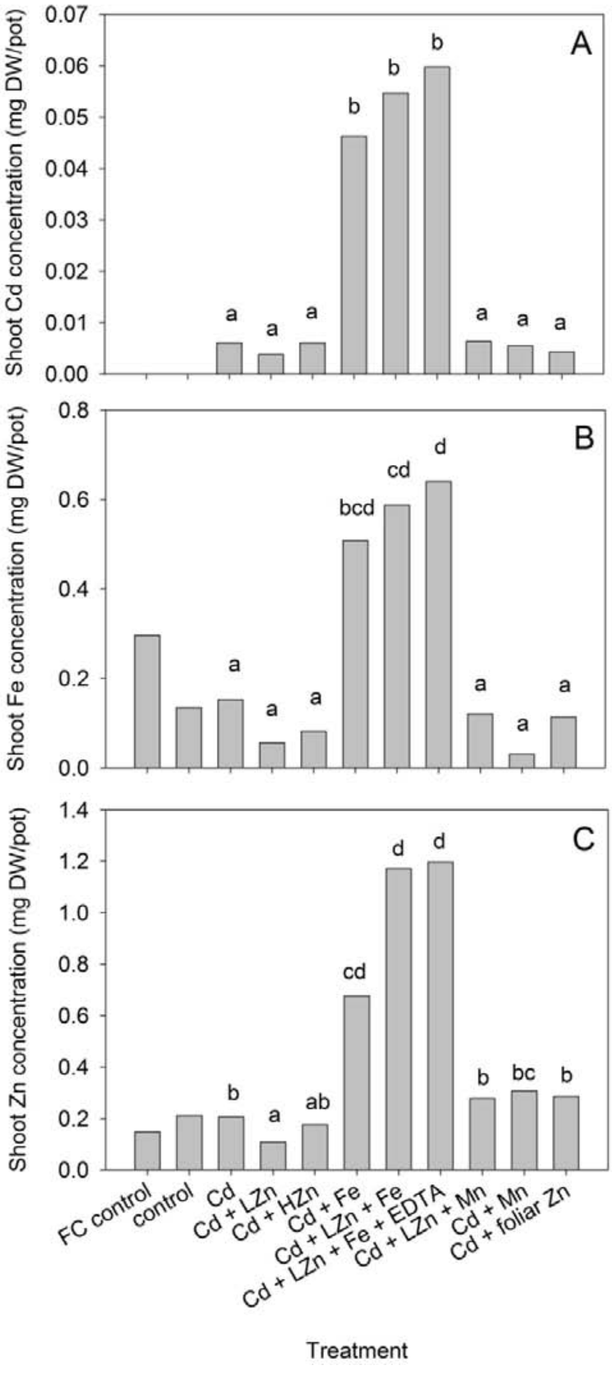

Figure 5-4. Cadmium, iron and zinc uptake into plant shoots. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar). Analysis of variance letters are such that where treatments share a letter they are not significantly different. Panel A: cadmium; Panel B: iron; Panel C: zinc.

Manganese addition to soils had no significant effect on Cd, Fe or Zn concentrations in shoots, which may be expected because there was no significant net influence of Mn addition to soils on soil solution concentrations (Table 5-3; Figure 5-4). Soil spiked with EDTA had no significant effect on the concentrations of Cd, Fe or Zn in the shoots, despite there being significantly higher Fe and Zn concentrations in the soil solution. This finding is in contrast to the results of other work, where EDTA has been shown to enhance Zn accumulation in rice shoots (Karak *et al.*, 2006) and to depress Cd accumulation in rice shoots (Shao *et al.*, 2008). Foliar treatments of Zn or EDTA also did not significantly affect Cd, Fe or Zn concentrations in shoots (Figure 5-4).

5.4.5 Metal concentrations in rice grains

Spiking Cd soils with Zn resulted in significantly lower grain yield (Appendix C). This is not likely a result of phytotoxicity from the higher concentrations of Zn in soil solution, as shoot Zn concentrations were not indicative of phytotoxicity (Reuter and Robinson, 1997). The result is unexpected as shoot yield was not significantly reduced along with the grain yield.

The Cd concentration in rice grain was significantly higher in the Cd + HZn treatment compared to the Cd-only treatment (Figure 5-5). This is in contrast to the data for shoot Cd concentrations, where there was no significant effect on shoot Cd or Zn concentrations with the addition of high concentrations of Zn to soil (HZn). The increased Cd in grain may be attributed to higher soil solution concentrations of Cd during the drainage phase in the Cd + HZn treatment, possibly due to competition for binding sites on solid phases (Table 5-3and Figure 5-2). The fact that Cd was increased in the grain but not the shoot is reflective of the higher Cd concentrations in soil solution during the drainage phase but not during the flooded phase. Shoot uptake of Cd likely occurs during both flooding and drainage, whereas grain uptake of Cd is likely to predominantly occur during drainage when the grains are developing. The finding of increased Cd accumulation in rice grain grown on soil spiked with Zn is similar to the findings of Zhou *et al*. (2003), who found that spiking soils with 200 mg Zn/kg (total soil Cd = 1.5 mg/kg) enhanced Cd accumulation in rice grains from 0.056 mg/kg to 0.096 mg/kg in a greenhouse experiment under flooded cultivation. The concentration of Cl^- was also higher in the Cd + HZn treatment compared to the Cd and Cd + LZn treatments, which may have also enhanced plant uptake of Cd as shown by other workers (Ohtani *et al.*, 2007; Smolders *et al.*, 1998). In contrast, Li *et al*. (2009) found that Zn spiking decreased the accumulation of Cd in rice grain, which was attributable to the competition between Zn and Cd during translocation within the plant as available Cd in soil (0.01 M $CaCl_2$ extraction) and Cd taken up by rice roots was not significantly different between Zn treatments and the control. Zinc concentrations in rice grains were not reported.

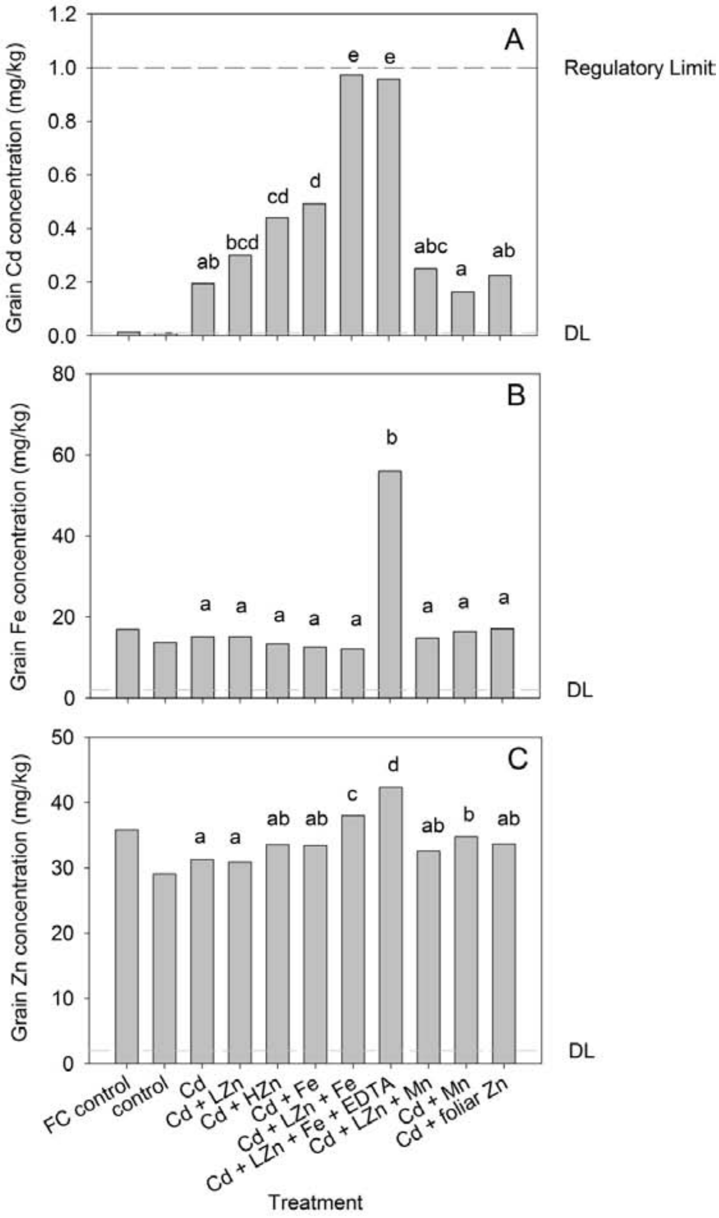

Figure 5-5. Cadmium, iron and zinc concentrations in rice grain. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA). Only Cd + foliar Zn is shown as it was representative of both foliar treatments. Analysis of variance letters are such that where treatments share a letter they are not significantly different. DL denotes detection limit. Panel A: cadmium; Panel B: zinc; Panel C: iron. Significant effects of soil treatments are indicated by different letters of the alphabet such that a is significantly different from b.

The Cd soil treatments spiked with Fe (e.g. Cd + Fe and Cd + LZn + Fe) were found to have significantly higher grain yield (Appendix C), likely through better plant nutrition with Fe (Tandon, 1996). Iron-spiking increased the Cd:Fe ratio by increasing the concentration of Cd in rice grain while the Fe concentration was constant (Figure 5-6). The additional Fe nutrition was not expressed through higher Fe concentrations in grains, as Fe does not readily translocate to non-photosynthetic organs of the rice plant (Simmons *et al.*, 2003). This indicates that addition of Fe to soils may increase the human health risk associated with Cd accumulation in paddy rice grain. This treatment effect cannot be explained by the effects on soil solution concentrations during soil drainage, as Cd concentrations in soil solution were unaffected (despite lower total Cd in soil for Fe treatments) whereas Fe concentrations were significantly higher in Fe-spiked soils. However, Cd accumulation in rice shoots and grain in Fe-spiked treatments was significantly higher even though there was a lower initial Cd concentration in these soils. The soil Cd in Fe-spiked treatments therefore underwent greater solubilisation to the soil solution than in non-Fe spiked treatments, possibly a result of the more acidic pH with Fe spiking (Degryse *et al.*, 2004). The higher uptake and accumulation of Cd in plant tissues may also be due to the higher chloride concentration (Ohtani *et al.*, 2007), and as a consequence the greater partitioning of solution Cd as $CdCl_2$ in soil solutions (Smolders *et al.*, 1998).

The Cd:Zn ratio in grain was also significantly higher in Fe-spiked soils (Figure 5-6); when Fe was spiked without Zn this was due to higher concentrations of Cd in grain, while Zn was constant, whereas when Fe was spiked in the presence of Zn it was due to higher concentrations of Cd and Zn in grain, but a greater increase for Cd.

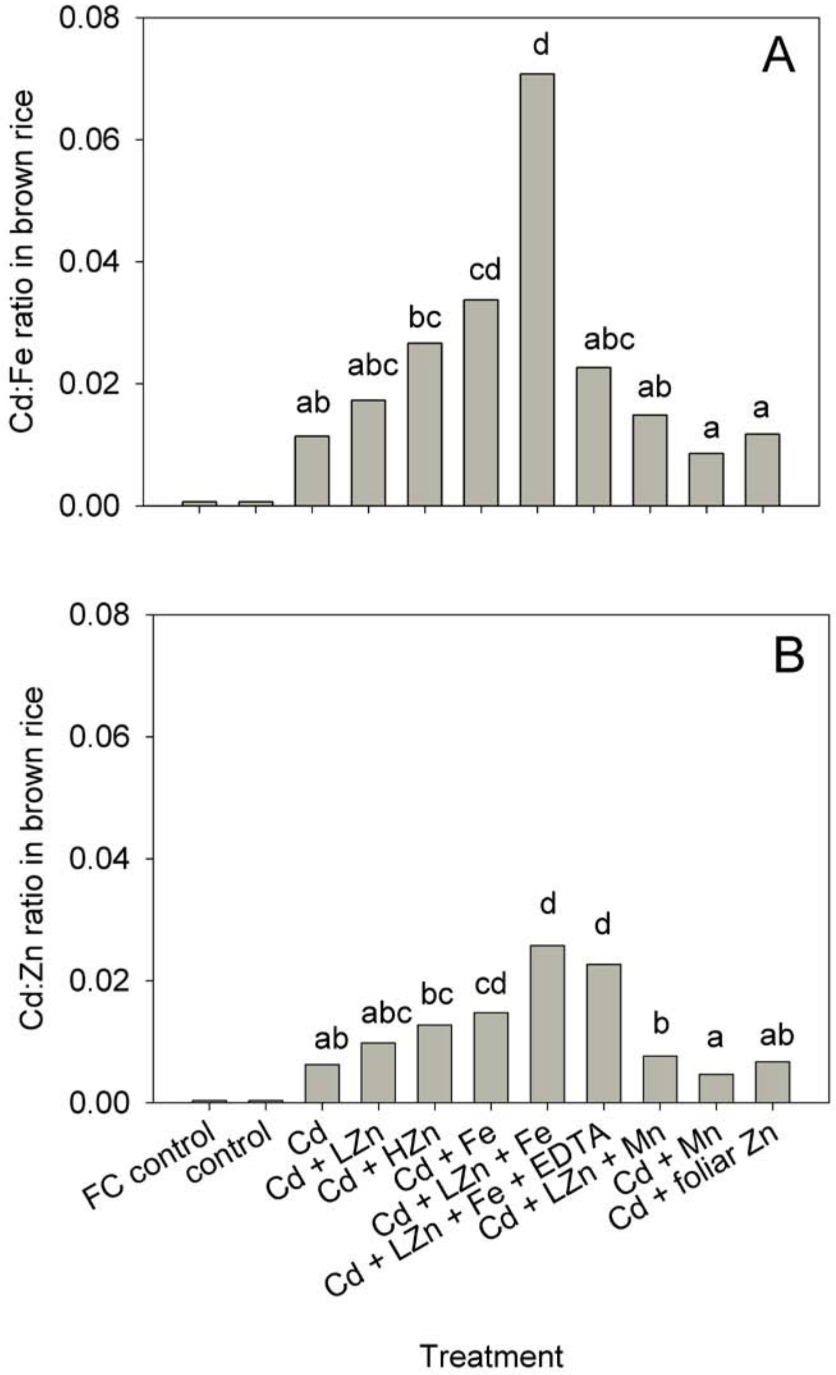

Figure 5-6. Cd:Fe and Cd:Zn ratios in brown rice grain. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA). Only Cd + foliar Zn is shown as it was representative of both foliar treatments. Analysis of variance letters are such that where treatments share a letter they are not significantly different. Panel A: Cd:Fe; Panel B: Cd:Zn.

Soil spiked with Mn gave significantly higher grain yields which may be due to better plant nutrition with Mn (Fageria, 2002), although plants were not found to be deficient in Mn. Spiking soil with Mn had no significant effect on the accumulation of Cd in rice grains (Figure 5-5), which may be expected as the Cd concentration in soil solutions was also unaffected. Soil spiked with Mn had no significant effect on Fe or the Cd:Fe or Cd:Zn ratios in rice grains. However, it must be noted that the Mn-spiked treatments (and all non Fe-spiked treatments) were Fe deficient, and the effect of Mn may be different under Fe sufficient conditions and should be investigated further.

Spiking soil with EDTA was found to significantly increase the concentrations of Fe and Zn in rice grains, whereas Cd concentrations were not significantly affected. As a result, the Cd:Fe ratio in grains was significantly decreased, but the Cd:Zn ratio was not significantly affected. The observed effect of EDTA is in agreement with other work that found addition of EDTA to soils enhanced the accumulation of Zn (Karak *et al.*, 2006) and Fe (Shao *et al.*, 2008) in rice grain. The higher Fe and Zn concentrations in rice grain observed in the present study are correlated to the significantly higher concentrations of Fe and Zn in the soil solution during the drainage phase. Shoot concentrations of Fe and Zn were not similarly increased with EDTA addition to soil, which may indicate that the Fe and Zn accumulated in the grain were translocated directly from soil solution, rather than being derived from storage in leaves, a phenomenon suggested previously by (Jiang *et al.*, 2007b). This may occur by direct uptake of the EDTA-metal complexes as this mechanism has been demonstrated in plants (Schaider *et al.*, 2006). Because EDTA decreased the Cd:Fe ratio, this treatment may be a promising candidate for further investigation to decrease grain Cd bioavailability to humans.

Cadmium, Fe and Zn concentrations in rice grains were found to be unaffected by Zn or EDTA foliar treatments (Figure 5-5). This indicates that these treatments were ineffective in decreasing the uptake and translocation of Cd from soils.

5.5 Conclusions

Rice grown in Cd-contaminated soil with added Fe and/or Zn in the presence of Cl^- may increase the risk to human and animal health from Cd in the grain. In the present study, Cd spiked soil with added Fe was found to have significantly higher Cd:Fe and Cd:Zn ratios in rice grain by increasing the concentration of Cd without affecting Fe or Zn concentrations. Similarly, Cd-spiked soils with added Zn significantly increased the Cd:Fe and Cd:Zn ratios in rice grain by increasing the concentration of Cd in the grain without affecting Fe or Zn concentrations. The increase in grain Cd concentrations was likely due to the presence of higher Cd concentrations in soil solutions with these treatments due to greater competition with Zn and Fe for surface exchange sites as well as enhanced Cd bioavailability by Cl^-.

In this study, Mn added to Cd spiked soil increased the concentration of Zn in rice grain significantly, likely through increased Zn uptake by rice and greater translocation within the plant, but there was no effect on concentrations of Cd and Fe, and no significant effect on the Cd:Fe and Cd:Zn ratios. However, the observations of the present study were made under conditions of Fe deficiency and further investigation is required.

Spiking soil with EDTA may help to decrease the risk to human health posed by Cd accumulation in paddy rice grain. Although the concentration of Cd in rice grains was unaffected, the Fe concentration was significantly higher and resulted in a significantly lower Cd:Fe ratio in rice grain, and therefore may decrease the bioavailability of the Cd present in grain (Reeves and Chaney, 2008). The mechanism behind this appeared to be a direct soil solution to grain pathway, rather than shoot to grain. Spiking with EDTA also significantly increased the Zn concentration in rice grain although the decrease in the Cd:Zn ratio was not significant, without affecting the Cd concentration or grain yield.

Foliar treatments of Zn or EDTA had no effect on Cd, Fe or Zn concentrations in paddy rice grain, nor on the Cd:Fe and Cd:Zn ratios. These treatments are not promising methods of limiting the risk to human health from Cd accumulation in paddy rice grain.

6 Conclusions and Future Directions

6.1 Conclusions

Rice is one of the most important crops grown for human consumption in the world, and contamination with Cd poses a potential health risk to humans. Subsistence rice farmers are most at risk from Cd accumulation in paddy rice grain due to poor micronutrient nutrition, and their staple food may be grown on agricultural soils which have been polluted with Cd through the land application of Cd-rich biosolids and contamination with mine waste (Hutton, 1982). Research into Cd uptake and translocation in rice plants was sparked by the 'Itai-Itai' disease that struck subsistence rice farmers in Japan in the 1960s who were also deficient in Fe and Zn (Nordberg, 2004). There have been no similar reports of health problems like this in western society, possibly due to the recognition of Cd as a contaminant of concern in the environment and better micronutrient nutrition minimizing bioaccumulation of Cd by Western consumers from their diets (McLaughlin *et al.*, 1999b). It is often not economically feasible to remediate Cd-contaminated soils for paddy rice production.

Paddy rice grains accumulate more Cd than the nutrients Fe and Zn, based on their relative soil concentrations (Chaney *et al.*, 1996). This combination of greater accumulation of Cd relative to Fe and Zn in rice grain can contribute to enhanced Cd bioavailability (McLaughlin *et al.*, 1999b). It is therefore important to investigate the mechanism(s) by which Cd becomes enriched in paddy rice grain relative to Fe and Zn.

The enrichment of Cd in paddy rice grain occurs during soil oxidation, which accompanies pre-harvest drainage of the flooded paddy (Iimura, 1981b; Inahara *et al.*, 2007). Pre-harvest drainage is typically initiated 2 weeks before maturity, coinciding with the grain-filling phase of rice growth. Therefore it is during this time that soil Cd becomes more available to the plant than soil Fe and Zn (or Fe and Zn become less available than Cd), and these elements can compete with Cd in terms of root uptake and translocation to grain (Honma and Hirata, 1977; Saito and Takahashi, 1978a), i.e. the Cd:Fe and Cd:Zn ratios in the soil solution become higher.

Cadmium therefore accumulates in paddy rice grain and becomes enriched relative to Fe and Zn during pre-harvest drainage, but the mechanism by which this occurs remains unclear. The risk to human health posed by Cd enrichment in paddy rice grain cannot be mitigated without an adequate understanding of this phenomenon.

There were two main objectives of this thesis:

1. To elucidate which soil processes are likely responsible for Cd accumulation and enrichment relative to Fe and Zn in paddy rice grain, and;
2. To determine whether soil amendments to paddy fields or foliar sprays of EDTA or Zn to rice plants could be used to limit Cd accumulation and enrichment in paddy rice grain.

Objective 1 was addressed in part through testing the mechanism that is currently proposed to be responsible for the enrichment of Cd in paddy rice grain; differential rates of mineral sulfide oxidation.

The release of Cd, Fe and Zn from co-precipitated sulfide minerals was investigated through the synthesis and oxidation of sulfide minerals containing Fe as well as Cd and Zn at environmentally relevant molar ratios (Chapter 3). The unknown effect of mineral crystallinity on the relative release of Cd, Fe and Zn was also explored by comparison between freeze-dried (poorly crystalline) and oven-dried (OD) (more crystalline) materials.

The possible role of galvanic interactions between mineral particles in controlling the relative release of Cd, Fe and Zn was explored through comparison of mixtures of pure sulfides (MPS) with co-precipitated sulfides (SS) having similar molar ratios.

Cadmium release during oxidative dissolution was close to 100 % for CdS and CdS OD, but less than 5 % for 1.2 % Cd SS, 0.6 % Cd SS, 1.2 % Cd MPS, 0.6 % Cd MPS or 0.6 % Cd SS OD. This indicates that when Cd is present as pure CdS in isolation from other metal sulfides, it is released to a much greater extent than when present under more environmentally realistic scenarios. The result is in agreement with previous work on Cd co-precipitated in sphalerite at less than 50 molar percent of cations by Barrett and McBride (2007).

When the sulfide minerals were oxidized, the Cd:Fe and Cd:Zn molar ratios in solution were less than unity (expressed in Chapter 3 article in press as Fe:Cd and Zn:Cd molar ratios in solution greater than unity), indicating that Cd was not released in preference to Fe or Zn from co-precipitated (Cd, Fe, Zn) sulfide minerals. This finding was in line with those of Barrett and McBride (Barrett and McBride, 2007). It is therefore highly unlikely that the differential oxidation of sulfide minerals is the process responsible for Cd enrichment in paddy rice grain. It was also shown that the Zn:Cd ratio in solution derived from the oxidation of a mixture of pure sulfides was higher than in solution from oxidation of the corresponding co-precipitated (Cd, Fe, Zn) sphalerite, which may be an indication that galvanic interactions can protect Zn from oxidative release.

The greater crystallinity of sulfide minerals may result in preferential release of Cd relative to Fe and Zn from co-precipitated (Cd, Fe, Zn) sphalerite. Oven-drying of the co-precipitated (Cd, Fe, Zn) sphalerite (0.6 % Cd SS OD compared to 0.6 % Cd SS) gave rise to higher Cd:Fe and Cd:Zn ratios in solutions (expressed in Chapter 3 article in press as lower Fe:Cd and Zn:Cd ratios in solutions). This is in contrast to freeze-dried co-precipitated (Cd, Fe, Zn) sphalerite, where Fe and Zn were released in preference to Cd (higher Fe:Cd and Zn:Cd ratios). These results could indicate that when co-precipitated (Cd, Fe, Zn) sphalerite minerals are present in nature, greater crystallinity prior to oxidation may result in more Cd released relative to Zn and Fe. This result has not been previously documented in the literature and further investigation is required into whether this is due to surface oxidation of starting materials or crystallinity. There was no consistent difference between 1.2 % Cd MPS and 1.2 % Cd SS, indicating that the possibility of galvanic interactions in the 0.6 % Cd MPS treatment may have been anomalous, or that the effect is dependent upon the Cd concentration.

Nanoparticles were identified as representing up to 8 % of ‘dissolved S’ in the oxidizing solutions of CdS, 0.6 % Cd MPS and 1.2 % Cd MPS, and 37 to 50 % of ‘dissolved S’ in the oxidizing solutions of FeS, ZnS, 1.2 % Cd SS and 0.6 % Cd SS. However, these nanoparticles were shown to be comprised only of sulfur rather than metals or metal-sulfur compounds. I therefore conclude that differential release of metals as nanoparticles was not likely an important factor in the enrichment of Cd in paddy rice grain.

The key finding from the research presented in Chapter 3 was that sulfide mineral oxidation does not result in greater Cd release to solution than Fe or Zn from mixed mineral phases at environmentally relevant concentrations. This suggests that although sulfide oxidation likely occurs during pre-harvest drainage of rice paddies, it is not likely that this mechanism alone results in Cd accumulation and enrichment in paddy rice grain. Other mechanisms (e.g. sorption), are likely to contribute to the control of Cd solubility in submerged soil environments. The outcome of this research addressed objective 1 in part, by eliminating one potential mechanism for Cd enrichment in paddy rice grain.

In order to address Thesis Objective 1 more completely, it was necessary to verify this finding in a soil environment, and to elucidate alternative mechanisms for the phenomenon of Cd enrichment in paddy rice grain.

The dominant processes controlling Cd solubility (relative to Fe and Zn) in flooded and pre-harvest drained paddy soil (Objective 1) were further investigated through the experimental simulation of paddy soil flooding and drainage (Chapter 4). The influence of sulfide mineral oxidation on relative release of Cd, Fe and Zn to solution was investigated for the first time in a soil environment, in comparison to a sulfate-limited control. It was shown that when sulfide minerals form under reducing conditions and then oxidize, this process in fact limits Cd solubility relative to Fe and Zn. In the absence of any similar research from the soil science literature, this finding is supported by the results of Cantwell *et al*. (2008), where the oxidative release of both Cd and Zn was found to be limited when higher amounts of sulfide were present in reduced sediments over a range of metal:sulfide molar ratios comprising 5, 2, 1, 0.5 and 0.2.

The result addresses Thesis Objective 1 and verifies the outcome of Chapter 3 for a soil system; metal sulfide precipitation during flooding and re-oxidation during drainage is not likely the cause of Cd enrichment in paddy rice grain. This conclusion brings to light a new hypothesis, that sulfide formation (prior to oxidation) is in fact the best-case scenario in terms of limiting Cd enrichment in paddy rice grain.

To better understand the phenomenon of Cd enrichment in paddy rice grain, the worst-case scenario for Cd enrichment in paddy rice grain was considered; conditions of sulfate-limitation. Under sulfate-limitation, it was demonstrated that the Cd:Fe and Cd:Zn ratios in soil solution increase during soil oxidation by virtue of decreasing Fe and Zn concentrations while Cd remained unchanged. This would indeed give rise to greater plant uptake of Cd relative to Fe and Zn per unit Cd in solution, in comparison to soil that does not undergo pre-harvest drainage. Sulfide oxidation is clearly not the mechanism responsible for this, but rather the lack of protection through sulfide formation during submergence seemed to be the plausible mechanism for this observation.

To enhance our understanding of the phenomenon and to address Objective 1, the possible influence of cation competition was tested with a Zn amendment to the paddy soil, which resulted in lower Cd:Fe and Cd:Zn ratios in soil solution through increased concentrations of Fe and Zn and no effect on solution Cd concentrations. This finding elucidated the previously unknown effect of competitive ions (Fe and Zn) on Cd solubility in soil solution under sulfate-limiting and variable-redox conditions. Addressing Objective 1, it was postulated that relative metal concentrations were affected mainly by differential adsorption to soil constituents during oxidation. Leading to Objective 2, the observations of decreased Cd:Fe and Cd:Zn ratios in soil solution suggested that Cd accumulation in paddy rice grain relative to Fe and Zn may be lower in soils high in Zn and that Zn soil amendments may be a feasible method of lowering Cd:Fe and Cd:Zn ratios in soil solution, thereby limiting the risk to human health from Cd enrichment in paddy rice grain.

The findings that addition of Zn to soil lowers the Cd;Fe and Cd:Zn ratios in soil solution and that Cd, Fe and Zn concentrations in soil solution were most likely controlled by differential adsorption to Fe/Mn oxyhydroxides, were then investigated in a soil-plant system to ascertain whether Zn may be a promising additive for agricultural soils to lower the Cd concentration and the Cd:Fe and Cd:Zn ratios in paddy rice grain.

A pot study was undertaken in which paddy rice was cultivated under flooded conditions incorporating a pre-harvest drainage period (Chapter 5). For the soils tested, it was shown that Zn addition to soil actually enhanced Cd accumulation in the grain without affecting Fe or Zn, and that this was due to increased concentrations of Cd in the soil solution. This result was just the opposite to that of the solubility study in Chapter 4, where Zn addition increased Fe and Zn concentrations in soil solution without affecting solution Cd concentrations. Absolute concentrations of Cd and Zn were similar between the two studies, so the result highlights the importance of undertaking plant-based studies, and indicates that addition of Zn may not always reduce Cd accumulation in paddy rice grain.

The effects of Fe or Mn added to soils were also tested to address Objective 2 and evaluate whether adsorption to oxyhydroxide minerals formed in the soil (during oxidation) would influence Cd and Zn solubility/bioavailability, as indicated from the soil solution results of Chapter 4. If Zn were strongly adsorbed in preference to Cd and Fe, soils high in Fe and Mn may actually pose a higher risk for Cd accumulation in paddy rice grain, a possibility which had not been formerly investigated. It was found that Fe addition to soil increased the Cd:Fe ratio in rice grain by increasing Cd accumulation in the grain without affecting Fe or Zn, and that this was likely due to increased concentrations of Cd and residual Cl^- in the soil solution. Therefore, rice grown in soil amended with soluble Fe salts may pose a greater risk to human health in terms of Cd accumulation in rice grain. Soil spiked with Mn had no significant effect on the accumulation of Cd in rice grains, which may be expected as the Cd concentration in soil solutions was also unaffected. Soil spiked with Mn had no significant effect on Fe or the Cd:Fe or Cd:Zn ratios in rice grains. However, it must be noted that the Mn-spiked treatments (and all non Fe-spiked treatments) were Fe deficient, and the effect of Mn may be different under Fe sufficient conditions and should be investigated further.

The effect of EDTA added to soil on relative accumulation of Cd, Fe and Zn in rice grain was also tested to address Objective 2 and the knowledge gap arising from conflicting reports in the literature. It was shown that application of EDTA to soil was found to increase the Fe and Zn concentrations in paddy rice without affecting the Cd concentration, thereby likely decreasing human Cd bioavailability. This result was in agreement with other work where EDTA enhanced the uptake of Zn (Karak *et al.*, 2006) and Fe (Shao *et al.*, 2008) in rice grain.

Further to Objective 2, it was shown that foliar sprays of EDTA or Zn had no effect on relative accumulation of Cd, Fe or Zn in paddy rice grain. The result is in contrast with the work of Shao *et al*. (2008) who previously demonstrated that EDTA could decrease Cd accumulation in paddy rice grain. The discrepancy between results may indicate that the results of Shao *et al*. (2008) were confounded by the effect of applying Fe to the plants as a part of the EDTA treatment.

In summary, the outcomes of the pot trial addressed Objective 2 by demonstrating that soil additions of EDTA or Mn may be promising methods of limiting the risk to human health posed by Cd accumulation in paddy rice grain. Soil additions of Fe or Zn were not found to be effective methods to reduce Cd accumulation in rice grain.

There was no discernible consistent relationship between metal concentrations in soil solutions, shoots and grains, highlighting the importance of plant-based testing where plants are grown to maturity and grains harvested. Relative metal concentrations in shoots cannot be confidently used to predict metal concentrations in the grain. On a broader level, the conclusions of Chapter 5 as compared with those of Chapter 3, demonstrate that Cd accumulation and enrichment relative to Fe and Zn in paddy rice grain is likely significantly modified by plant-based mechanisms rather than simple soil solution concentration ratios.

6.2 Limitations and Future Directions

The findings of this research indicate that differential sulfide oxidation may not be the major mechanism in all soils behind Cd enrichment in paddy rice grain, and suggests an alternative hypothesis; the consideration that the precipitation of sulfide minerals during flooding is actually protective against Cd enrichment in paddy rice grain, even when pre-harvest drainage occurs. Future research should investigate whether this phenomenon is indeed operative in most soils. This finding was verified using a closed vessel reaction system where it was shown that metal sulfide precipitation during the reduced phase lowers the Cd:Fe and Cd:Zn ratios in soil solution during the oxidized phase. Therefore, sulfate addition to paddy fields may in fact be a viable method of limiting Cd enrichment in paddy rice grain where necessary. However, this requires verification in rice cultivation experiments and/or field trials.

The effect of addition of Zn to soil on Cd:Fe and Cd:Zn ratios in soil solution observed in the solubility experiments were different to the effects observed in the soil solution of the pot trial. This may be due to the effect of plant roots and the chemistry of the soil-root interface, and therefore highlights the importance of plant-based studies in this area. However, the discrepancy may also indicate a limitation of the ‘reaction cell’ apparatus used to carry out the experiments. The soil:solution ratio used to facilitate sampling and mixing meant that it was much lower than would be found in a paddy soil, even under flooded conditions. Additionally, an unexpected and large pH rise occurred during oxidation, which does not occur in a typical paddy field, nor did it occur in the pot trial using the same soil. This was likely an artifact of the reaction cell system, and may have affected metal solubilities, particularly as high pH conditions favour adsorption of the metals of interest to the solid phase.

The promising effect of soil EDTA addition on increasing the accumulation of Fe (relative to Cd and Zn) in paddy rice grain was significant, and would benefit from verification in a field trial. The soil amendment with EDTA may provide a viable method of enhancing both the nutritional profile of paddy rice as well as decreasing the risk to human health from Cd. When the Fe concentration is higher in rice grain, subsistence rice farmers would receive better Fe nutrition from the crop, and would therefore be better protected against the health effects of Cd that may also be present in the grain. It has been previously established that there is no correlation between soil Fe and grain Fe for paddy rice, and that therefore most soil factors do not influence grain Fe, so this EDTA application to soil may provide a unique method of increasing grain Fe.

The indication that soils amended with Fe and Zn salts may increase Cd accumulation in paddy rice grain relative to Fe and Zn requires further investigation. The observation for Zn addition was made under conditions of Fe deficiency, while the Fe added treatment resulted in high concentrations of residual Cl^- in the soil and Fe-sufficient plants which were compared to Fe-deficient control plants. This risk may be unique to scenarios where Fe amendment overcomes Fe deficiency and/or where high residual Cl^- is present, but this should be verified with further work. Additionally, these observations were made under the ‘worst-case scenario’ of cultivation under conditions where sulfate-reduction did not occur to an observable extent. Where conditions are strongly reducing enough to precipitate sulfide minerals, Cd may be so limited to the plant during drainage that additional Fe and/or Zn in the soil may not affect Cd accumulation relative to Fe and Zn in the grain at all.

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8 Appendices

8.1 Appendix A - Chapter 4 reaction cell method development

8.1.1 Design

The first Controlled Atmosphere Stirred Flask (Stirred flask) was designed using a 1000 mL rigid plastic container. Holes were drilled in the lid to fit a pH electrode, a combination redox potential electrode, a sampling port, a gas inlet tube and a gas outlet tube (Figure 8-1). A 6 cm long stir bar was placed on the bottom of the stirred flask along with 100 g of soil and 500 mL DI water. The stirred flask was placed on a stirring plate. Several methods were tried to seal the holes in the lid including: nonporous plastic wrap (Saran Premium Wrap, Johnson & Co, Inc., USA); sticky tape (3M Tartan 200 tape, Canada) and; silicone sealant (Selleys Wet Area, NSW). Silicone sealant was the only sealing method in which gas could be seen consistently bubbling through the gas outlet tubing into a beaker of water. Silicone sealant was hence deemed to be the most effective method of keeping the flask airtight, and was used in all subsequent versions of the stirred flask.

Figure 8-1. Version 1 of the stirred flask

The airtight seal in version 1 was not completely reliable (gas did not bubble through consistently), so version 2 was tried. Version 2 featured a smaller size (500 mL flask) and different style of lid. It was hoped that this might make the stirred flask more airtight and eliminate the need for silicone sealant (Figure 8-2). This was not the case, however, an improved seal around the gas and sampling tubing was observed. The gas and sampling tubes were 2 mm holes into which 10 mm rubber stoppers were inserted (Figure 8-2). The rubber stoppers formed a reliable seal with the lid and no silicone sealant was required. The basic design and sealing method are unchanged from Version 2 to Version 4, and other modifications are described in sections 8.1.2 to 8.1.7.

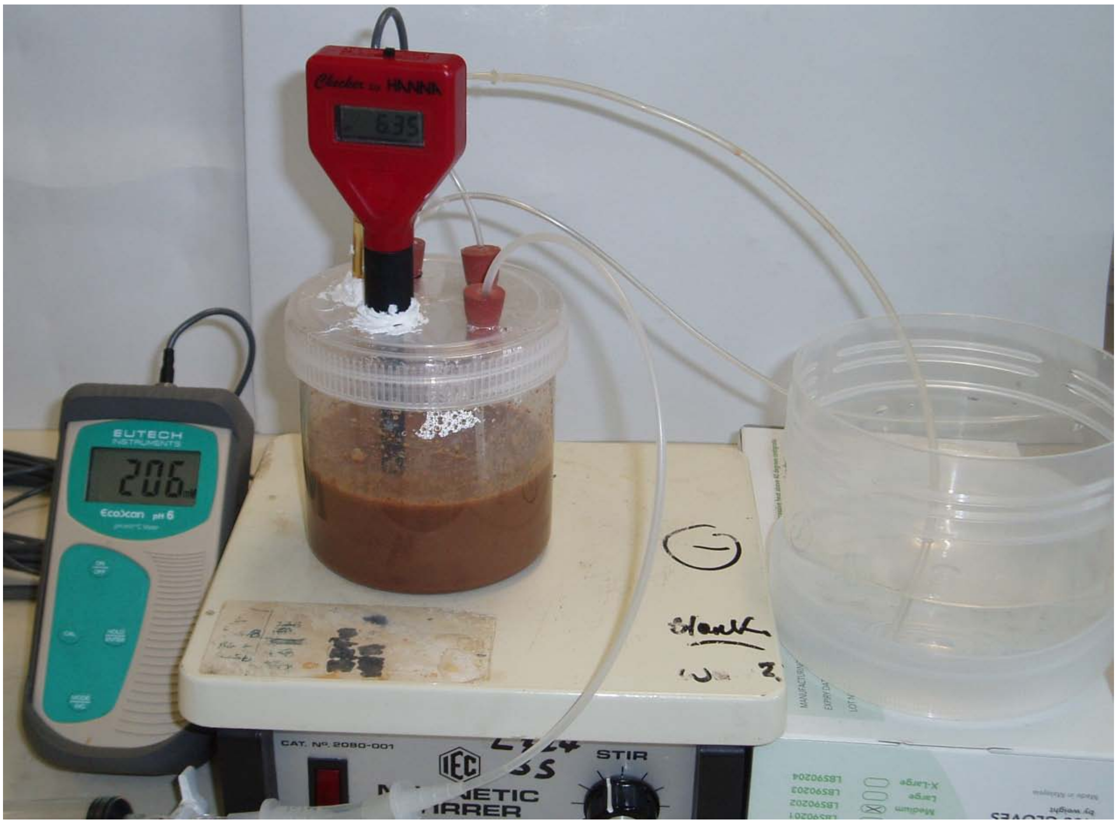

Figure 8-2. Version 2 of the stirred flask.

8.1.2 Stirring Mechanism

The stirred flask version 1 utilized a 6 cm stir bar in a 1000 mL flask filled with 100 g soil and 500 mL water (Figure 8-1) and was ineffective at keeping the soil in suspension. Version 2 of the stirred flask used the same 6 cm stirrer bar in a 500 mL flask filled with 50 g soil and 250 mL water (Figure 8-2), which gave the stir bar a better fit in the bottom of the flask. It was also hoped that the decreased mass of soil would improve mixing. Version 2 also was inadequate in keeping the soil in suspension.

A stirred flask version 3 was then examined and differed from the previous versions by modifying the stirring method. This comprised a self-stirring flask made from polystyrene plastic, purchased from Sigma-Aldrich (Corning, ‘disposable spinner flask’) and originally designed for cell culture work (Figure 8-3).

Figure 8-3. Version 3 of the stirred flask

The stir bar in the flask is encased in plastic and suspended from the lid by a plastic rod so that it does not touch the bottom of the flask. This plastic assembly allows the stir bar to move freely using a stir plate. Additionally the plastic assembly has ‘wings’ attached to the plastic rod to improve the stirring (Figure 8-3). These wings prevented the insertion of the electrodes to the midway depth in the stirred flask without interfering with the stirring. The plastic assembly was modified by removing half of the ‘wings’ to allow the insertion of the electrodes to proper depth (Figure 8-4).

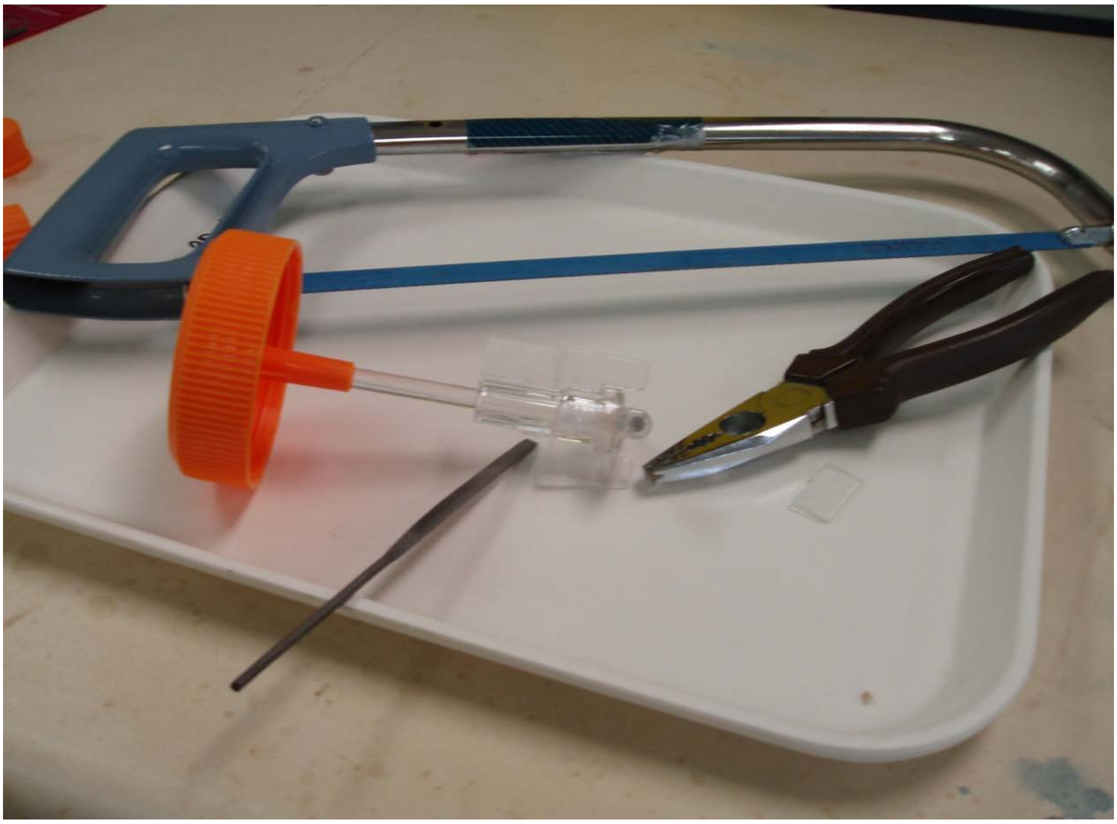

Figure 8-4. Modifications to stirred flask used in Version 3

To test this version, 50 g of soil and 200 mL of water was added to the stirred flask. The stirring was better than versions 1 and 2 but still not ideal. Reducing the amount of soil to 7.5 g in 200 mL water gave better results, and reducing it further to 5 g in 200 mL gave consistent, smooth stirring. Version 3 of the stirred flask was tested for approximately 1 month with continuous stirring during a preliminary experiment. The results showed there were no problems with the seals however the stirring assembly was noticeably abraded due to constant contact with moving soil. Abrasion was present on the ‘wings’ and the plastic rod which suspended the moving part from the lid. The abrasion had caused the moving parts to drop and the assembly was resting on the bottom of the flask. The abrasion was occurring on the bottom of the flask and after approximately one week a hole formed on the bottom of the flask and the contents leaked out. This version of the stirred flask was therefore unsuitable as would be prone to catastrophic failure.

With the inadequacies of the previous versions of the stirred flask a final version, version 4 (Figure 8-5), was developed using a stirring wand (Figure 8-6). The wand, SpHinner (Gerard Scientific, Adelaide), was installed in a hole drilled into the lid of a 400 mL plastic flask. The wand is powered by electricity and is capable of variable speeds and can keep the soil in suspension even at low speed settings. The wand required no additional modification and only comes into contact with the lid of the flask. The moving part of the stirrer is replaceable and can be replaced if wear is observed. Version 4 of the stirred flask used 5 g of soil in 200 mL water and smooth, consistent stirring was observed.

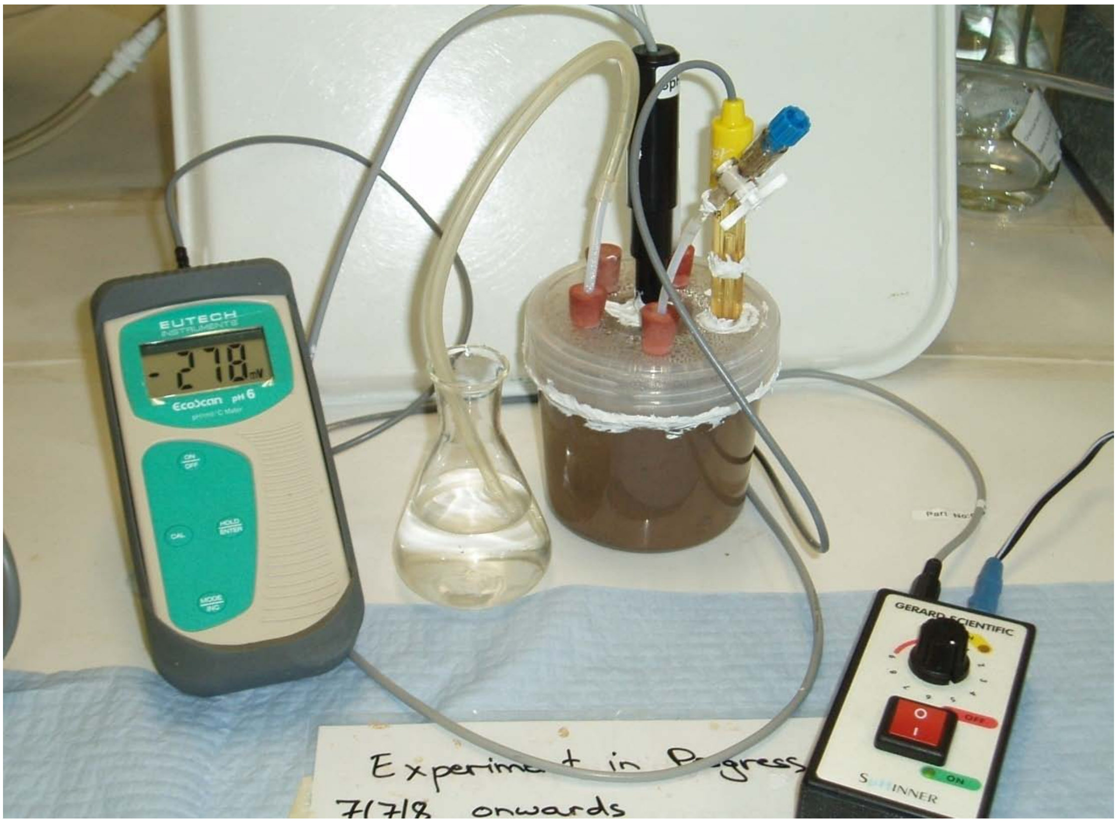

Figure 8-5. Version 4 (final version) of the stirred flask

Figure 8-6. SpHinner used in Version 4 of the Stirred Flask

8.1.3 Gas Delivery

In order to control the redox potential of the soil suspension gases needed to be supplied to the stirred flask. We needed to be able to exclude oxygen from the flask to drive the redox potential down to negative values to simulate a flooded paddy soil, and then be able to raise the redox potential by reintroducing oxygen to the system. Other workers have achieved this using air and nitrogen (Patrick *et al.*, 1973).

To expedite the experimentation time eight stirred flasks were concurrently run. The method of gas delivery is critical and went through three prototypes. The nitrogen was supplied from a gas bottle set up next to the bench so that the stirred flasks were guaranteed an uninterrupted nitrogen atmosphere when required. The air was supplied from a compressed air tap permanently installed in the laboratory. The gas flow for each gas and each stirred flask was controlled using a flow controller (micro, Gilmont, USA) prior to entering the stirred flasks.

Initially the gas flow to the flasks were in series (Figure 8-7) but even using needles inside the tubing to equalize the pressure did not result in equal gas distribution to each of the three flasks.

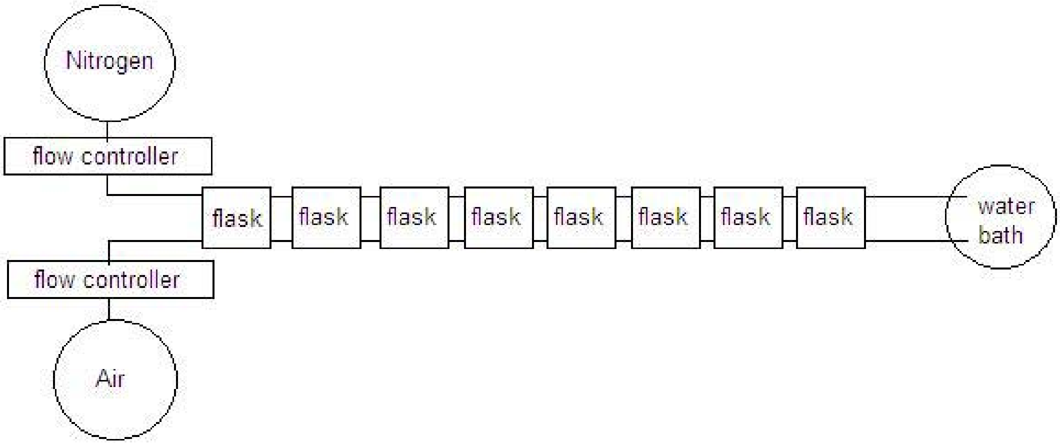

Figure 8-7. Gas flow to flasks in series, nitrogen and air

Subsequently, the gas delivery was redesigned so that the flow was in parallel and each flask was directly connected to two flow controllers, one for air and one for nitrogen (Figure 8-8). The gas flow could be easily kept equal in the three flasks using this method.

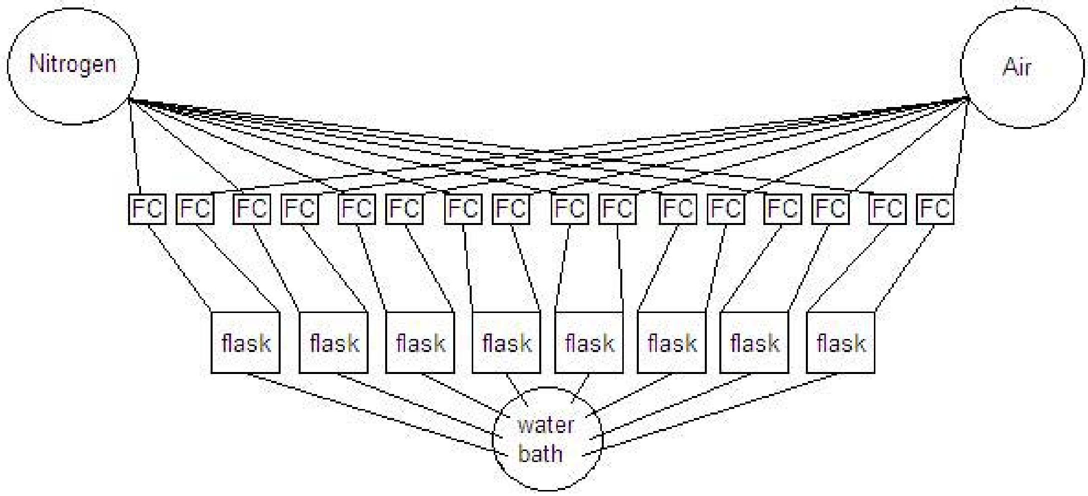

Figure 8-8. Gas flow to flasks in parallel, nitrogen and air

However, it was not possible to poise the redox potential by manipulating the ratio of gases (Section 8.1.7), so it became redundant to have separate nitrogen and air systems. The atmosphere of the flask would simply need to change from nitrogen only to air only, as the experiment required oxidation to follow a prolonged reduced state. The gas flow was then redesigned to one flow controller per flask, with both nitrogen and air tubes (equipped with stopcocks) feeding into each flow controller (Figure 8-9).

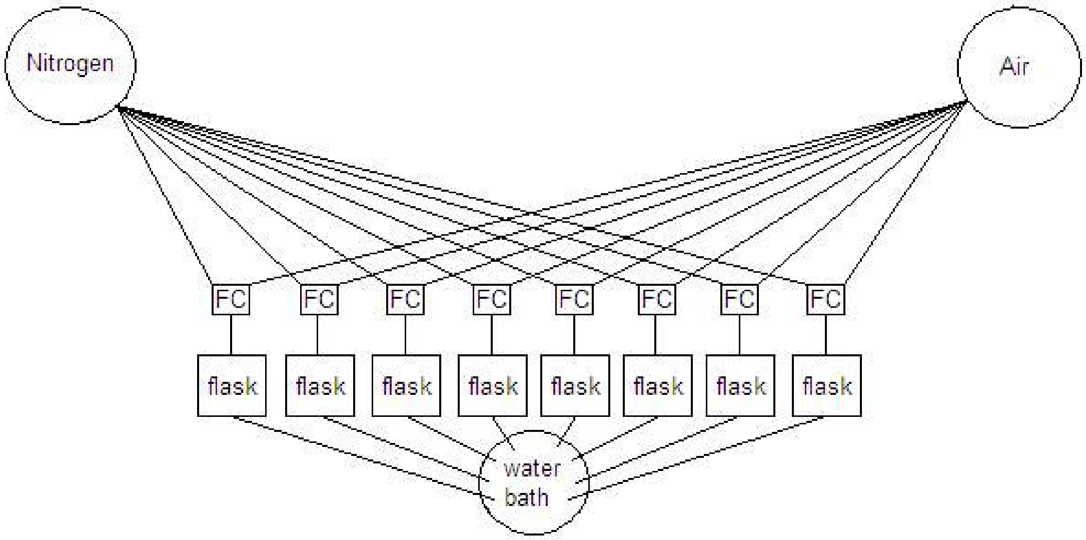

Figure 8-9. Gas flow to flasks in parallel, nitrogen or air

The gas delivery setup underwent one final change. It was found that oxidation occurred too quickly when the atmosphere was changed from nitrogen to air, making it difficult to obtain samples at the required redox potential. The air supply was removed from the gas delivery system and a small hole in the lid of the flask would be opened to allow oxygen exposure to occur more slowly.

8.1.4 Organic matter addition

It was found that the Griffith soil could not be reduced to an Eh below +358 mV (initially +366 mV) without the addition of organic matter in preliminary experiments. In order to promote reduction, organic matter addition was used to stimulate the action of the soil microbes. Dried green rice straw (organic matter) was added to the Griffith soil to promote reduction. It was found that 0.4 g organic matter was needed to drive 50 g Griffith soil in 250 mL water to -200 mV in 2 days. When using this same straw with the ChangSha soil however, 0.6 g was required to drive the redox potential to approximately -150 mV after 2 days. Yellow wheat straw was also tried, but addition of 3.0 g yellow wheat straw only able to drive the redox below -51 mV after 7 days. Green grass was also tried, but addition of 1.0 g green grass was unable to move the redox below +218 mV after 1 day.

8.1.5 Sampling

Initially samples were obtained using a ‘Rhizon sampler’ (Figure 8-10). The advantage of the Rhizon sampler is that it has an inbuilt 0.1 μm filter. The hydrophilic porous polymer was inserted into the stirred flask, leaving the luer-lock connector external to the flask. To take the sample, 1 mL of solution was withdrawn and discarded using a syringe, before a needle was connected to the luer lock and inserted immediately into an evacuated test tube (Vacutainer, BD). The vacuum induces the solution to be drawn into the evacuated test tube, filling approximately 8 mL in an hour or two.

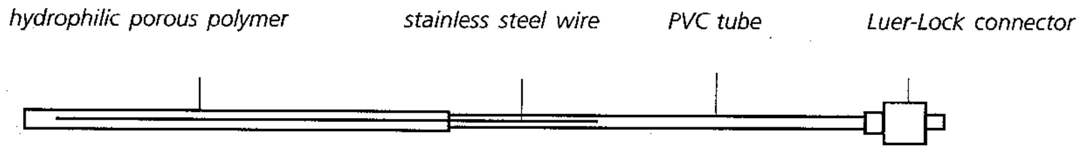

Figure 8-10. Rhizon sampler

However, this method did not work and only a few drops of solution could be obtained. The use of a syringe attached to the luer-lock and manually drawing the solution through the Rhizon was also tried. This method also gave a poor result. We hypothesized that the soil suspension was coating the rhizon thereby blocking the filter.

The next sampling method trialed was 5 mm tubing connected to the luer-lock. It was assumed that the soil suspension would travel freely through the 5 mm tubing and there would be minimal exposure to air. This method was problematic as soil particles easily blocked the 1 mm internal diameter of the tubing.

Another alternative was also tried with the 5 mm tubing inside the flask attached directly to a 3-way stopcock with luer-lock connectors outside. When sampling, the first 3 mL of solution was drawn through one arm of the stopcock using a syringe and discarded. Next, a 20 mL sample was collected through the other arm of the stopcock using a needle attached to the luer-lock and plunged immediately into an evacuated test tube. Again, this did not work as soil particles clogged the needle after approximately 1 mL of sample was collected. To overcome this problem, a luer-lock syringe with a two-way stopcock fixed to the nose was used instead to draw the sample through (Figure 8-11).

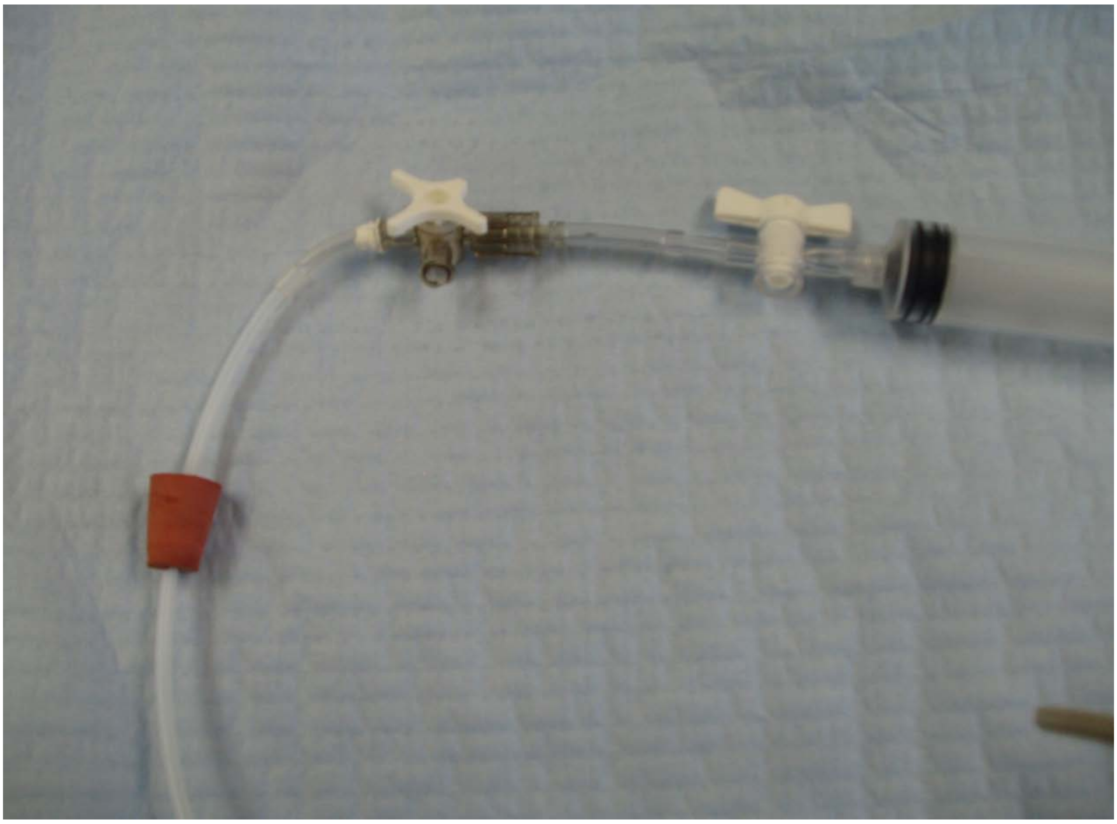

Figure 8-11. Sampling apparatus

The two-way stopcock is connected to the three-way stopcock using a small piece of tubing. Having a two-way stopcock attached to the syringe means that air contact with the sample is minimized during the journey to the anaerobic glovebox (glovebox). The two-way stopcock is closed before the syringe and stopcock are disconnected from the flask and taken directly to the glovebox. Inside the glovebox the two-way stopcock is removed from the syringe and the sample can be processed.

8.1.6 pH control and measurement

A preliminary experiment in keeping the pH of the suspension constant was undertaken. This was unsuccessful, as soil buffering made it difficult to control or manipulate the pH using acids and bases. It was decided that the pH will be allowed to change naturally with changes in the redox potential, and will be monitored with each sample.

For stirred flasks versions 1 to 3 a pH electrode was installed permanently in the flask so that the pH could be monitored in situ during the course of the experiment. Preliminary investigation conducted with stirred flask version 3 showed that the bulb of the pH electrode became highly abraded due to constant contact with moving soil particles, making the electrode difficult to calibrate and slow to respond to changes in pH. Inserting a freshly calibrated electrode for each measurement was tried and resulted in an undesirable addition of air to the reduced system. Additionally, damage to the bulb caused pH readings to differ by up to 1.0 pH unit between an electrode which had been installed in the flask for 1 hour, compared to a new electrode. Stirred flask version 4 does not measure the pH in the stirred flask, due to the unreliability of pH measurements taken in this way. Instead the pH is measured in the suspension sample once it has reached the anaerobic chamber.

8.1.7 Eh control and measurement

Attempts to poise the redox potential of the suspension (i.e. -100 mV, 0 mV) were undertaken. Poising the solution was attempted by adding nitrogen, air or a specific mixture of nitrogen and air into the flask. The flow of each gas was controlled by a flow meter and the flows were between 0 and 10 mL/min for each individual gas. It was not possible to poise the redox potential using this method. The redox potential would decrease quickly if nitrogen was slightly higher than air, and would increase quickly if air was slightly higher than nitrogen (Figure 8-12).

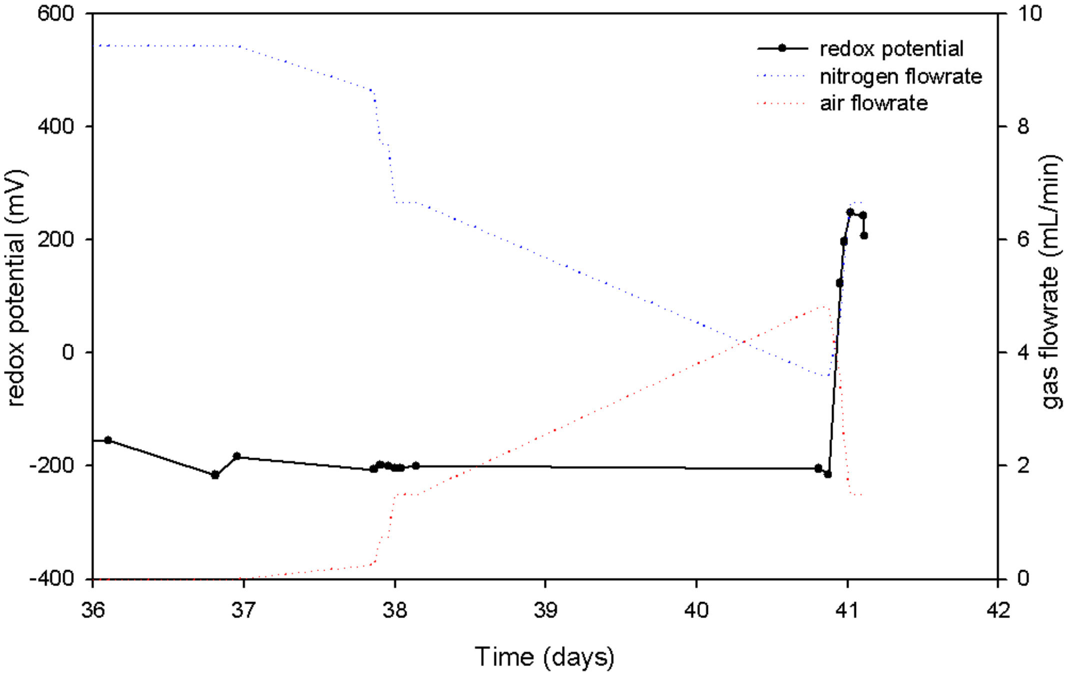

Figure 8-12. Attempt to poise redox potential by varying gas composition

It was decided that trying to poise the redox potential would be abandoned. Instead nitrogen alone is added until the reactor reached a reduced state and then maintained until the oxidation is initiated. When oxidation is initiated, nitrogen flow is stopped and one of the holes in the lid is opened as stirring continues.

8.1.8 Iron(II) Colorimetry

Iron(II) colorimetry was carried out using the phenanthroline method (Sparks, 1996). Immediately following sampling, 1 mL of sample was transferred into a 25 mL volumetric flask in an anaerobic chamber and color was developed using 1 mL of 3 g/L 1,10-phenanthroline. 2 mL of 5 M ammonium acetate was then added followed by 1 mL of 6 M HCl to bring the pH into the 3 to 5 range. The sample was then removed from the glovebox and poured into a 1 cm path-length cuvette. The spectrophotometer was set to 510 nm and standards covering the range 0 to 5 mg/L used to make a calibration curve before sample absorbances were read.

The iron (II) colorimetric method states that the color should be stable for up to 15 days after development (Sparks, 1996). This was confirmed for 100 minutes with no change in absorbance (Figure 8-13).

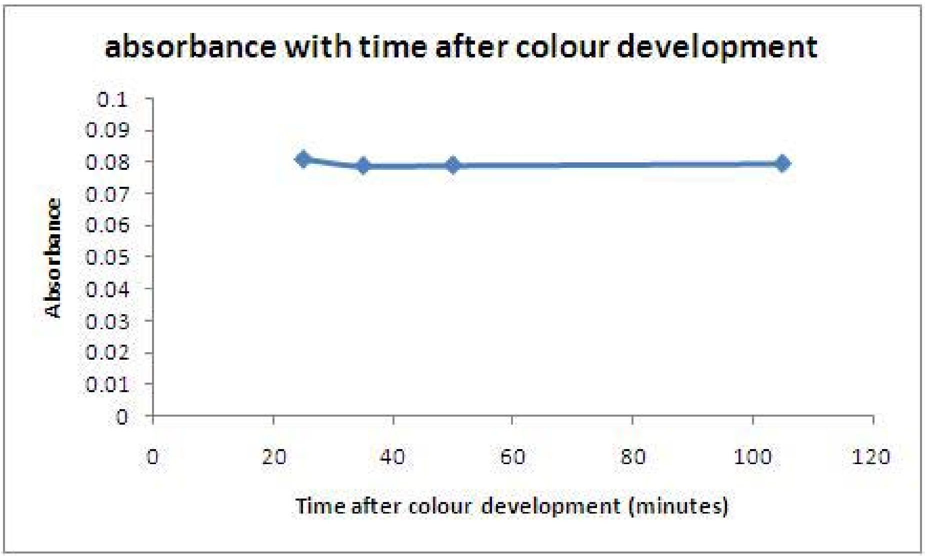

Figure 8-13. Absorbance at 510 nm with time after color development for 0.5 mg/L ferrous ammonium sulfate standard solution

The reproducibility of the iron (II) colorimetric measurements was verified by measuring the absorbance of each of the five standards in triplicate (Figure 8-14). The percentage error was at most 5.71 %, and standard error calculated to be a maximum of 0.0030 absorbance units.

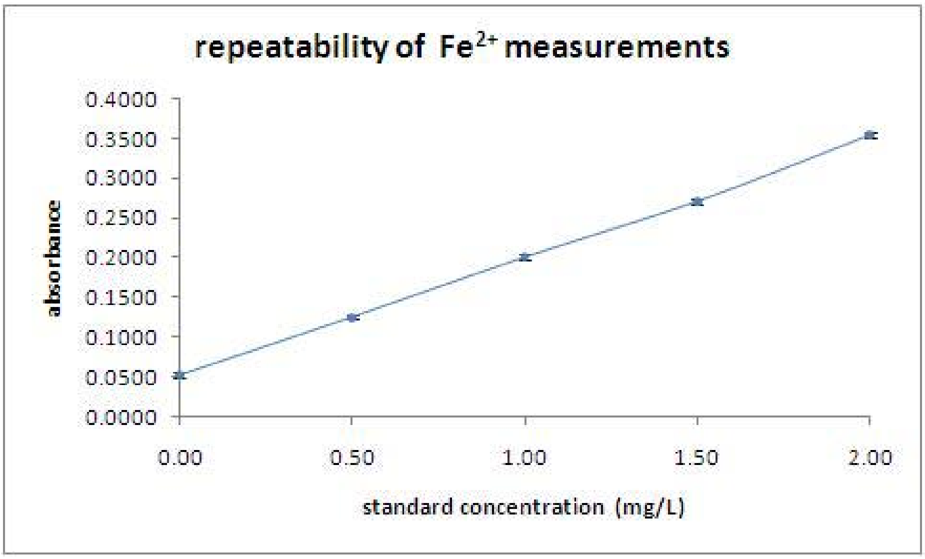

Figure 8-14. Variation in absorbance at 510 nm for three replicates of each standard ferrous ammonium sulfate solution used in colorimetric determination of iron method; 0, 0.5, 1.0, 1.5, 2.0 mg/L

The detection limit and quantification limit were obtained by measuring the absorbance of five blanks and calculating the standard deviation of the positive values. The standard deviation was multiplied by three and added to the mean to obtain the detection limit of 0.018 absorbance units. The quantification limit was obtained by multiplying the standard deviation by five before addition to the mean, to give 0.058 absorbance units.

8.1.9 Sulfide Colorimetry

Sulfide colorimetry was carried out using the methylene blue method (Greenberg *et al.*, 1992). Immediately following sampling, 5 mL of sample was transferred into a container in an anaerobic chamber and color was developed using Hach Sulfide Reagents 1 and 2. The sample was then removed from the glovebox and poured into a 1 cm path-length cuvette. The spectrophotometer was set to 664 nm and standards covering the range 0 to 2 mg/L used to make a calibration curve before sample absorbances were read. Exact concentrations of standards were cross-checked by analyzing stock Na_2S solution by ICP-OES. Sodium was used as the most reliable measure of molarity.

To determine the range of standards to use for this method, standards ranging 0 to 160 mg/L (Figure 8-15) were analyzed. It was found that the absorbance is linear up to approximately 3.2 mg/L, after which it plateaus before decreasing from the maximum observed absorbance at approximately 32 mg/L to no absorbance at all by 160 mg/L. The decrease at higher sulfide concentrations is likely due to the high color intensity inhibiting the spectrophotometer detection and/or the color development reagents being insufficient for reaction with the high concentrations of sulfide in solution. (Hach, 2008).

8-16).

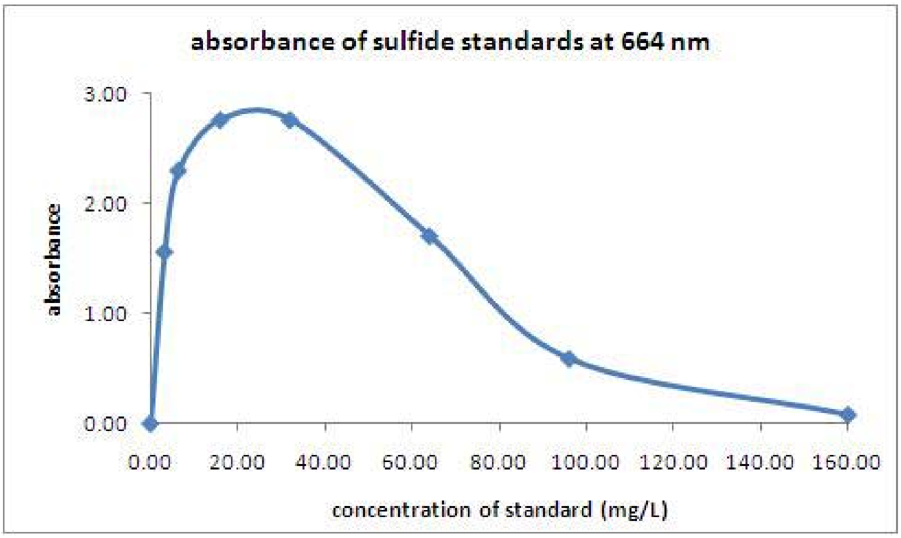

Figure 8-15. Absorbance at 664 nm of sulfide standards ranging from 0 to 160 mg/L

To ensure that low absorbances at high concentrations were not due to loss of gaseous sulfide formed by oxygen in the dilution water, standards made up using de-gassed doubly deionised water, and standards made up using conventional doubly deionised water. There was no difference in absorbance between these samples (Table 8-1).

Table 8-1- Effect of dilution water on absorbance of 32 mg/L Fe(II) standard at 510 nm

Degassed MQ	**MQ**
2.8341	2.8341

Recommended color development times from the literature range from 3 to 15 minutes (Greenberg *et al.*, 1992; Hach, 2008). To study the color complex stability the absorbance of a freshly prepared 32 mg/L standard after color was studied to from 5 to 95 minutes. The results demonstrated that there was no change in absorbance at 664 nm with time (Figure 8-16).

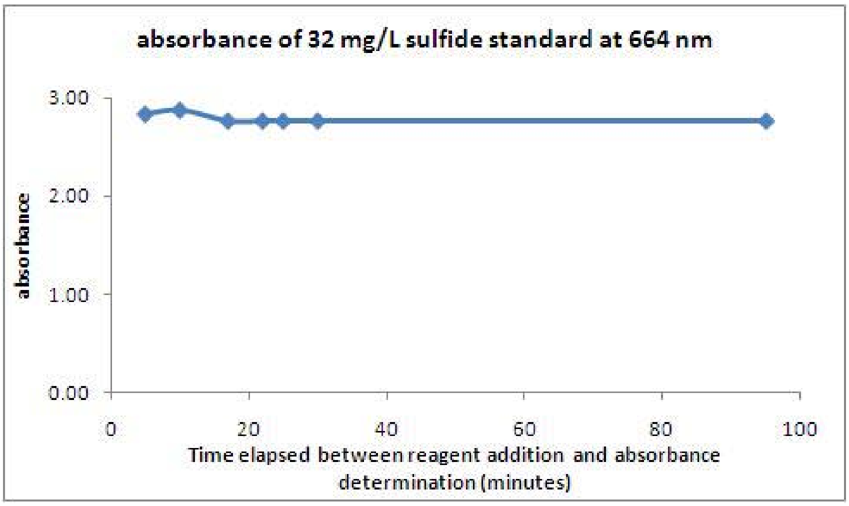

Figure 8-16. Change in absorbance at 664 nm with time elapsed after reagent addition to 32 mg/L sulfide standard prepared 15 minutes previously

To determine whether sulfide could have been volatile loss from the prepared standard (sodium sulfide) with time the absorbance of 32 mg/L standards allowed “rest” for 0 and 25 minutes before reagent addition. All standards were given 5 minutes of color development time before absorbance was measured at 664 nm. There was no change in absorbance of standards with ‘resting time’ (Figure 8-17).

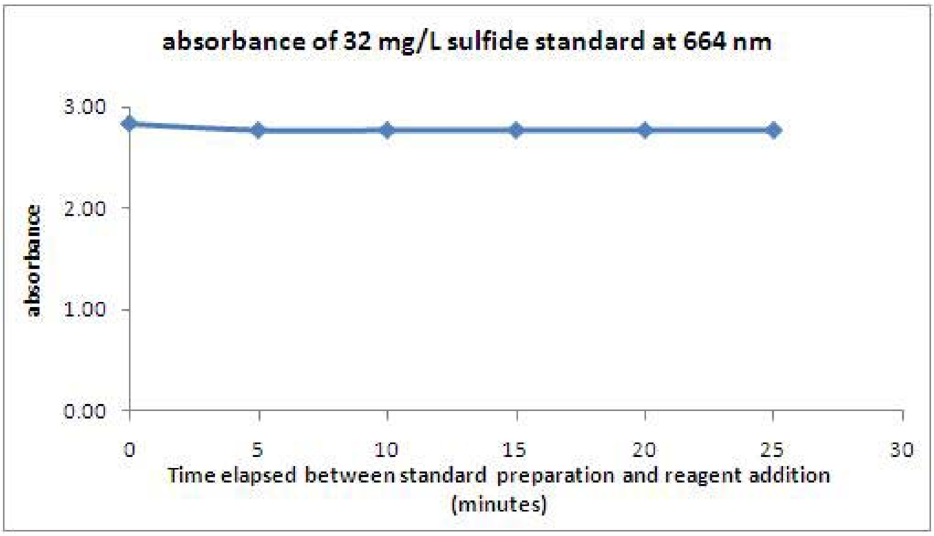

Figure 8-17. Change in absorbance at 664 nm with time elapsed after standard preparation for 32 mg/L sulfide standard

The reproducibility of the sulfide colorimetric method was determined by measuring the absorbance of 4 standards in triplicate (Figure 8-18). The error was no greater than 1.3 %, and the maximum standard error observed was 0.0065 absorbance units.

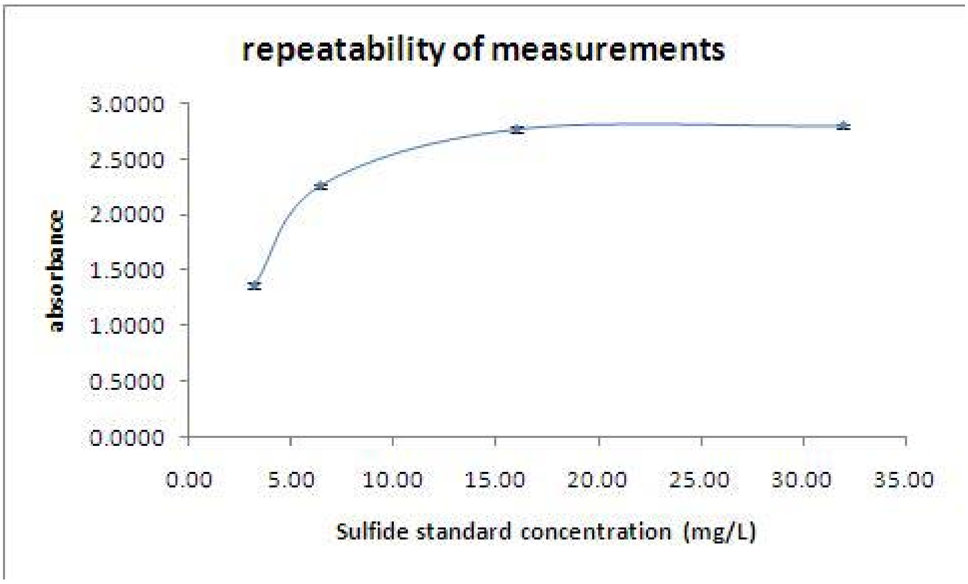

Figure 8-18. Variation in absorbance at 664 nm for three replicates of each sodium sulfide standard used in colorimetric determination of sulfide method; 0, 3.2, 6.4, 16, 32mg/L

The detection limit and quantification limit were obtained by measuring the absorbance of five blanks and calculating the standard deviation of the positive values. The standard deviation was multiplied by three and added to the mean to obtain the detection limit of 0.015 absorbance units. The quantification limit was obtained by multiplying the standard deviation by five before addition to the mean, to give 0.049 absorbance units.

8.1.10 Leaching spiked Griffith soil

The uncontaminated paddy field soil from Griffith (NSW) was spiked with solutions containing varying concentrations of Cd, Fe and Zn (Table 8-2) as chloride salts. Solutions were prepared using reagent-grade chemicals. The EC of the spiked soils was determined and leaching was deemed necessary as the high Fe spikes had an EC > 7,500 µS/cm compared with < 300 µS/cm for the other treatments. Leaching was carried out 6 times with artificial rainwater before the EC came down to a more comparable level (459 µS/cm) and began to plateau off (Figure 8-19). The EC is still elevated in the high Fe spikes relative to the other spikes though, and this may affect metal solubility. Salinity has been found to enhance Cd solubility and phytoavailability in pot experiments with wheat plants (Khoshgoftar *et al.*, 2004). All spiked soils were aged for 4 weeks by keeping the soil at field capacity. Soil moisture content at field capacity is 37.12 % as determined by the Tension plates method at 100 cm.

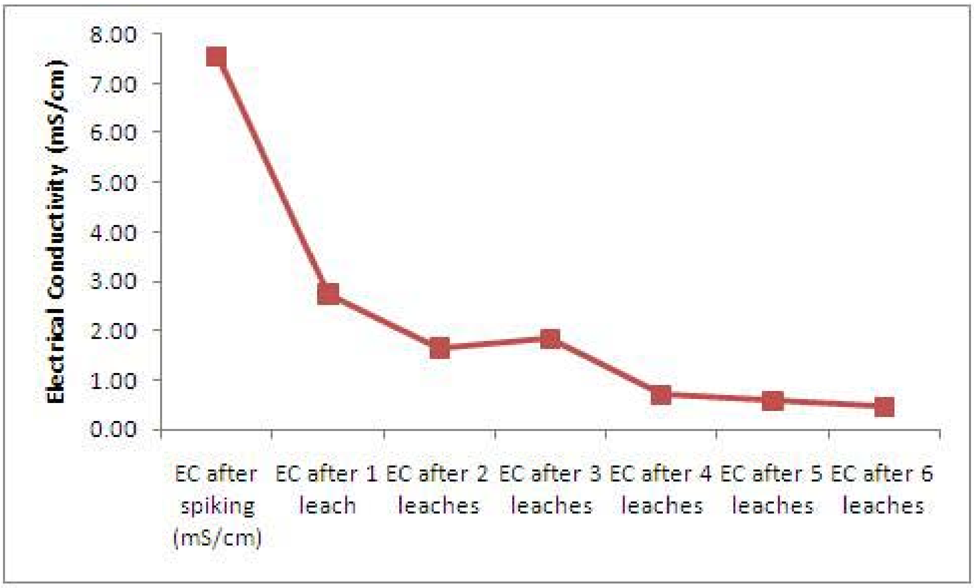

Figure 8-19. Change in EC with leaching for Griffith soil spiked with 6,702 mg/kg Fe

8.1.11 Determination of timeframe for reduced incubation

The time period for the stirred flask experiments was determined in preliminary experiments using ChangSha soil. Keeping one cell reduced for a period of 14 days (total experiment time was 16 days) was not long enough for iron in solution to reach a steady concentration (Figure 8-20).

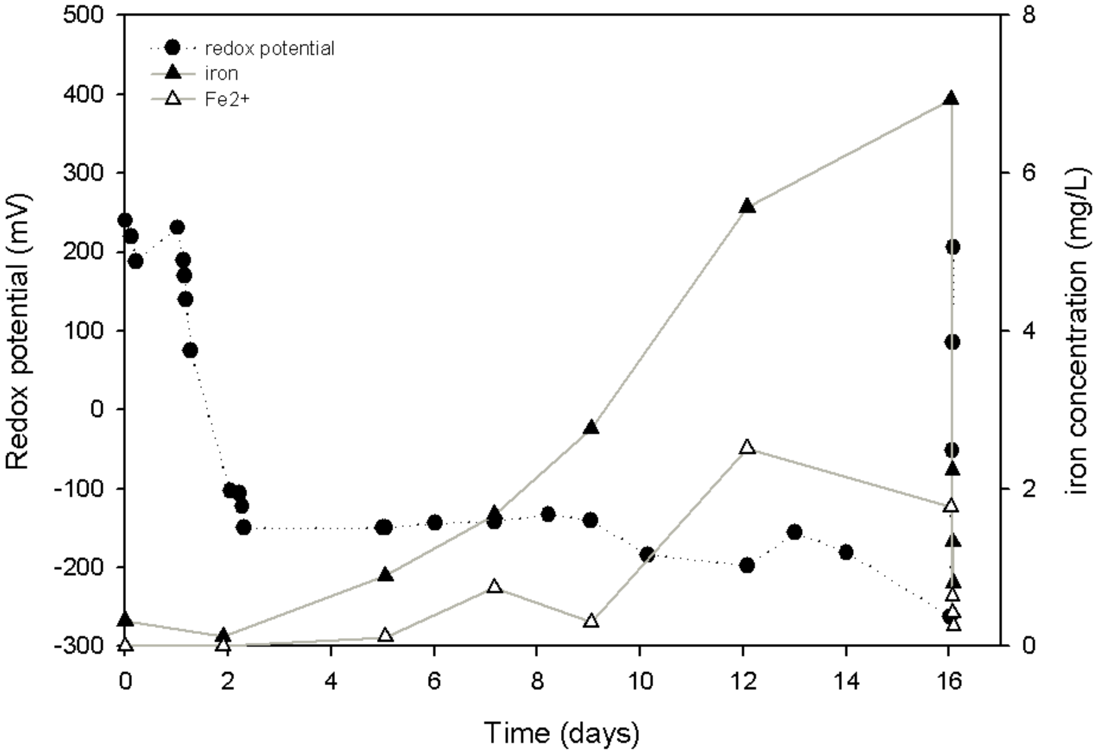

Figure 8-20. Iron concentration in solution for ChangSha soil subjected to redox changes over 16 days

Further experiments with ChangSha soil in triplicate showed that 30 days is the minimum timeframe for the reduced phase (Figure 8-21). All cells show a relatively stable iron concentration between 20 and 42 days. 30 days was therefore adopted as the timeframe for the reduced phase in experiments using the uncontaminated soil.

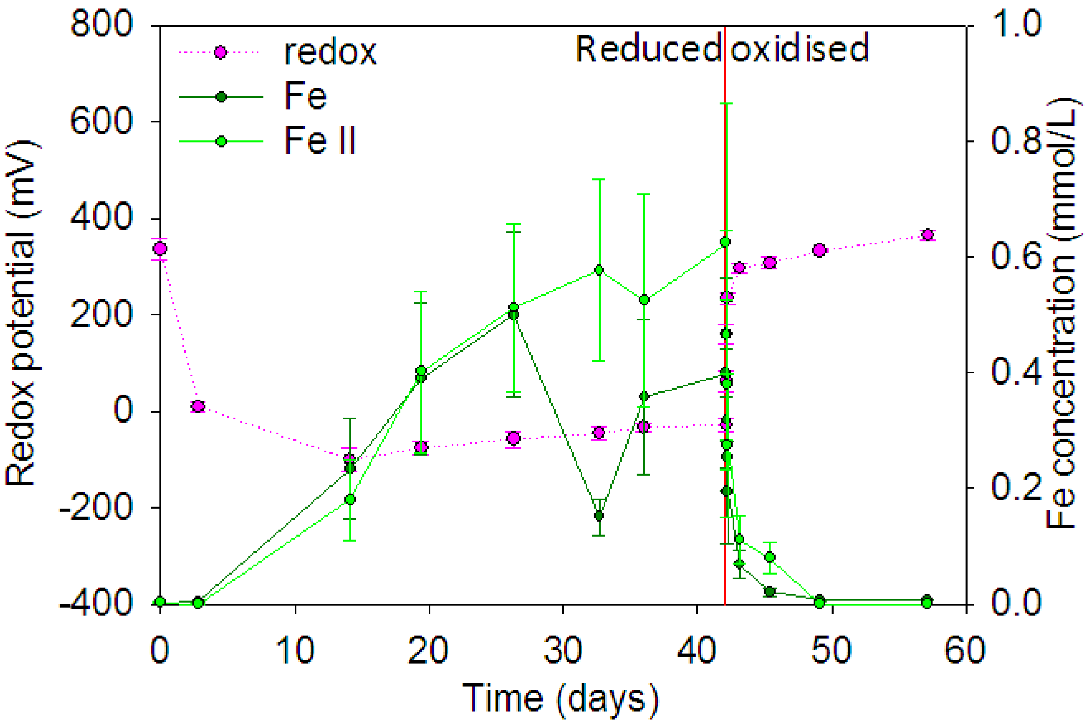

Figure 8-21. Iron concentration in solution from ChangSha soil subjected to changes in redox over 60 days

8.1.12 Determination of sulfate-spiking rates

The amount of sodium sulfate added to each soil was equimolar to the sum of the maximum concentrations of Fe, Mn, Zn and Cd, minus maximum concentration of S observed in previous experiments.

For Griffith paddy soil spiked with 2.7 mg/kg Cd and 98 mg/kg Zn:

0.55 mmol Fe/L + 0.1 mmol Mn/L + 0.01 mmol Zn/L + 0.00008 mmol Cd/L – 0.16 mmol S/L = 0.50 mmol/L S to be added.

For ChangSha soil:

0.75 mmol Fe/L + 1.45 mmol Mn/L + 0.70 mmol Zn/L + 0.00008 mmol Cd/L – 0.30 mmol S/L = 1.15 mmol/L S to be added.

8.1.13 Visual observations of sulfate-spiked soil slurries

Figure 8-22. Griffith (flask C) and ChangSha (Flask D) soil solutions at day 10 with sulfate addition. Blackened ChangSha soil solution indicates likely presence of sulfide minerals.

8.1.14 Correlations between solution components for ChangSha soil

Pearson's linear correlation coefficients – r

* probability of significance is > 95 %

** probability of significance is >99%

***probability of significance is >99.9%

Source	Time period	Cd/Zn	Cd/Ca	Zn/Ca	Cd/Mg	Zn/Mg	Cd/Mn	Zn/Mn
Cornu *et al*.	0 to 6 days	0.89**	0.95**		0.95**		0.92**	
	9 to 21 days	0.94**	-0.81**		-0.82**		-0.23	
The present study	0 to 3 days	0.42	0.46	0.90*	0.32	0.94**	0.62	0.91*
	0 to 42 days	0.29	-0.36	0.19	-0.46*	0.23	-0.33	0.37
	42 to 56 days	-0.37	0.11	0.40	-0.51*	0.55**	-0.53*	0.74***

Table 8-2 - statistics - correlations between solution components

		Cd	pH	redox	DOC	DIC	Fe	FeII
Reduced	Cd		0.23	0.14	-0.48*	-0.10	-0.36	-0.43*
phase	FeII	-0.43*	-0.56**	-0.32	0.77***	0.12	0.83***	n.a.
	Zn	0.29	-0.06	-0.42*	0.38	-0.09	0.37	0.35
Oxidised	Cd		0.39	-0.02	-0.26	0.52*	-0.53*	-0.50*
phase	FeII	-0.50*	-0.59*	-0.32	0.77***	-0.56**	1.00***	n.a.
	Zn	-0.37	-0.84***	-0.21	0.86***	-0.48*	0.73***	0.76***

		S	Mn	Ca	Mg	Na	Cu	Ni
Reduced	Cd	0.61**	-0.33	-0.36	-0.46*	-0.40	0.39	-0.45*
Phase	FeII	-0.51*	0.51*	0.38	0.51*	0.04	-0.37	0.44*
	Zn	0.35	0.37	0.19	0.23	-0.11	0.27	-0.08
Oxidised	Cd	-0.01	-0.53*	0.11	-0.51*	-0.01	-0.03	-0.49*
phase	FeII	-0.27	0.57**	0.15	0.39	-0.58**	-0.27	0.42
	Zn	-0.27	0.74***	0.40	0.55**	-0.62**	-0.50*	0.56**

8.2 Appendix B - Chapter 5 Pot trial method development

8.2.1 In situ redox measurements

In a preliminary pot experiment the use of platinum electrodes connected to a datalogger was trialed. The effect of electrode installation depth on Eh values was assessed for depths of 0-1, 5 and 15 cm. The response of the platinum electrodes to prolonged flooding and short drainage periods was also evaluated (Figure 8-23). The electrode at surface depth (0 – 1 cm) gave the most inconsistent readings, with Eh values obtained from 5 and 15 cm being more reliable. In subsequent experiments platinum electrodes were installed at 10 cm depth, as preliminary findings indicated that readings from this depth should be reliable, and the rhizon samplers had to be installed at 10 cm depth. This method for monitoring redox potential of the soil gave Eh data which was expected based on the conditions imposed. Initially we observed the redox potential drop as the soil was flooded, then during periods of oxidation the redox potential responded by rising rapidly before dropping again when flooding was reinstated. Platinum electrodes were hence found to provide a reliable indication of the redox state of soil pots planted with rice.

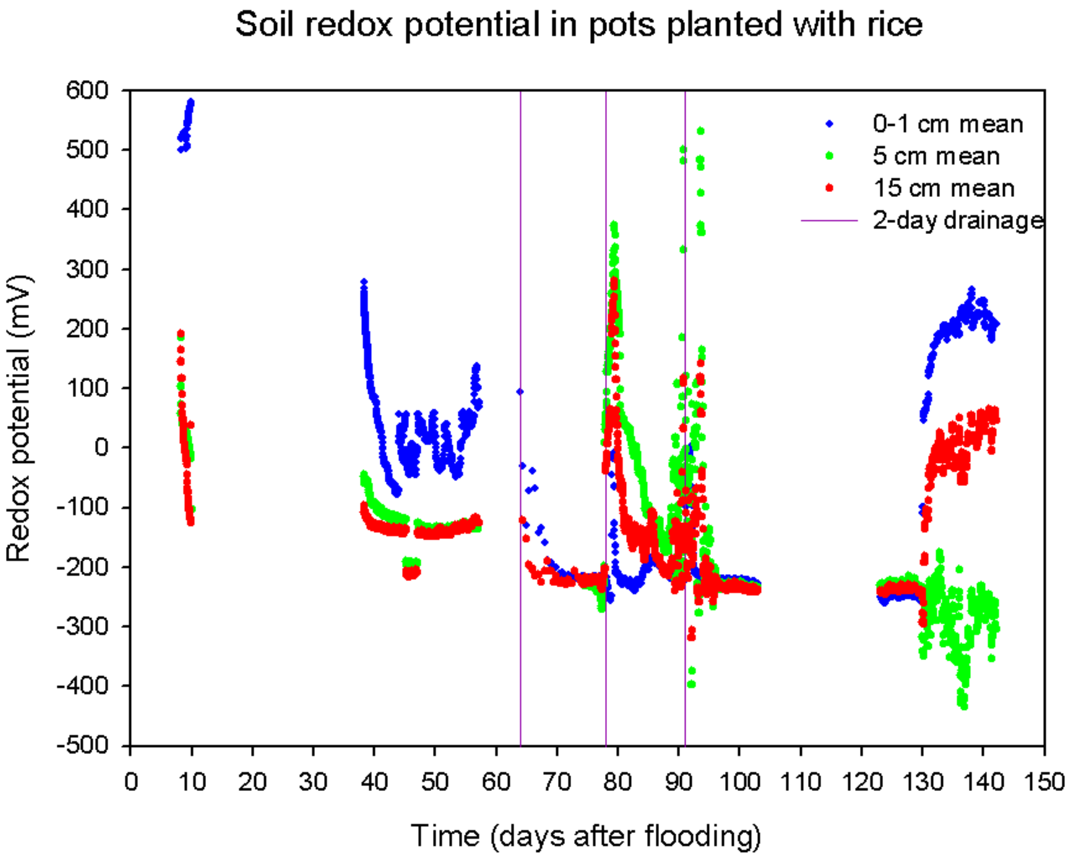

Figure 8-23. Redox potential of soil planted with rice; measured using platinum electrodes installed at either 0-1, 5 or 15 cm depth

The potential influence of rice roots on redox potential also warranted investigation. Rice plants pump oxygen from aerial parts down to the roots making the rhizosphere relatively oxygen-rich (Ponnamperuma, 1964) . This could potentially cause the overall redox potential measurements to be higher as electrodes would likely make contact with rice roots in the pots. To determine the influence of rice roots on Eh readings, the redox potential was also measured using platinum electrodes connected to a datalogger in pots without rice planted. It was thus determined that the presence of rice roots did not influence the redox potential measurements (Figure 8-24).

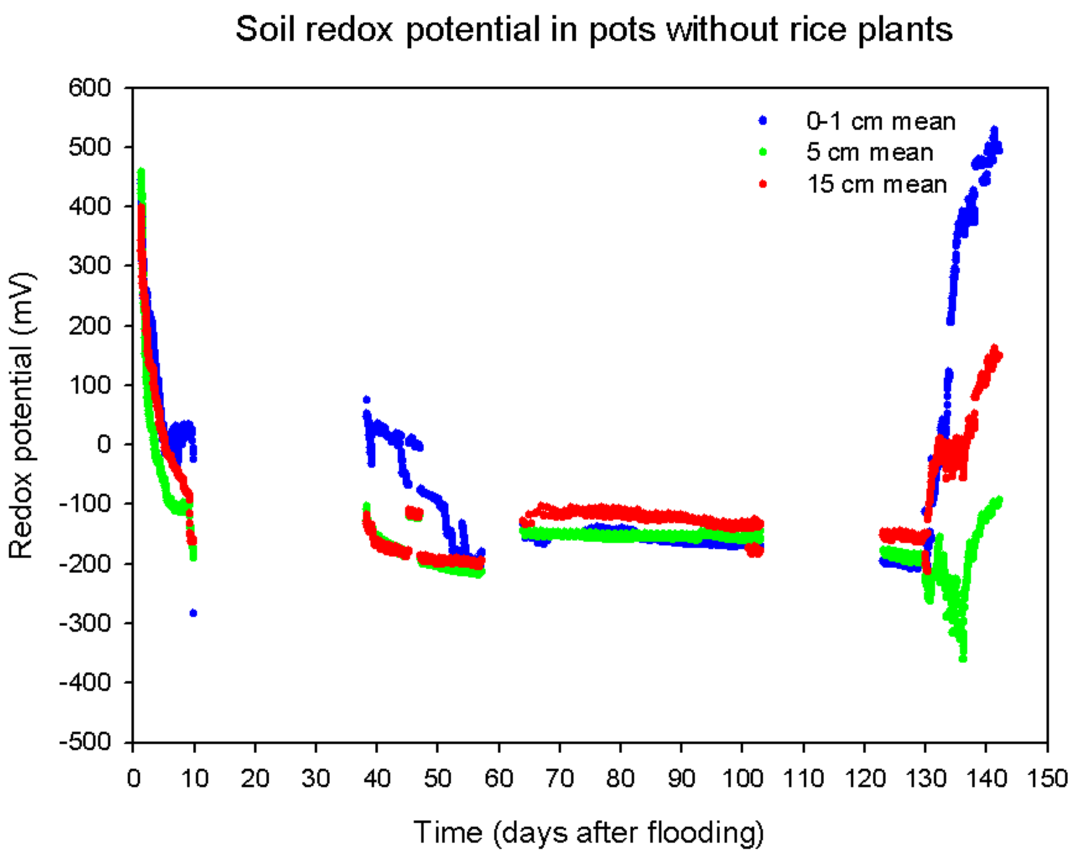

Figure 8-24. Redox potential of soil without rice planted; measured using platinum electrodes installed at either 0-1, 5 or 15 cm depth

8.2.2 pH measurements

pH was measured in the glovebox in soil solutions obtained by rhizon samplers. Initially *in situ* measurements were tried using a permanently installed perforated tube with a pH electrode inserted, but we were unable to prevent oxygen-exposure to the solution in the tube or be confident that the tube solution was a true representation of ‘soil solution’ since it was denied full contact with the soil due to obstruction by the body of the tube. A comparison between standing water (supernatant) in the flooded pots, *in situ* pH and pH measured in the glovebox from solution obtained using a rhizon sampler showed that pH measured *in situ* was more acidic than the other two methods (Figure 8-25). This indicates that it was more oxidized (Ponnamperuma, 1972) and therefore a less reliable indication of the pH of soil solution at depth.

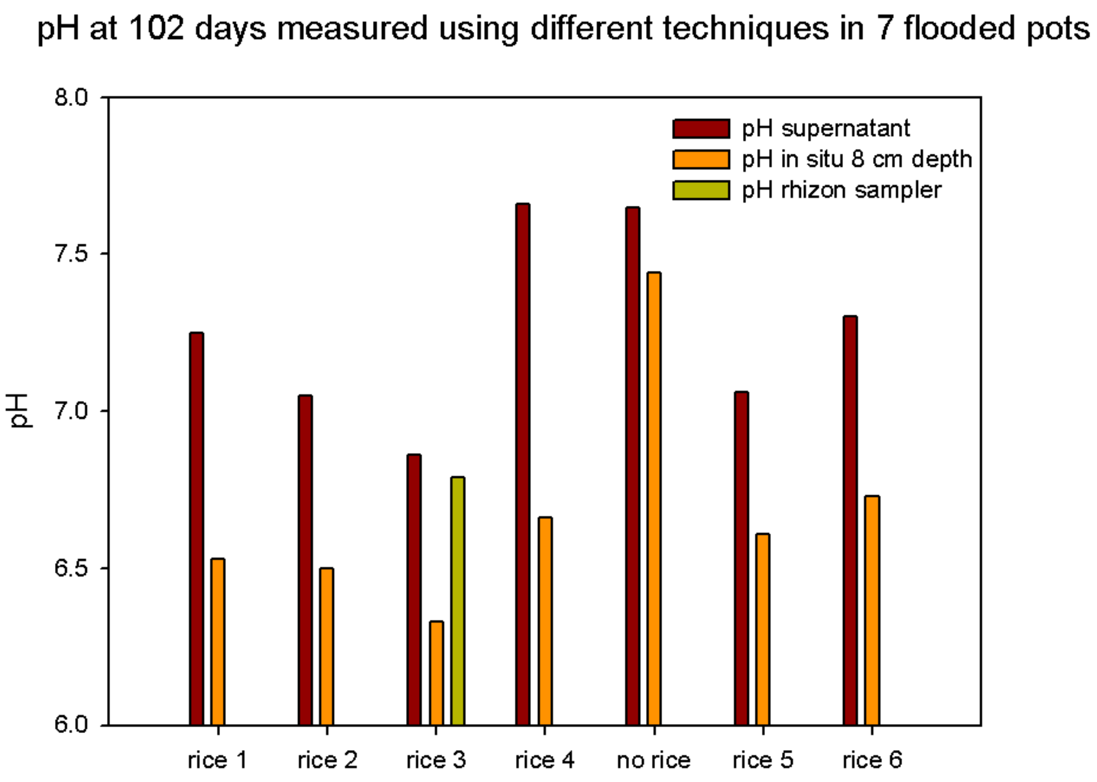

Figure 8-25. pH at 102 days measured using different techniques in 7 flooded pots

8.2.3 Soil solution sampling

Soil solution sampling using ‘Rhizon samplers’ was trialed in pots planted with rice throughout the growth period. We evaluated whether this sampling method could satisfactorily keep soil solutions at the Eh conditions of the pot by comparing trends in Eh, pH and element concentrations of soil solution with observed changes in Eh imposed by alternate flooding and drainage cycles. Soil solution samples obtained using Rhizon samplers were taken directly to an anaerobic chamber where the Eh was measured using a combination electrode, and samples were processed for various analyses. The Eh values obtained by measurement in the anaerobic chamber were all much higher than those collected from the platinum electrodes and datalogger (Figure 8-23, Figure 8-26). This indicated that some oxidation of the solution samples may be occurring during sample collection and/or transport to the anaerobic chamber. However, the expected trend of higher Eh and lower pH

(Ponnamperuma, 1972) during drainage events was still observed in soil solution (Figure 8-26). Also, soil solutions contained reduced species such as Fe(II) (Figure 8-27) which indicates that the redox potential measurements should be used only as a guide.

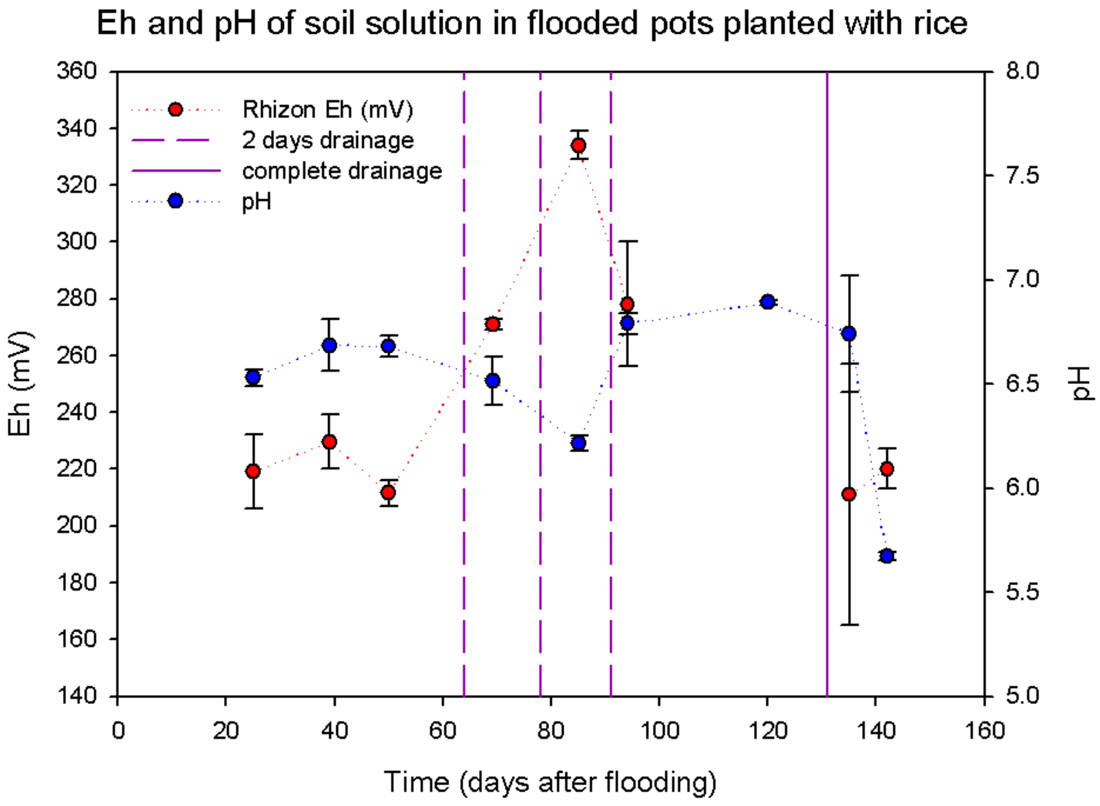

Figure 8-26. Eh and pH in soil solution obtained using rhizon samplers

The relationship between total Fe and Fe(II) in soil solution was also investigated. Fe(II) was measured using the colorimetric method whereas total Fe was measured using ICP-OES. Concentrations were similar (Figure 8-27) as was expected; total Fe detected by ICP-OES should be in the reduced form as Fe(III) generally forms insoluble precipitates.

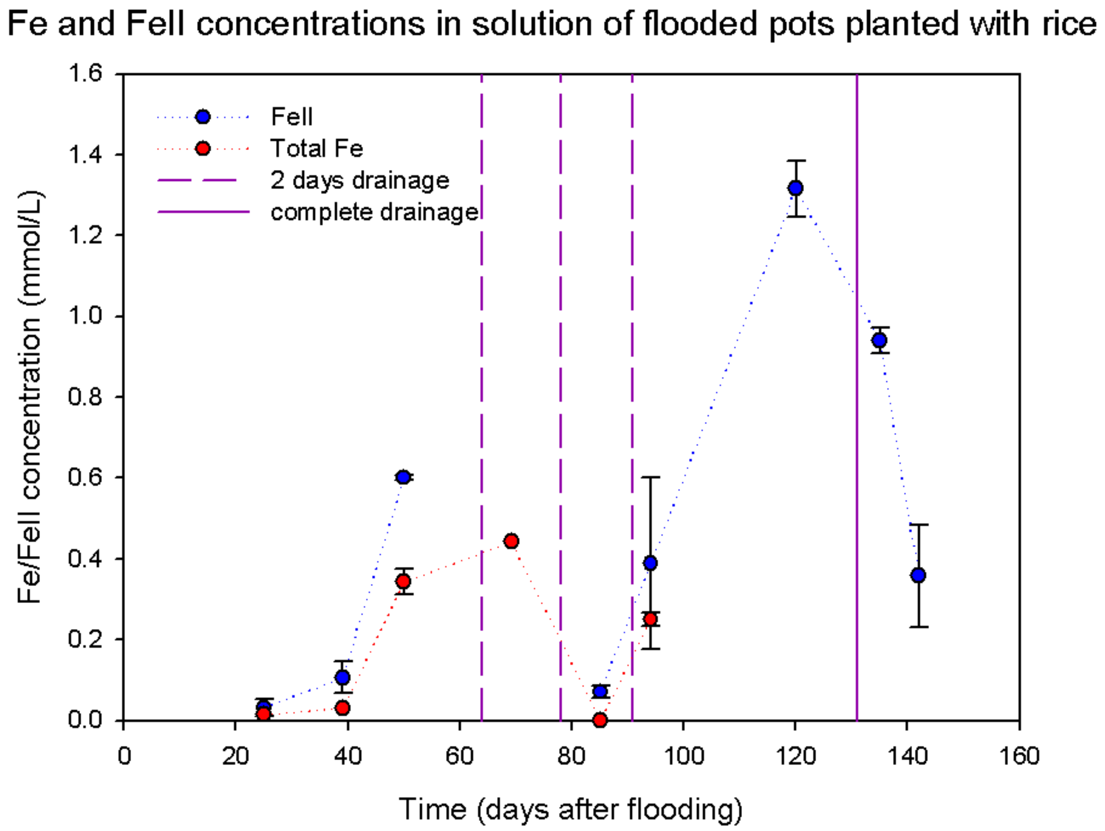

Figure 8-27. Fe(II) measured using colorimetry and total Fe measured using ICP-OES in soil solution

Concentrations of other elements in soil solution also responded to the imposed changes in water management as expected (Figure 8-28, Figure 8-29, Figure 8-30). DOC increased with prolonged flooding and was lower during drainage events. This was expected as microbes consume organic matter and produce DOC during their anaerobic respiration (Ponnamperuma, 1972). Exchangeable cations Ca, Mg, Na appeared to respond more slowly to drainage. Their concentrations increased with time regardless of drainage events (Figure 8-28), perhaps indicating that release from exchange sites upon flooding is a quicker process than readsorption to newly precipitated Fe oxyhydroxides during brief drainage periods. Potassium and Mn showed no clear trends with flooding/drainage (Figure 8-29). Sulfur showed the expected response to changes in water management; increasing upon drainage and decreasing with flooding (Figure 8-29). This is most likely due to oxidative dissolution/reductive dissolution of sulfide minerals (Ponnamperuma, 1972). Cadmium and zinc were found to respond differently to one another with changes in redox potential (Figure 8-30). Both metals increased with flooding, but Zn increased much more than Cd. The increase is likely due to release from sorption sites on dissolving Fe oxyhydroxides.

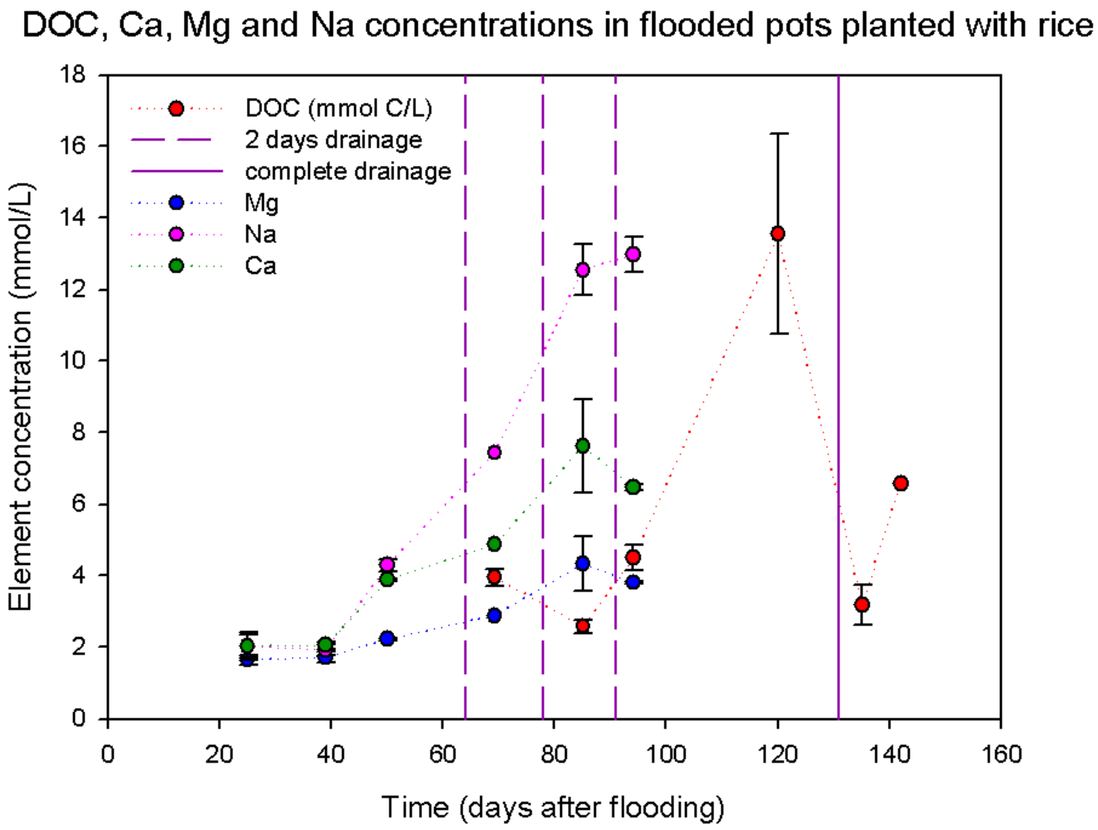

Figure 8-28. DOC, Ca, Mg and Na in soil solution obtained using rhizon samplers in flooded pots planted with rice subjected to periodic drainage events

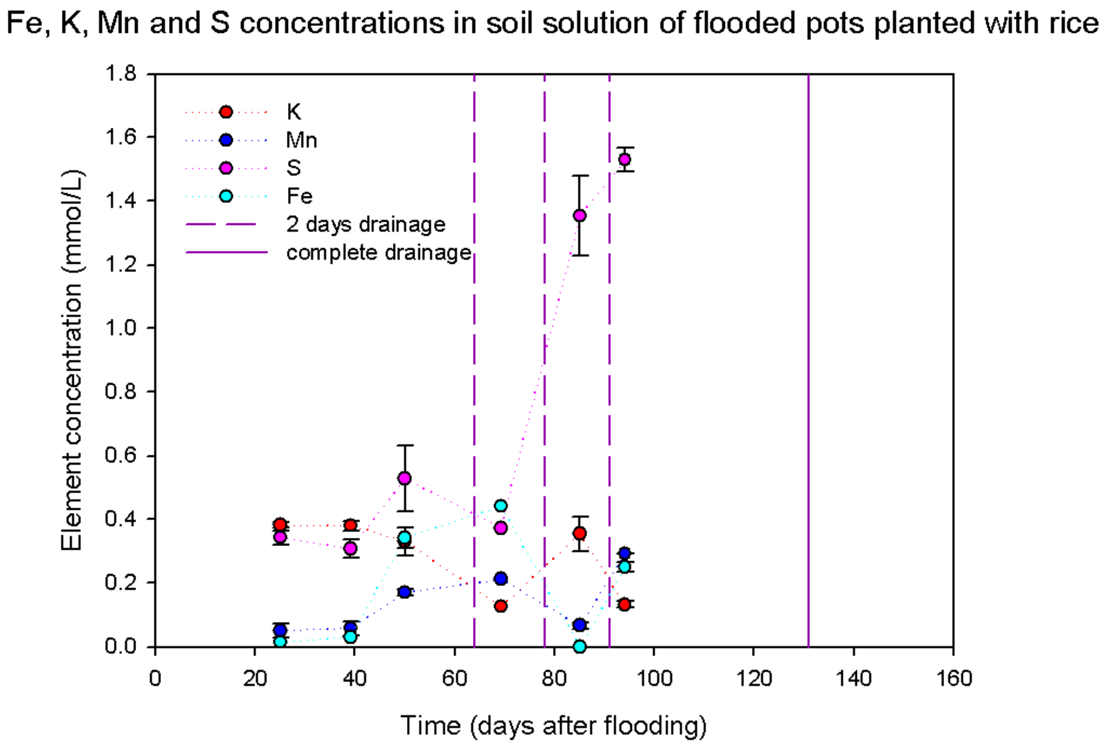

Figure 8-29. K, Mn, S and Fe in soil solution obtained using rhizon samplers in flooded pots planted with rice subjected to periodic drainage events

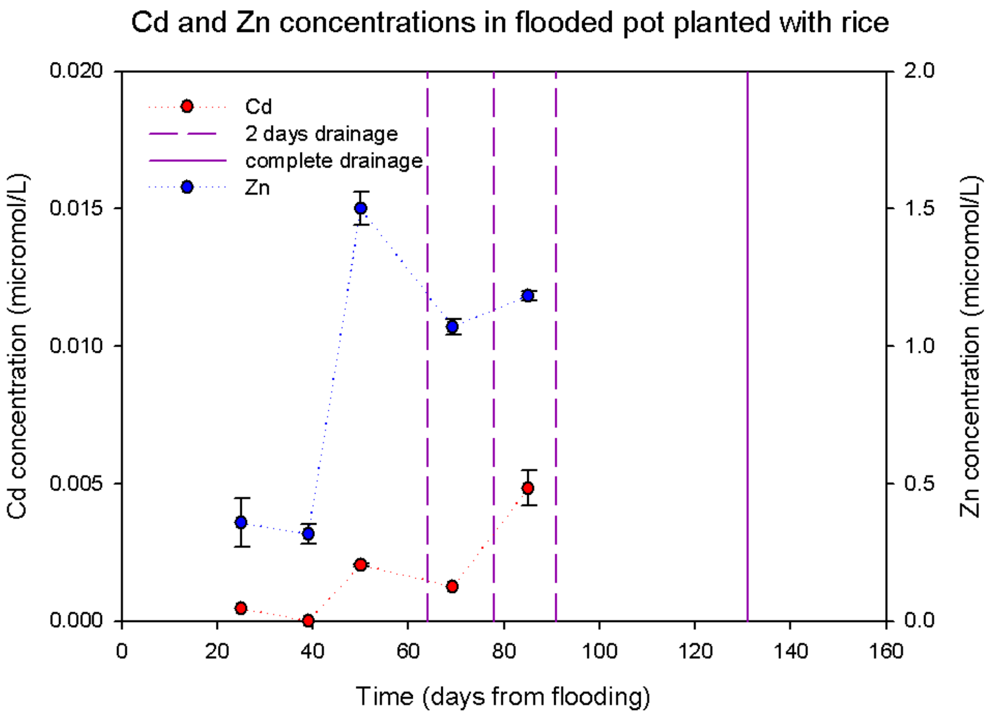

Figure 8-30. Cd and Zn in soil solution obtained using rhizon samplers in flooded pots planted with rice subjected to periodic drainage events

8.2.4 Rice cultivation

A preliminary experiment was conducted to learn about the agronomic aspect of growing rice plants in the growth chamber. It was necessary to determine how long the available rice varieties would take to reach maturity, which variety would be most suitable to use, as well as the appropriate number of plants to grow per 2 kg pot for optimal plant health. Pots were planted with 2, 4 or 8 plants per pot and grown to maturity under a 12 h 30°/24° day/night cycle. The plants took 148 days to reach maturity from germination, and the healthiest/largest plants grew in the 2-plant pots. Plants were removed periodically from the 8-plant pots to monitor changes in shoot dry weight with time for each cultivar (Figure 8-31). Although shoot dry weight was found to be similar between the two cultivars, Amaroo was slightly quicker to reach maturity so was favored for use in subsequent experiments.

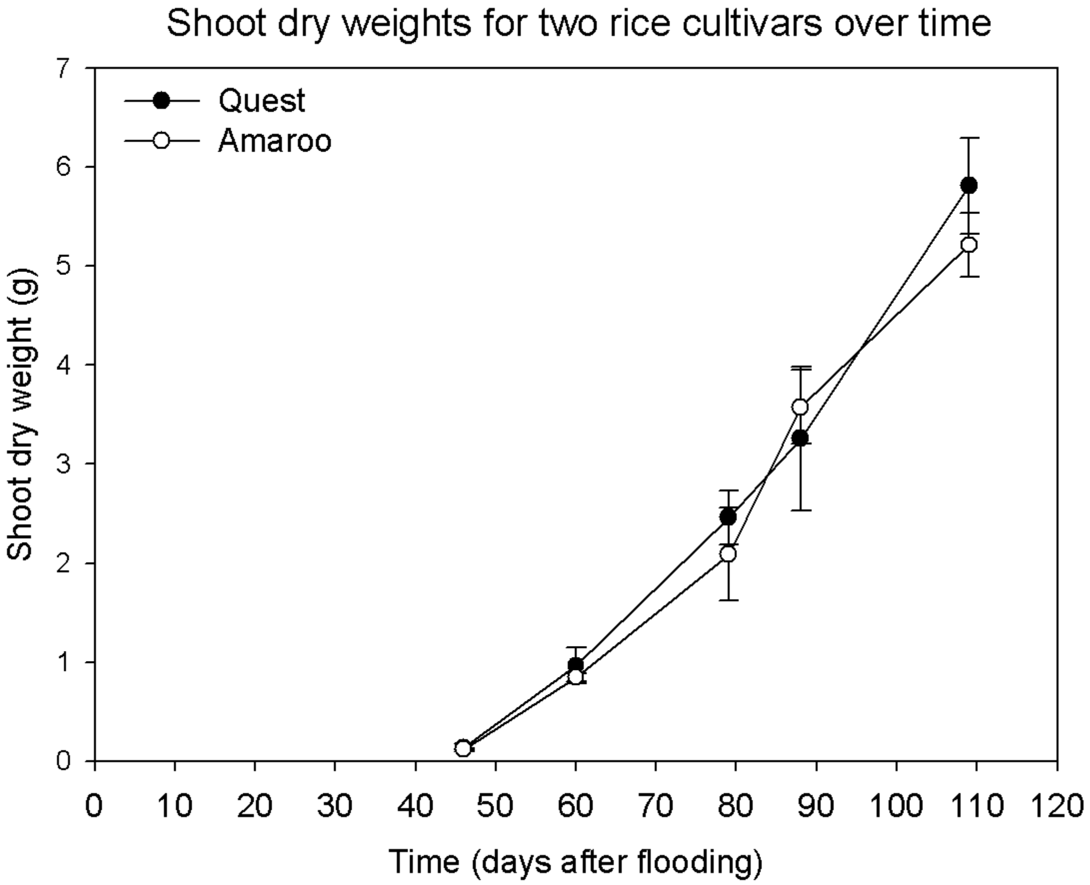

Figure 8-31. Shoot dry weights for Quest and Amaroo rice cultivars during 110 days of growth

It was also necessary to learn whether nutrient composition in shoots and/or grains would change with time in the lead up to harvest due to changes in water management, and whether nutrient composition would be different between cultivars. Accordingly, 2 weeks before maturity (still flooded) and at maturity (drained), some Amaroo and Quest plants were harvested and separated into shoots and grains (immature). Iron, S and Zn concentrations in shoots were relatively constant in the lead up to harvest (Figure 8-32). Nutrient concentrations were lower in Quest than in Amaroo, a finding which further validated the decision to use Amaroo in subsequent experiments.

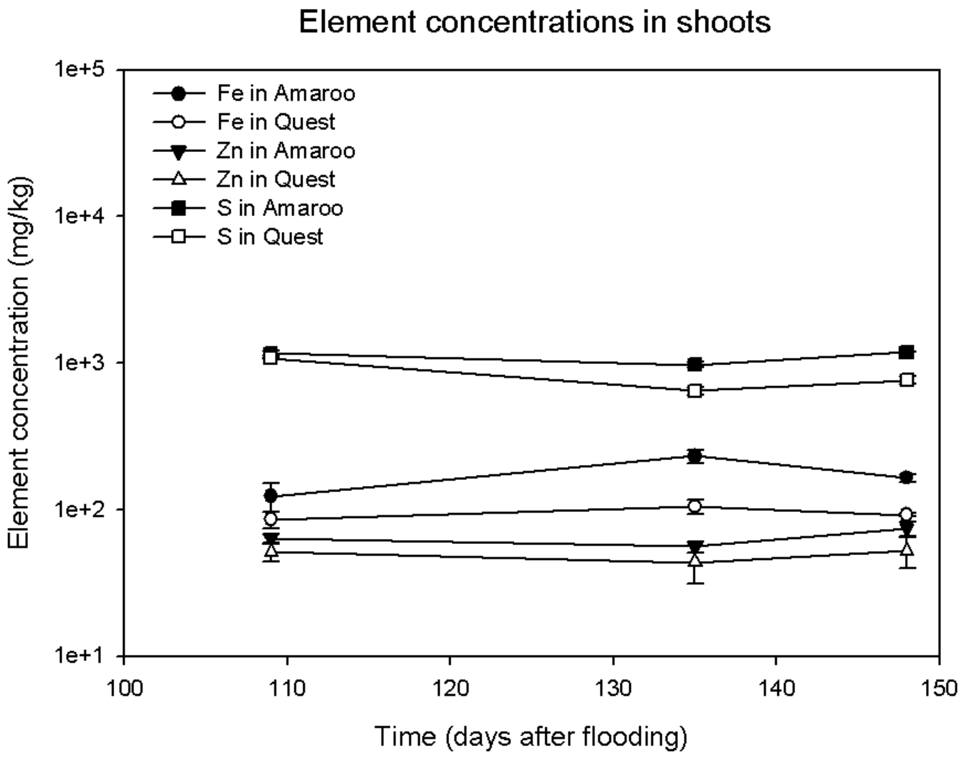

Figure 8-32. Fe, Zn and S concentrations in Amaroo and Quest shoots leading up to harvest

Nutrient concentrations in grains were also relatively constant in the lead up to harvest despite the change from flooding to drained cultivation (Figure 8-33). Unlike shoot concentrations, there was no difference between Amaroo and Quest cultivars.

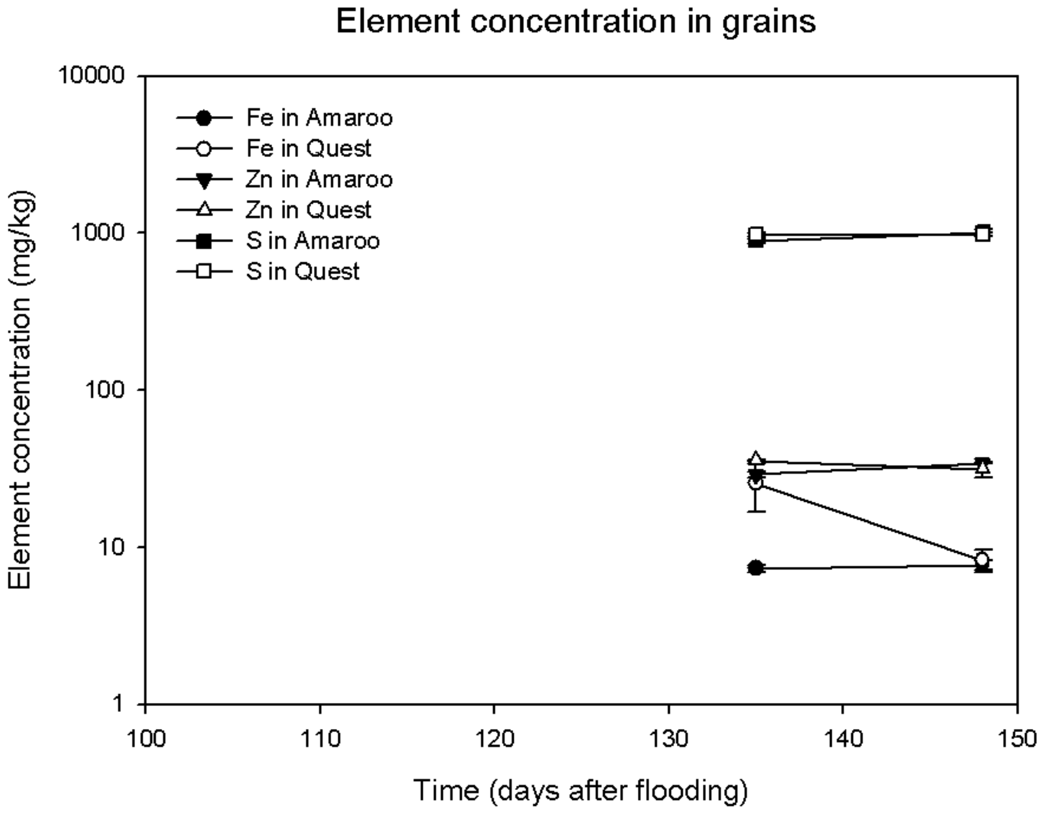

Figure 8-33. Fe, Zn and S concentrations in Amaroo and Quest grains leading up to harvest

A comparison of nutrient composition between the different plant parts at maturity was also of interest. Accordingly, upon maturity Quest plants were harvested and separated into roots, shoots and grains. Plant parts were digested and the extracts analyzed by ICP-OES. Iron and Zn concentrations were significantly different between plant parts (Figure 8-34, Figure 8-35). Roots contained the most Fe and Zn, followed by shoots then grains. Iron plaque was noticed on roots upon harvest and cleaning, so the high Fe concentration was expected due to the presence of plaque alone. Higher Zn in roots was also expected as the trend of roots>shoots>grains has been reported elsewhere for Cd and Zn (Ito and Iimura, 1976a). Sulfur was also highest in the roots, but appeared to be more readily translocated to grains the Fe or Zn, as more S was found in rice grains than shoots (Figure 8-36).

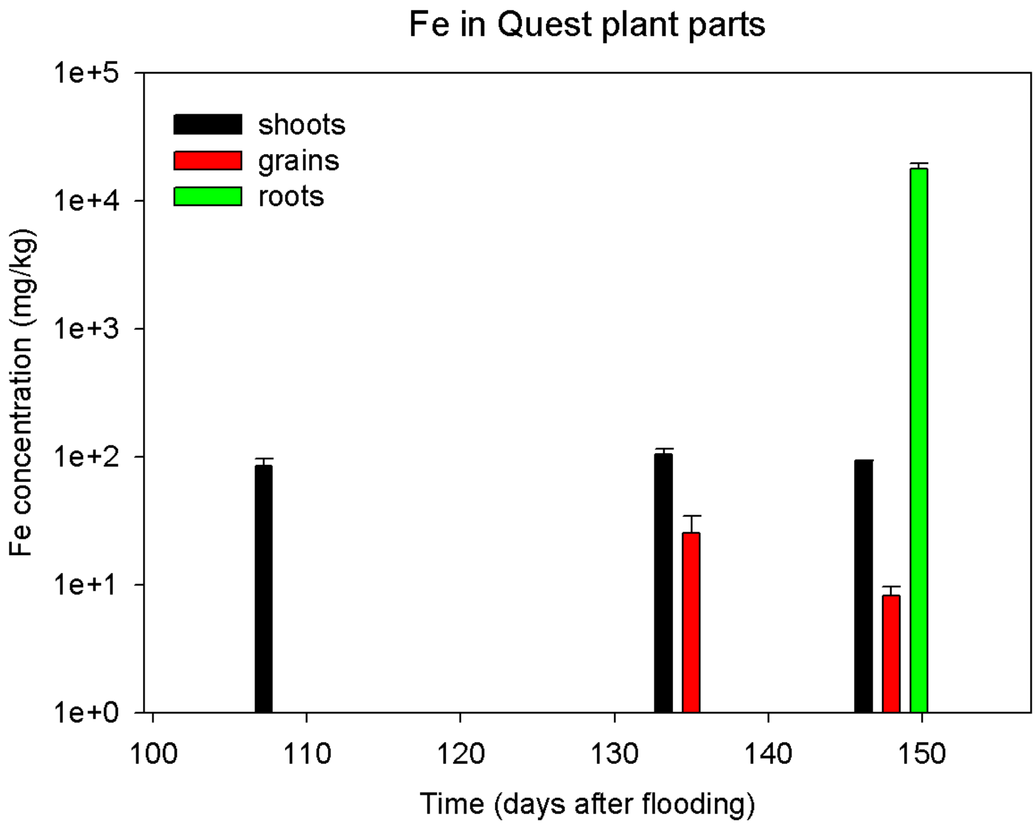

Figure 8-34. Fe in Quest roots, shoots and grains leading up to harvest

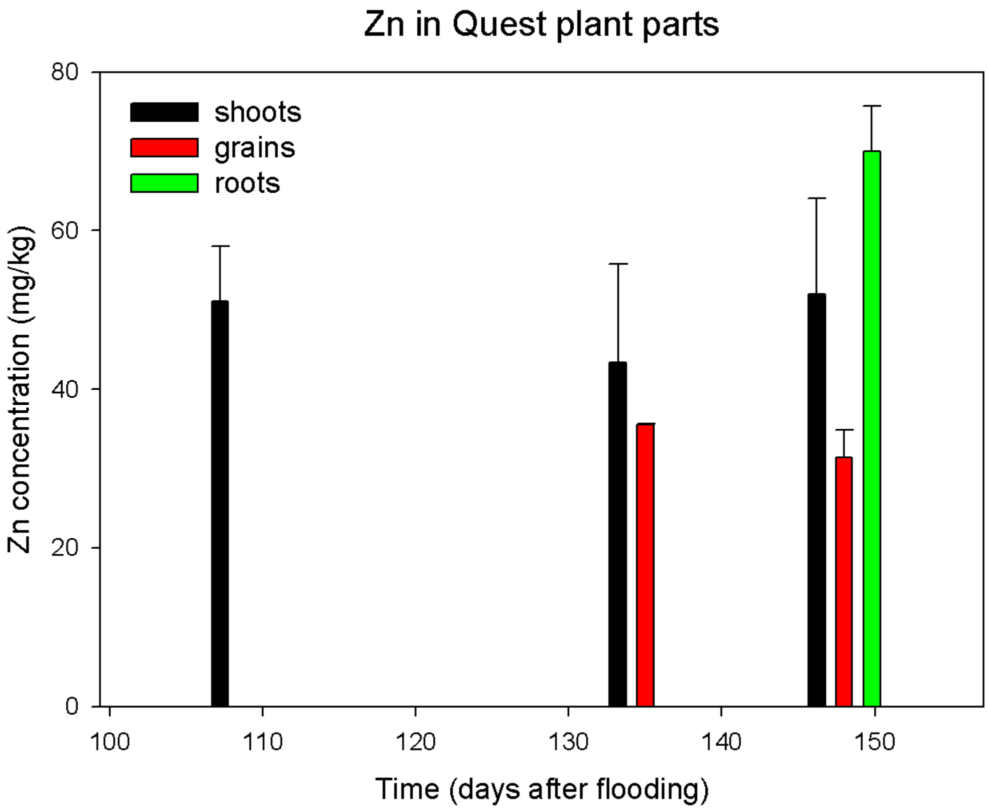

Figure 8-35. Zn in Quest roots, shoots and grains leading up to harvest

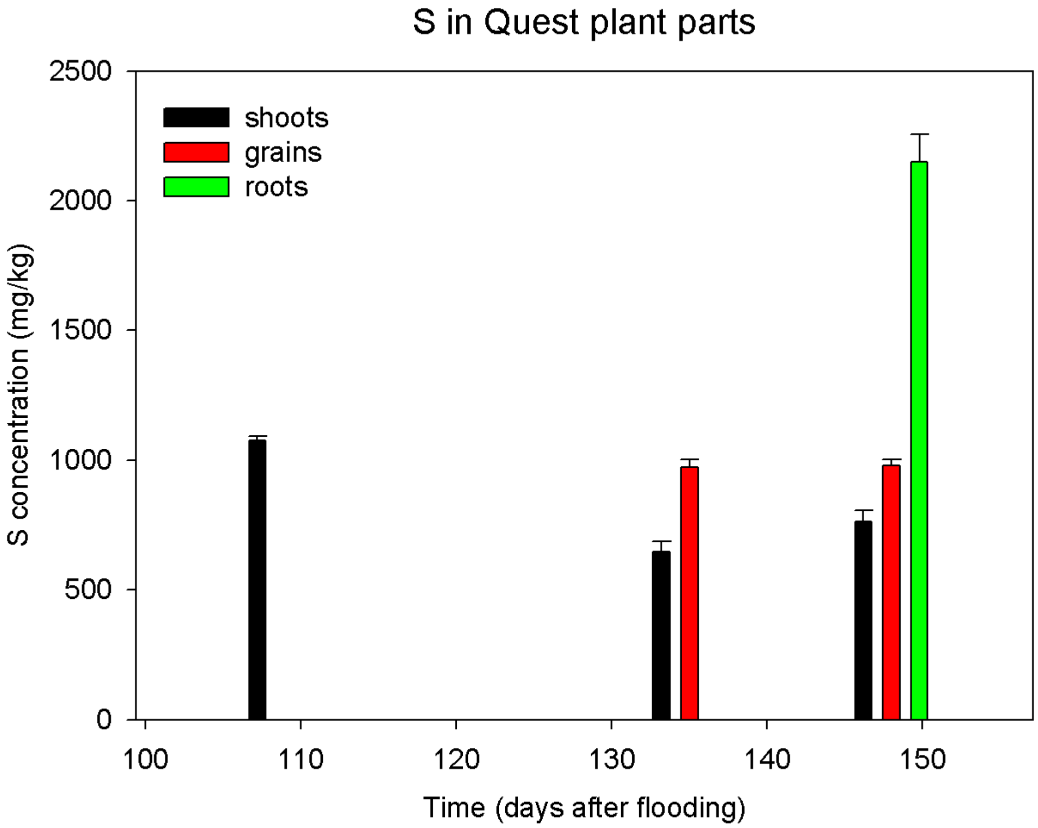

Figure 8-36. S in Quest roots, shoots and grains leading up to harvest

8.3 Appendix C – Supplementary data corresponding to Chapter 5

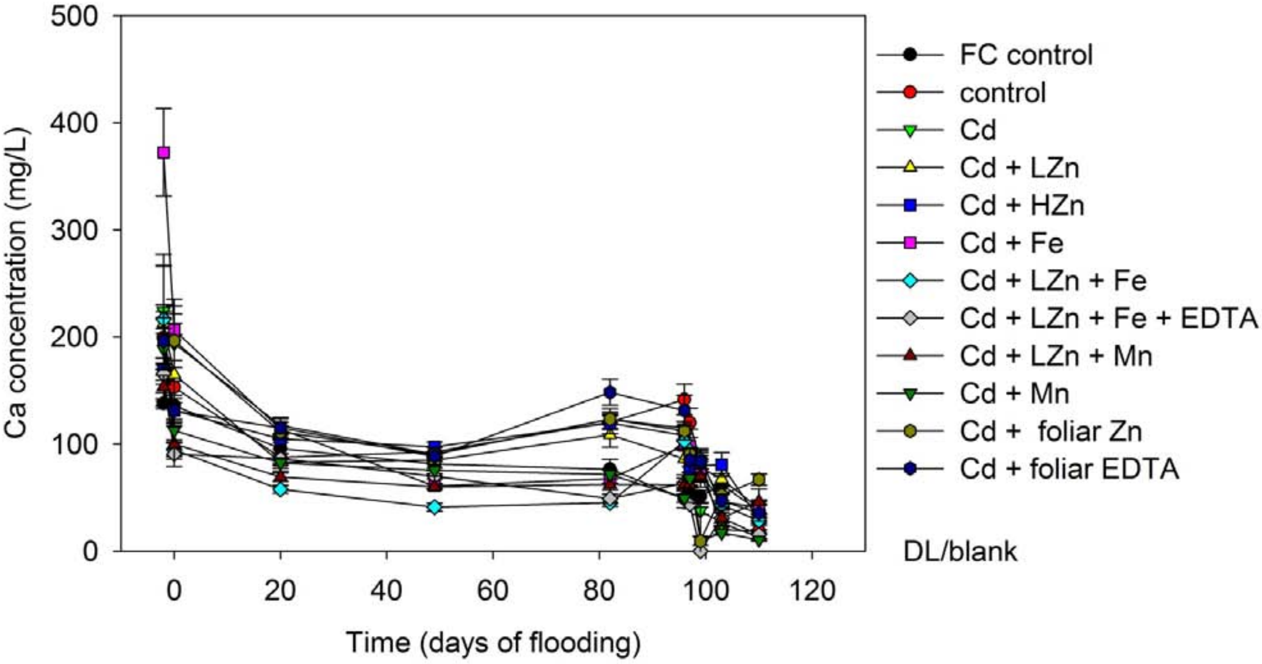

Figure 8-37. Calcium (Ca) concentrations in soil solution during rice cultivation. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA).

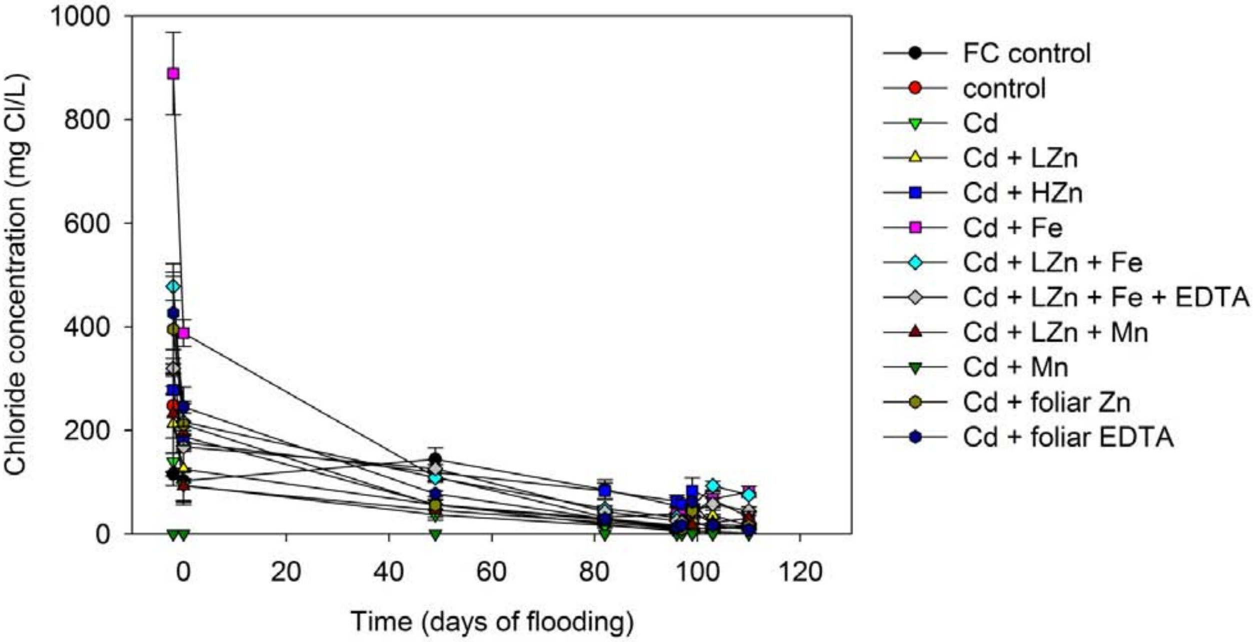

Figure 8-38. Chloride (Cl^-) concentrations in soil solution during rice cultivation. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA).

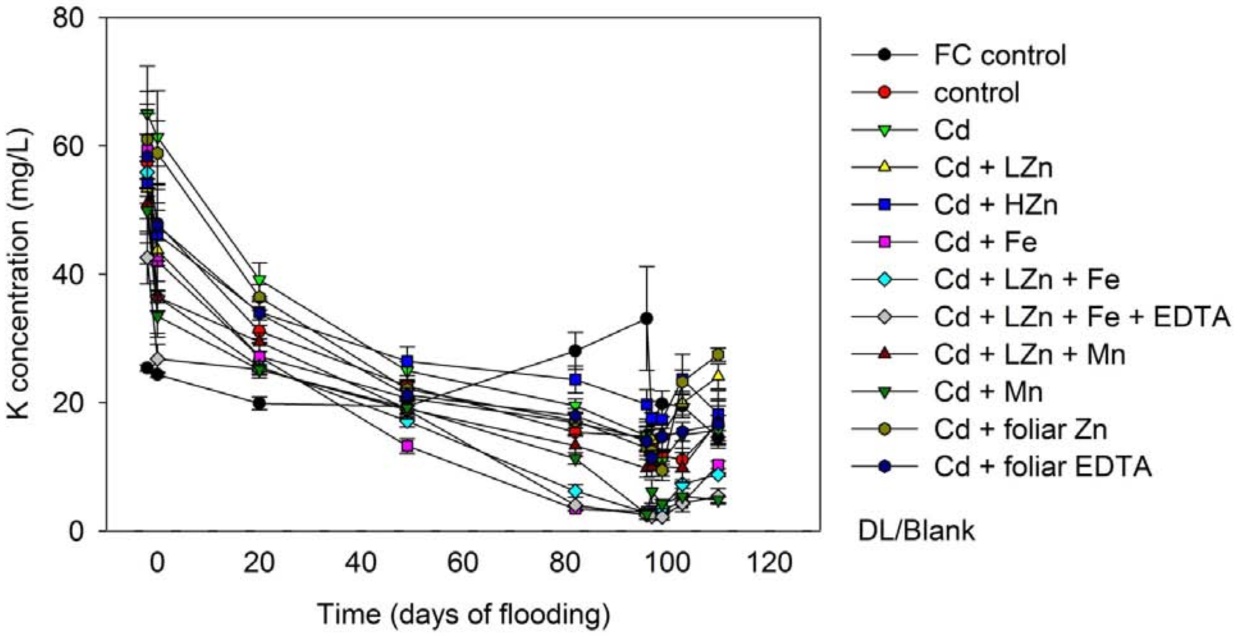

Figure 8-39. Potassium (K) concentrations in soil solution during rice cultivation. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA).

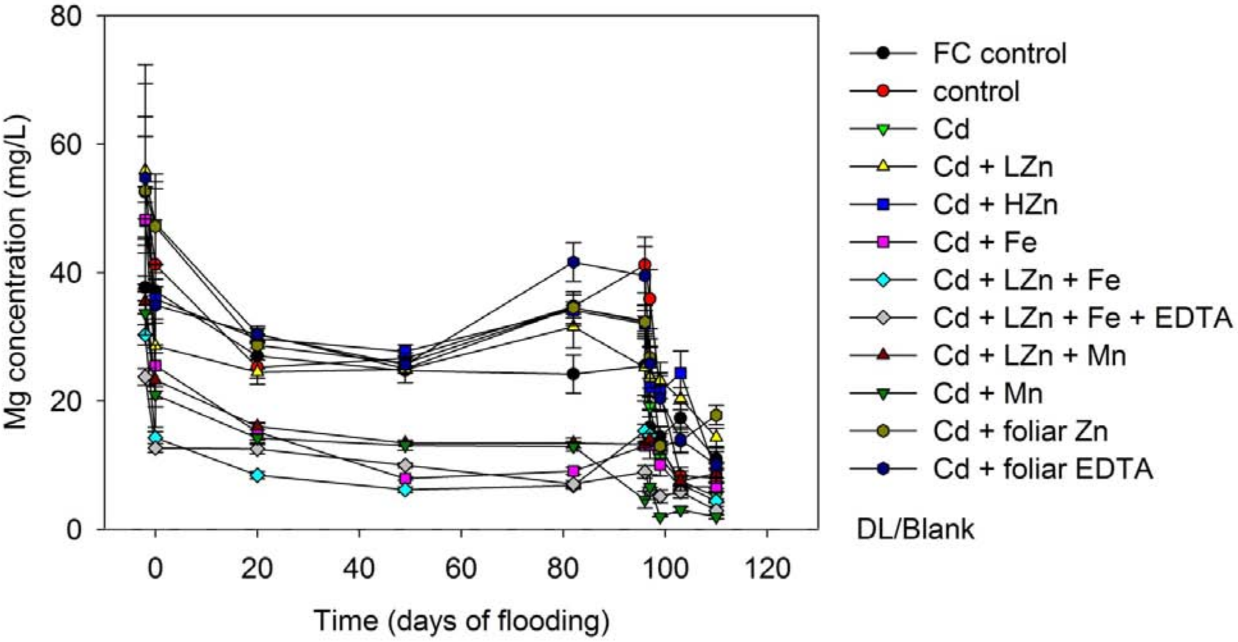

Figure 8-40. Magnesium (Mg) concentrations in soil solution during rice cultivation. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA).

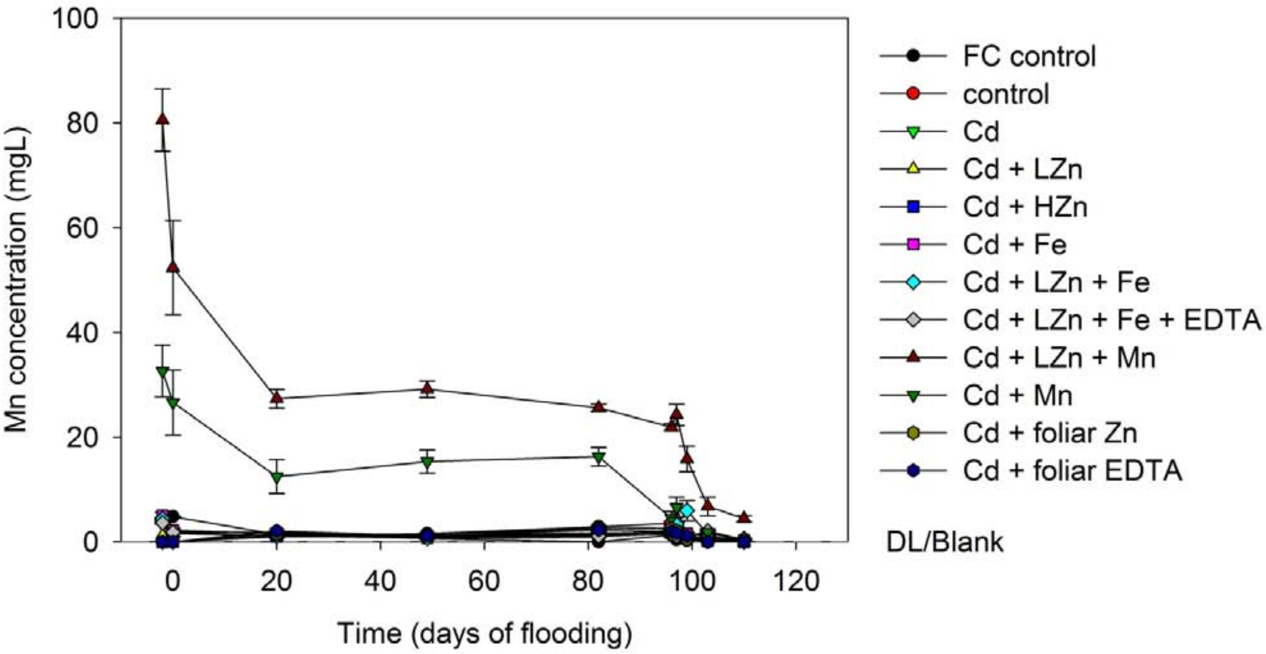

Figure 8-41. Manganese (Mn) concentrations in soil solution during rice cultivation. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA).

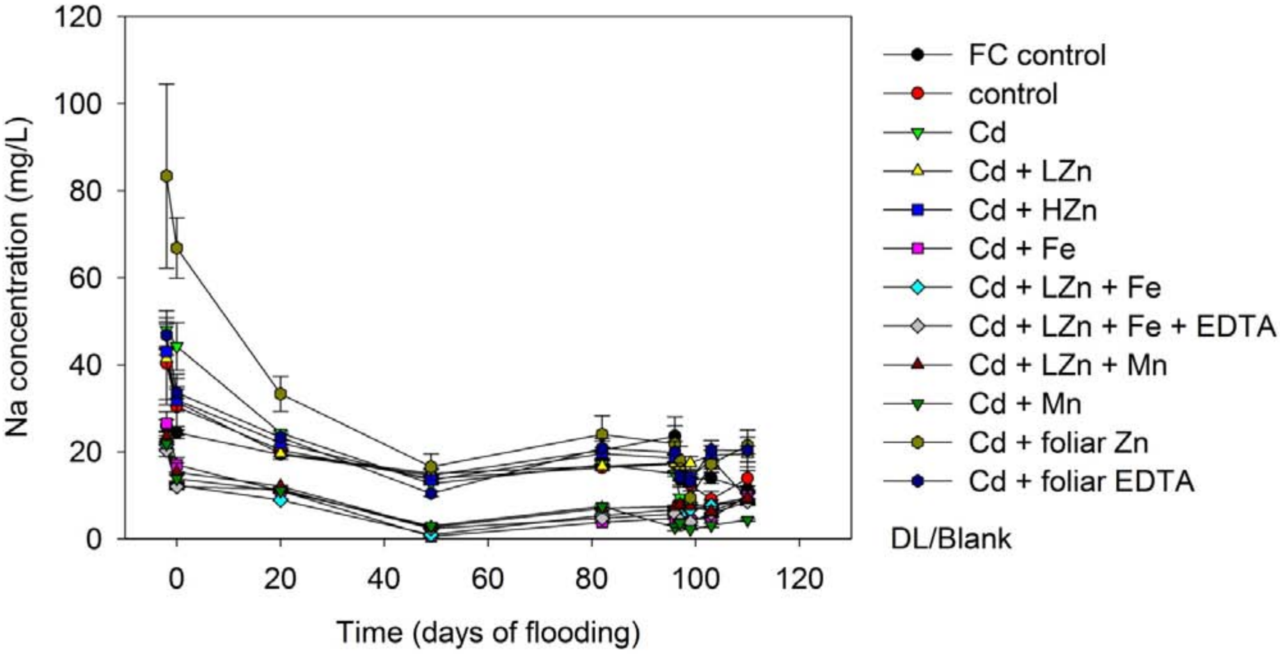

Figure 8-42. Sodium (Na) concentrations in soil solution during rice cultivation. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA).

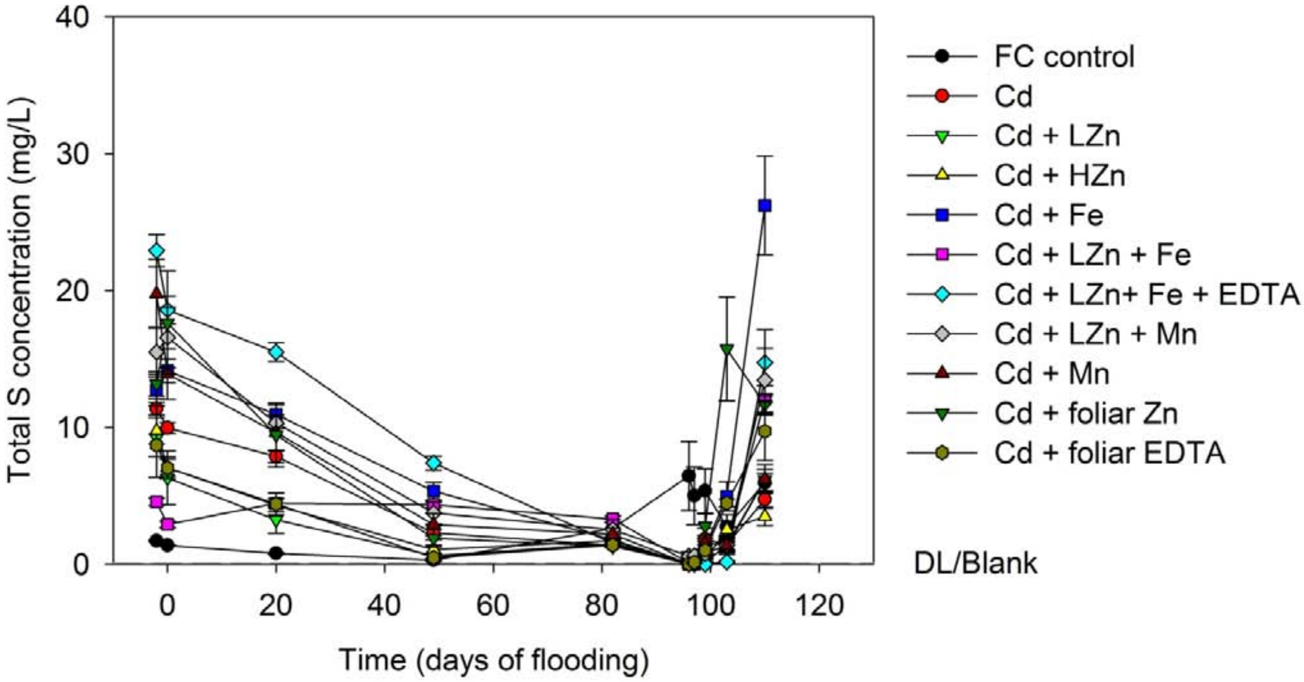

Figure 8-43. Sulfur (S) concentrations in soil solution during rice cultivation. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA).

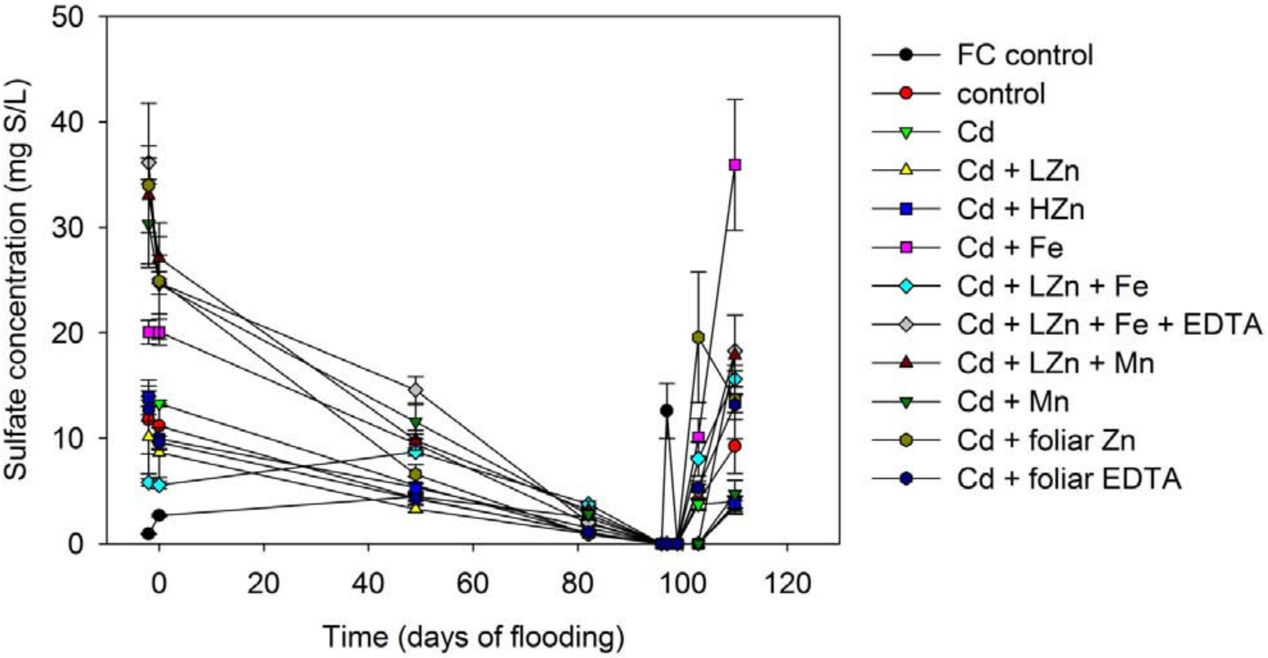

Figure 8-44. Sulfate (SO_4^{2-}) concentrations in soil solution during rice cultivation. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA).

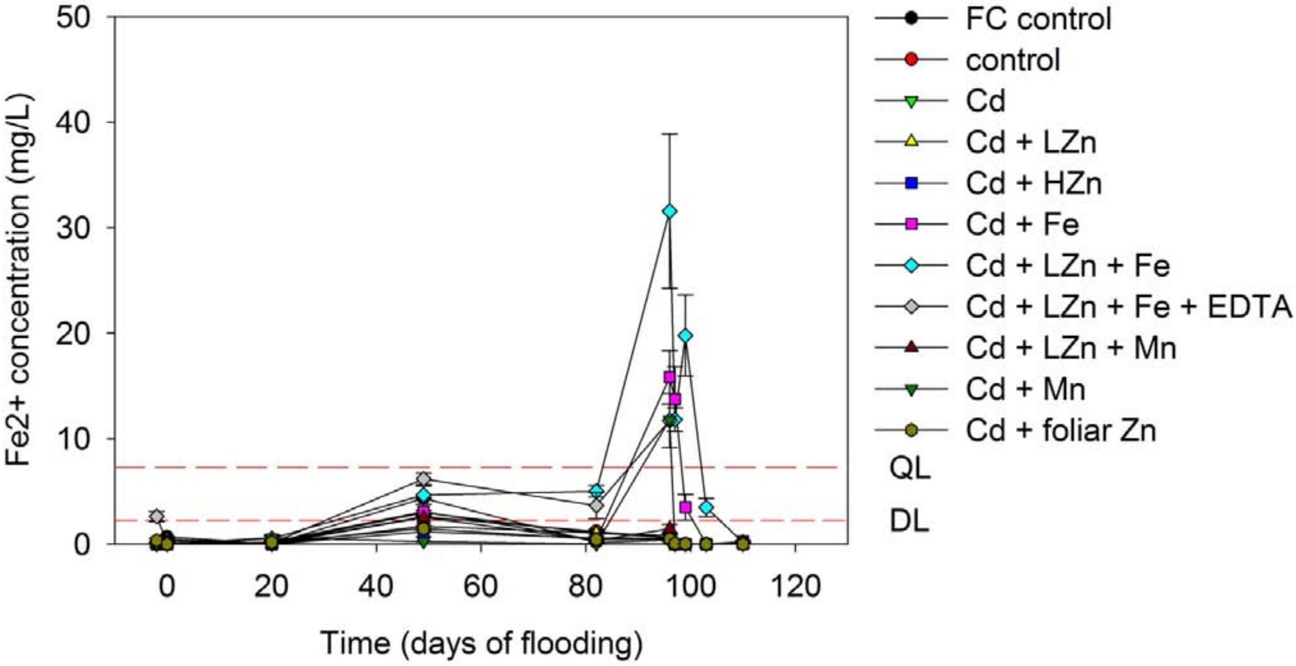

Figure 8-45. Iron(II) (Fe^{2+}) concentrations in soil solution during rice cultivation. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and Mn-spiked (Cd + Mn), Cd and low Zn with Mn-spiked (Cd + LZn + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA).

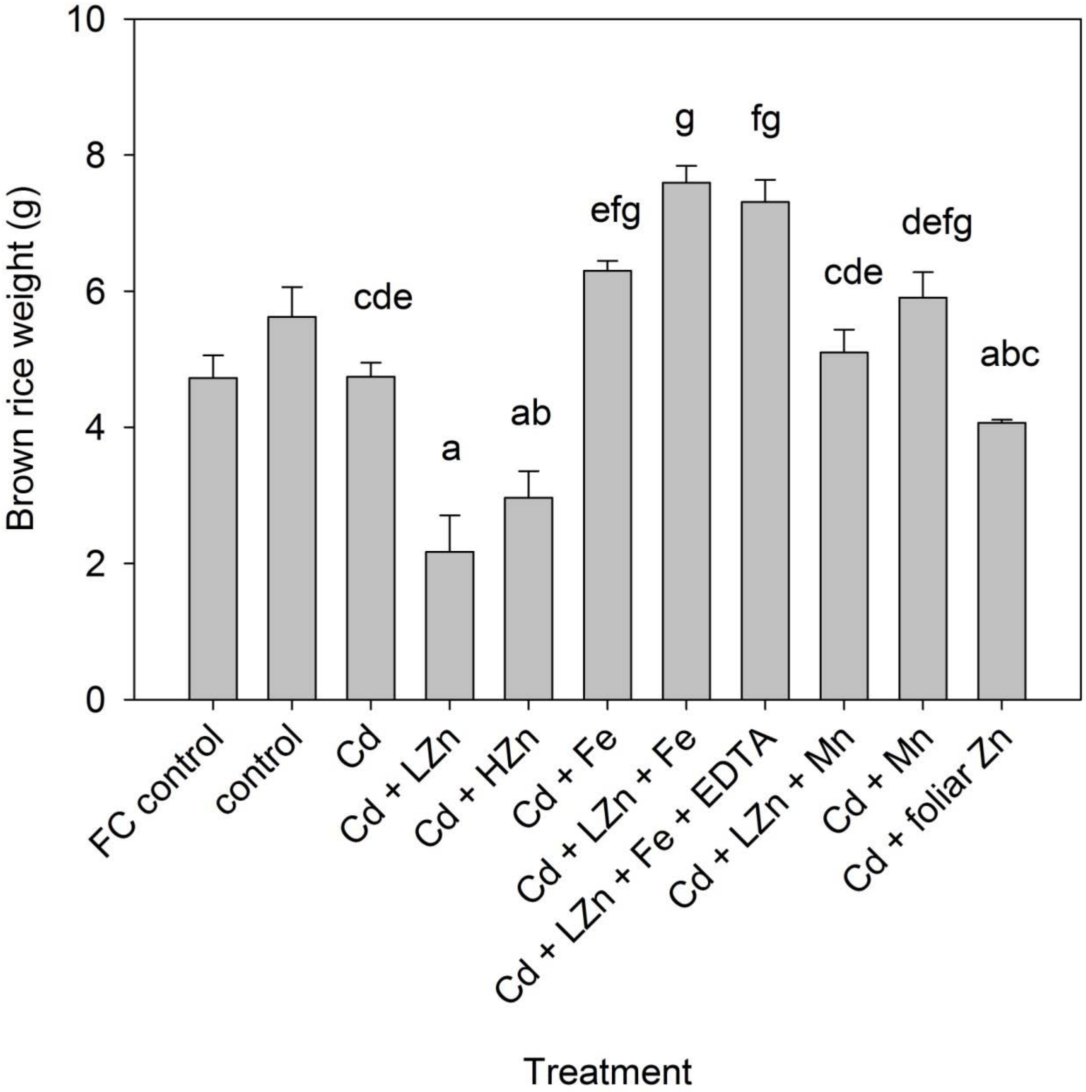

Figure 8-46. Brown rice yield in grams per pot at harvest. Soil treatments were: field capacity unspiked control (FC control), unspiked control (control), Cd-spiked control (Cd), Cd and low Zn-spiked (Cd + LZn), Cd and high Zn-spiked (Cd + HZn) Cd and Fe-spiked (Cd + Fe), Cd and low Zn with Fe-spike (Cd + LZn + Fe), Cd and low Zn with Fe and EDTA-spiked (Cd + LZn + Fe + EDTA), Cd and low Zn with Mn-spiked (Cd + LZn + Mn) and Cd and Mn-spiked (Cd + Mn). Foliar treatments were: plants grown in Cd-spiked soil receiving a Zn foliar spray (Cd + foliar Zn) and plants grown in Cd-spiked soil receiving an EDTA foliar spray (Cd + foliar EDTA). Cd + foliar EDTA not shown as there was no significant effect.