

Analysis of field-moist Cd contaminated paddy soils during rice grain fill allows reliable prediction of grain Cd levels

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Abstract Research undertaken over the last 40 years has confirmed that the long-term consumption of cadmium (Cd) contaminated rice contributes to human Cd disease. Rice is the staple of millions throughout South and Southeast Asia. Therefore, the ability to accurately assess the risk of rice grain Cd uptake in areas of elevated soil Cd would be a pre-requisite to protecting public health and regional export security. During 2001–2002, 308 concomitant soil and rice grain samples were collected from a Cd/

Zn co-contaminated site in Western Thailand and determined for *aqua regia* digested soil Cd and rice grain Cd. No significant relationship was observed between total soil Cd and rice grain Cd ($r^2=0.117$). This intuitively is to be expected since total soil Cd bears no relationship to phyto-available Cd. Similarly no relationship was observed between 0.005 M DTPA extractable soil (air-dry) Cd and rice grain Cd ($r^2=0.165$). Again this result could have been predicted as the phyto-availability of Cd in paddy soils is a function of the complex interaction between soil pH, redox conditions and the presence of competing ions. Consequently, in 2003 a further study was undertaken to assess the effectiveness of commonly utilized soil extractants namely, 0.1, 0.05 and 0.01 M CaCl_2 solutions at a soil extractant ratio of 1:5 and 1 M NH_4NO_3 for 2 h or 4 h extractions times at a soil/extractant ratio of 1:2.5. Soil samples were collected at the critical rice grain fill stage and sub-divided into Portion A which was subjected to conventional air-drying and sample preparation procedures and Portion B which was maintained at Field Condition (FC) and stored at $<4^\circ\text{C}$ until extractions were undertaken. Concomitant rice grain samples were collected at maturity. The results indicate that air-dried soil samples subjected to conventional soil preparation procedures were totally ineffective at predicting the uptake of Cd by rice stem, leaf or grain, regardless of extractant. Further, the results indicate that the Stepwise Regression model incorporating 0.1 M CaCl_2 extractable Cd and soil pH_w determined on

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field moist samples accounts for 63.8% of the variability in rice grain Cd.

Keywords Cadmium · Extraction methods · Rice grain cadmium · Uptake model · Zinc

Introduction

There is growing concern over the contamination of water, soil and agricultural produce by heavy metals throughout a rapidly changing and industrialized Asia, with the risk being highest in urbanized areas where solid and liquid urban wastes are used in a peri-urban agricultural context for the production of vegetables and rice. In addition, there are also isolated rural communities, particularly those dependent on irrigated rice-based agro-ecosystems in Southeast Asia, that face a significant health risk associated with the long-term consumption of cadmium (Cd) contaminated rice, resulting in human Cd-disease (Nogawa et al. 1983; Nogawa and Kido 1993; Tsuritani et al. 1992; Cui et al. 2004; Jin et al. 2002; Wu et al. 2001; Cai et al. 1995; Nordberg 2003; Simmons et al. 2005).

The complex interactions affecting the mobility of Cd, Zn and Fe in paddy soils and uptake to rice are not yet fully understood. However, what is clear is that the co-relationship between soil pH and redox is critical (Babich and Stotzky 1978; Bingham et al. 1980; Page et al. 1981; Iimura 1981; Chaney et al. 1996). In paddy soils, soil solution pH alters towards near neutral as the system moves from oxidizing to reducing conditions, which reduces Cd phyto-availability (Chaney et al. 1996). However, drainage of fields at the critical grainfill stage in order to optimize yields and facilitate ease of harvesting, decreases soil pH to antecedent conditions and increases Cd phyto-availability to rice plants (Chaney et al. 1996). In addition, increased acidity upon drainage and the development of oxidizing conditions results in the dissolution of secondary Al precipitates and the release of any sorbed Cd. The increased acidity also leads to desorption of Cd from soil organic matter (Zachara et al. 1992). Conversely, oxidation results in the formation of Fe and Mn oxides and subsequent co-precipitation of Cd (Alloway 1997). If reducing conditions reoccur, co-precipitated Cd becomes exchangeable and is sorbed onto surfaces of Al and organic matter. In tropical highly weathered soils the adsorption of Cd²⁺ may also be a function of

pH dependant charge associated with the edges of clays, humus polymers and oxides (Zachara et al. 1992). Further, in submerged paddy soil sulphate (SO₄²⁻) ions may be reduced to sulphide (S²⁻) resulting in the precipitation of the Cd and Zn sulphide minerals (Iimura 1981; Chaney et al. 1996). As stated, drainage, oxidation and acidification of paddy soils at the grain fill stage to maximize rice grain yield and facilitate harvesting results in the rapid transformation of CdS and ZnS to Cd²⁺, Zn²⁺ and SO₄²⁻.

The objective of this study was primarily to assess the applicability of the continued adoption of conventional air-drying of soils when undertaking research to predict the uptake of Cd to the rice plant particularly, rice grain. In addition this research was undertaken to evaluate various soil extractants as potential decision support tools for Cd risk assessment by evaluating their ability to explain the uptake of Cd by rice crops, in particular rice grain, on a Cd and Zn co-contaminated site in western Thailand.

Materials and methods

Site description

The co-contaminated Cd/Zn rice-based agricultural system used in this study is situated within Phatathai and Mae Tao Mai sub-districts, Mae Sot, Tak Province, Thailand. A comprehensive description of the sites characteristics has been previously described by Simmons et al. (2003, 2005). In brief the study area is situated on the western border of Thailand near Myanmar and consists of over 12,000 ha of cascade irrigated rice fields that source irrigation water from Mae Tao Creek. The upstream reaches of Mae Tao Creek pass through a Zn-mineralized zone that has been actively mined for the past 25 years. Mae Tao Creek is utilized for irrigation by eight communities with a combined resident population of >6,000 and an annual combined rice production of 7,592 t year⁻¹. The total area under paddy rice for the eight villages is 2,201 ha. Water is initially diverted from Mae Tao Creek to main irrigation canals and then through a series of in-field irrigation channels that carry water to the primary fields situated at the highest point in the topography. Water from the primary fields cascades to the next field in sequence (secondary), and continues downwards through sub-adjacent fields until it is

discharged into a drainage channel that subsequently re-connects to the main irrigation canal and/or Mae Tao Creek (Simmons et al. 2005).

Survey and sampling strategy

An initial extensive survey of the Zn/Cd co-contaminated site was undertaken from 2001 to 2002 in which a total of 308 surface soil samples (0–20 cm) were collected from irrigated fields that source waters from Mae Tao Creek (Simmons et al. 2003). Soil samples were collected from 10–20 points within each field at physiological maturity of the rice crop prior to harvest, thoroughly mixed and a composite sample of 500 g retained for laboratory analysis.

At the same time for each field sampled, panicles of mature grain were collected in the area from which soil samples were taken and returned to the laboratory for drying at 65°C for 48 h before de-husking. Soil samples were air dried and sieved to pass a 2 mm mesh in preparation for chemical analysis. All samples were geo-referenced using a hand held GPS and placed within a GIS platform that demarcated individual field boundaries and allowed an assessment of the spatial distribution of Cd.

The rice samples collected from the fields were made up of two distinct varieties, namely Glutinous and Jasmine (KDM105) rice. A simple *t* test statistical analysis between the two population groups revealed that there was no significant ($p > 0.05$) differences between the two varieties with respect to Cd concentration in the grain (mean Cd concentrations: 1.36 ± 0.14 and 1.10 ± 0.07 mg kg⁻¹ for Glutinous and KDM105 respectively). Consequently all further statistical analysis was undertaken on the combined group of samples.

Second survey

Cadmium uptake to rice grain occurs during the critical grain fill stage. In order to capture possible changes in the phyto-available Cd during this period, soil samples were collected in November 2003. Twenty fields that had been sampled in the previous survey were selected and soil samples collected during the grainfilling period. The selection of the 20 fields from the initial survey was based on identifying adjacent fields that were sampled in 2002 that had a range of Cd concentrations and pH

values that were representative of those previously observed. Soil samples were collected as previously described and thoroughly mixed in field before being divided into two 500 g portions. Portion A was subjected to conventional air-drying and sample preparation procedures as previously discussed. Portion B was maintained at Field Condition (FC) and stored at <4°C until extractions were undertaken.

Soil and plant analysis

For the initial sampling event, total soil Zn and Cd were digested in *aqua regia* (3:1 HCl/HNO₃) using an open tube digestion method (McGrath and Cunliffe 1985). Soil pH_w was determined on a 1:5 soil/water suspension (Rayment and Higginson 1992). DTPA-extractable Cd and Zn were determined by tumbling soils at 30 rpm for 2 h in 0.005 M DTPA using a soil/extractant ratio of 1:5. The modification of the soil/extractant ratio from 1:2 as proposed by Lindsay and Norvell (1978) follows Norvell (1984) who suggested that for metal enriched soils, a soil to extractant ratio of 1:5 is required.

Plant samples were digested in 2:1, HNO₃/HClO₄ using an open tube digestion technique and a pre-calibrated Thermolyne HPA 2330M-26 Hotplate and 40-tube (100 ml) Al block (300×300×70 mm). Prior to digestion, plant samples were pre-digested overnight at an ambient temperature ranging from 26–34°C. To assess within-batch and between-batch precision, two reagent blanks, three replicates of an appropriate In-House Standard Reference Material (I-HSRM), and three duplicates from the previous analytical batch were included in each batch of samples analyzed. In addition, test portions were analyzed in triplicate. Element concentrations were determined using a Perkin Elmer Analyst 3000 Atomic Adsorption Spectrophotometer with deuterium lamp background correction. Analytical accuracy was further assessed through the ‘real time’ comparison of I-HSRM results with, element specific control charts and the use of Continuous Verification Standards (CVS) at regular intervals. Chemical analysis of rice grain samples was undertaken on unpolished or “brown” rice samples as this is the form predominantly consumed by communities within the affected area.

Two extraction methods commonly adopted as indicators of Cd and Zn ‘bioavailability’ were evaluated on the 20 field samples collected in the second

survey on both air dried and field moist samples. CaCl₂-extractable Cd, Zn and Fe were determined by extracting soil portions A and B for 4 h in 0.01 M CaCl₂, 0.05 M CaCl₂ and 0.1 M CaCl₂ at a soil/extractant ratio of 1:5 (Houba et al. 2000). In addition, soil portions A and B were extracted in 1 M NH₄NO₃ for 2 h or 4 h at a soil/extractant ratio of 1:2.5 (Hall et al. 1998). Soil pH_w was measured on a 1:5 soil/water suspension (Rayment and Higginson 1992). Organic carbon and particle size analysis was determined of the 20 field samples following the Walkley-Black Method (Walkley 1947; Nelson and Sommers 1996).

Moisture content was measured for the field collected soils and subsequently expressed on an air-dry basis in order to facilitate comparability of results between the field moist and air-dry soil samples. This was achieved by multiplying the field moist soil sample weight (F_{FM}W) by the moisture factor. For each field moist soil sample collected, a 20 g sub-sample was oven dried at 105°C for 48 h. The moisture factor was determined following standard methods.

In addition, the volume of entrained soil solution in the field moist samples was also taken into account by adding the entrained soil solution volume (For example, a F_{FM}W of analytical sample (10 g) × Moisture factor of 0.706 equates to a mass of entrained solution of 2.94 g. Assuming a density of 1.00 this results in a volume of entrained solution of 2.94 ml) to the volume of extracting solution.

In field moist soil samples Cd (and Zn) concentration was determined by the equation

$$\text{Cd concentration (mg kg}^{-1}\text{)} = \frac{\text{rbc Cd concentration (mg l}^{-1}\text{)} \times \text{extractant volume}_{\text{adj}} \text{ (ml)}}{\text{MF adjusted sample weight (g)}}$$

Where

rbc Cd concentration	reagent blank corrected Cd concentration
extractant volume _{adj}	volume of extracting solution + volume of entrained soil solution
MF adjusted sample weight	moisture factor adjusted soil sample weight

In air-dry soil samples Cd (and Zn) concentration was determined by the equation

$$\text{Cd concentration (mg kg}^{-1}\text{)} = \frac{\text{rbc Cd concentration (mg l}^{-1}\text{)} \times \text{sample volume (ml)}}{\text{soil sample weight (g)}}$$

where

rbc Cd concentration	reagent blank corrected Cd concentration
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Results and discussion

Initial survey

The co-contamination of the site is confirmed through a regression of total digestible Zn and Cd from surface soil samples (Fig. 1). The highly significant ($p > 0.001$) correlation coefficient suggests that the two contaminants were co-transported in sediments carried in the irrigation water. Indeed, studies associated with the distribution of Cd and Zn within the irrigated field sequences would support the notion that contamination of these soil surface layers was due to fine sediments transported by the irrigated waters and deposited on the soil surface (Simmons et al. 2005). Through continual mixing of this surface layer (0–20 cm) the sediments have been evenly distributed throughout the tilled layer and do not occur at depth (Simmons et al. 2005). Similar significant correlations were observed between total soil Cd and Zn on both air-dry and FC samples for all soil

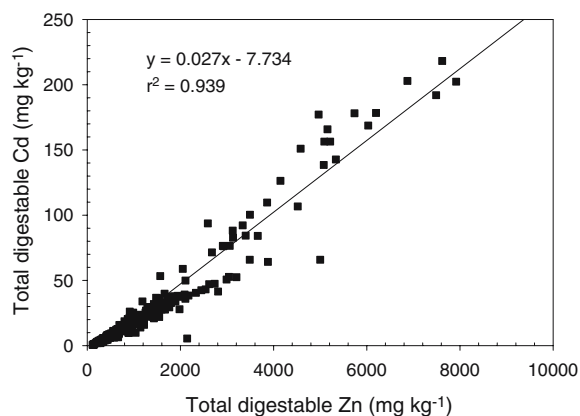


Fig. 1 Relationship between total *aqua regia* digestible Zn and Cd from surface soil (0–20 cm) samples collected from 308 rice fields in the Mae Sot area of Western Thailand

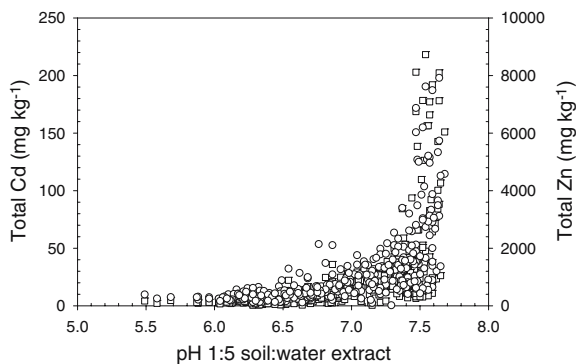


Fig. 2 Relationship between total *aqua regia* digestible Zn and Cd and soil pH (1:5w)

extractants evaluated. Further, the results indicate that in the current study area total soil Cd and Zn are directly related to soil pH with highest total Zn and Cd concentrations associated with soil pH >7.5 (Fig. 2). This is due to alkalinity associated with the source of Cd and Zn contamination.

In assessing the degree of Cd contamination, international guidelines and standards have been established that are based on total digestible concentrations (Table 1). The mean *aqua regia* Cd and Zn concentrations for the 308 samples collected in 2001–2002 were $26.2 \text{ mg Cd kg}^{-1} \pm 2.19$ (range: 0.59–217 mg Cd kg^{-1}) and $1,233 \pm 77 \text{ mg Zn kg}^{-1}$ (range: 115–7,918 mg Zn kg^{-1}) respectively. Placing these values within the context of the European Union (EU) Maximum Permissible (MP) total soil Cd and Zn concentration for sludge amended soils are 3.0 mg kg^{-1} and 300 mg kg^{-1} (Directive 86/278/EEC; European Economic Commission 1986), 89% and 86% of the samples collected exceed the aforementioned MP in Cd and Zn respectively. With respect to the Thai Investigation Level of $0.15 \text{ mg Cd kg}^{-1}$, all samples

collected exceed this standard (Pongsakul and Attajarusit 1999; Zarcinas et al. 2003). Further, Pongsakul and Attajarusit (1999) gave ‘background’ total Cd concentrations for Thai soils of $0.002\text{--}0.141 \text{ mg Cd kg}^{-1}$.

Internationally recognized Maximum Levels (ML) for contaminants in foods are established by the Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (CAC). Specifically, MLs are established by the Codex Committee on Food Additives and Contaminants (CCFAC) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2005). At the 64th JECFA meeting (JECFA Rome, 8–17 February 2005) polished rice was advanced to Step 5 at $0.4 \text{ mg Cd kg}^{-1}$. Subsequently at the 29th CAC (July, 2006) the ML for rice was finally decided (Step 8) at $0.4 \text{ mg Cd kg}^{-1}$. The mean concentration of Cd in the grain of unpolished rice was $1.15 \pm 0.06 \text{ mg kg}^{-1}$ (range: $0.02\text{--}7.75 \text{ mg Cd kg}^{-1}$). Over 67% of the rice grain samples exceeded the CAC ML of $0.4 \text{ mg Cd kg}^{-1}$ for the ‘safe’ lifetime consumption of rice with 40% of samples exceeding a Cd concentration $>1.0 \text{ mg Cd kg}^{-1}$.

To put this in context, proximal tubular renal dysfunction have been identified in populations consuming rice grain with Cd concentrations ranging from $0.3\text{--}>1.0 \text{ mg kg}^{-1}$ (Saito et al. 1977; Nogawa et al. 1983; Kido et al. 1988; Cai et al. 1995; Nakashima et al. 1997). In addition, 99% of the grain samples collected exceeded the ‘background’ mean Thai rice grain Cd concentration of $0.043 \text{ mg kg}^{-1} \pm 0.019$ (Pongsakul and Attajarusit 1999).

Of importance in assessing the risk of Cd entering the food chain and becoming a human health risk, is the ability to predict uptake by crops using routine soil extraction methods. Because total digestible Cd is

Table 1 International standards for Cd and Zn (were applicable) in agricultural soils based on soil total Cd and Zn values (mg kg^{-1})

Country	Statute	Cadmium (mg kg^{-1})	Zinc (mg kg^{-1})
European Union	Directive 86/278/EEC	1–3 ^a	300 ^a
Australia	Ecological Investigation level (EIL)	3 ^b	200 ^b
Canada	Environmental Quality Guidelines	1.4 ^c	200 ^c
Vietnam	VN Standard (TNVN 7209-2002)	2.0 ^d	200 ^d

^a EU maximum permissible (MP) levels for sludge amended agricultural soils

^b NEPC (1999) National Environment Protection (*Assessment of Site Contamination*) Measure 1999, Schedule B (1) Guideline on the Investigations Levels for Soil and Groundwater.

^c Canadian Council of Ministers of the Environment (2006) Soil Quality Guideline Values for Agricultural Soils

^d VN Standard (TNVN 7209-2002) for agricultural soils

commonly used as a standard method in establishing the degree of contamination (Table 1), one cannot imply that there should be a relationship between Cd in the rice grain and total Cd extracted (Fig. 3a). Indeed the relationship between the aforementioned parameters is not significant ($r^2=0.117$) which is to be expected since total Cd bears no relationship to bio-available Cd that is available for crop uptake. The *aqua regia* digestion extracts between 70–90% of all Cd pools including soluble, exchangeable, labile, and non-labile forms (McGrath and Cunliffe 1985).

The 0.005 M DTPA extractant has been used extensively to quantify what could be termed potential plant available Cd and Zn in a range of crop species including flax (Grant et al. 2002a), durum wheat (Grant et al. 2002b), and soybean (Simmons and Pongsakul 2004). Significant relationships between DTPA extractable and grain Cd have been observed in these crops that grow predominantly under well aerated soils that are primarily in an oxidizing state. In the

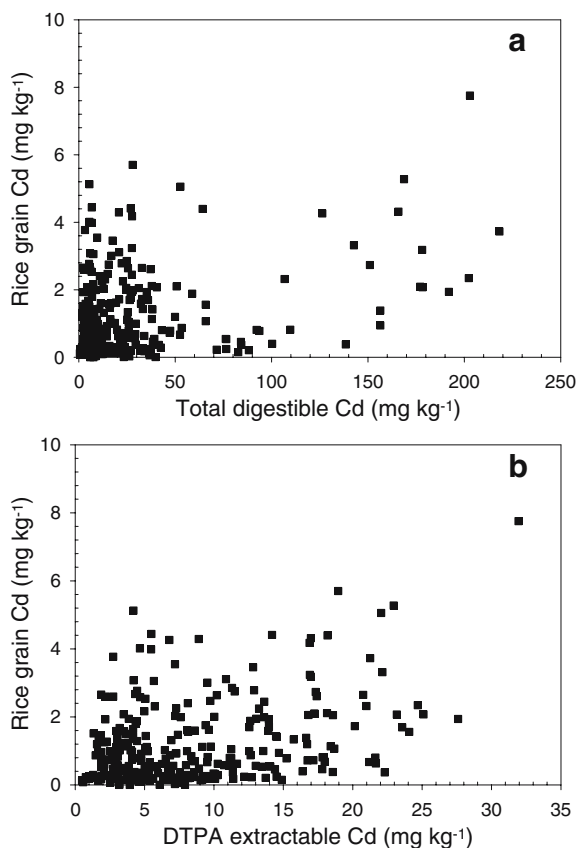


Fig. 3 Relationship between rice grain Cd concentration and **a** total *aqua regia* digestible and **b** 0.005 M DTPA extractable Cd from samples collected from co-contaminated Zn/Cd rice fields

current study a non-significant ($r^2=0.165$) relationship between DTPA extractable and rice grain Cd concentration was observed suggesting the inability of this extractant in accounting for Cd uptake by rice (Fig. 3b). Intuitively this result could have been predicted as the phyto-availability of Cd in soils is a function of soil pH and redox condition (Babich and Stotzky 1978; Bingham et al. 1980; Page et al. 1981; Iimura 1981; Chaney et al. 1996). Paddy soils undergo large fluctuations in soil solution pH with soils attaining a near neutral condition as the system moves from oxidizing to reducing conditions with a concomitant reduction in Cd phyto-availability (Chaney et al. 1996).

A standard practice that is adopted by rice farmers is to drain their fields prior to the critical grain filling stage in order to optimize yields and facilitate ease of harvesting. This results in a decrease in soil pH to antecedent conditions and increases Cd phyto-availability to rice plants (Chaney et al. 1996). The significance of the timing of pre-harvest drainage was demonstrated by Iimura (1981) whom observed un-polished rice grain Cd concentrations of 0.75 and 4.85 mg kg⁻¹ for rice grown on submerged soil and soil drained after tillering.

In addition, increased acidity upon drainage and the development of oxidizing conditions can affect the dissolution of secondary Al precipitates and the release of any sorbed Cd. Increased acidity also leads to desorption of Cd from soil organic matter (Zachara et al. 1992).

Apart from the lack of any relationship between DTPA extractable Cd and uptake to rice, there are practical limitations that make this method somewhat tedious and subject to significant error in laboratories that may not have a functional quality assurance program. In particular the number of samples that can be analyzed at any one time is significantly reduced due to deposits that accumulate on the burner of the AA that necessitates regular cleaning and recalibration. An advantage of using DTPA extractions is that it is a commonly used method for the determination of micronutrients and the fact that it extracts different components i.e. organic and exchangeable, results in higher values that are within the detection range of most laboratories in Southeast Asia.

However, the results from this study clearly demonstrate that there is no relationship between the uptake of Cd by grain and DTPA or *aqua regia* extracts bring into question the ability of these two methods in assessing phyto-availability.

Second sampling

In order to address the issue of phyto-available Cd and uptake by rice crops, a targeted survey of 20 selected fields was undertaken in 2003. Soil samples were collected during the grain filling stage of growth and split into two sets, one of which was air dried and the other kept at field moisture content. Extractions were undertaken on both sets of samples using neutral salts, namely CaCl_2 and NH_4NO_3 , at varying concentrations and extraction times. In addition, rice plant samples were collected at physiological maturity, split into leaf, stem and grain, and subjected to chemical analysis as described previously. The mean *aqua regia* Cd and Zn concentrations for the 20 samples collected in 2003 were $36.7 \text{ mg Cd kg}^{-1} \pm 5.26$ and $1,704 \pm 194 \text{ mg Zn kg}^{-1}$ respectively with a total of 18 samples exceeding the EU MP levels (Table 2). In addition, Org-C (%), pH_w (1:5) and clay content (%) ranged from 1.28–2.42%, 5.68–7.61 and 15.5–46.8, respectively (Table 2). Clay mineralogy for the study area is 20–40% kaolinite, 5–20% illite, 20–40% montmorillonite, 5–20% quartz and <5% goethite (Personal Communication: Dr. Orathai Sukreeyapongse, LDD).

In contrast to total soil Cd and Zn (Fig. 2) the highest concentrations of phyto-available Cd and Zn are associated with soil pH >5.5 and <6.0 as indicated for brevity, by field moist 0.1 M CaCl_2 -extractable Cd and Zn (Fig. 4a and b). This confirms the findings of Babich and Stotzky (1978), Bingham et al. (1980), Page et al. (1981) and Imura (1981). The observed increases in phyto-available Cd and Zn at pH >7.5 reflects the high total Cd and Zn concentrations (Fig. 2). A similar trend was observed for all extraction methods investigated irrespective of concentration, extraction time or moisture condition.

Critically, whereas <1.5% of total soil Cd is phyto-available at soil pH >7.5 over 65% of total soil Cd is phyto-available at soil pH >5.5–<6.0 (Fig. 5a). In contrast, <12% of total soil Zn is phyto-available at soil pH >5.5–<6.0 and <0.5% at soil pH >7.5 (Fig. 5b). This clearly demonstrates the comparative

phyto-availability of Cd as compared with Zn at the critical grain fill stage.

In an effort to predict the uptake of Cd by the rice crop the study was extended to include measurements of extractable Zn and pH. The justification for this is based on previous studies undertaken on durum wheat where the application of Zn to soils significantly decreased the Cd concentration in durum wheat grain, leaf, stem and root (Grant et al. 2002b) suggesting an antagonistic effect of Zn on Cd uptake. As the site is co-contaminated with Zn it was argued that the presence of high levels of available Zn in these soils could influence the uptake of Cd. With respect to soil pH, it is well recognized that both Cd and Zn availability are pH dependent. Oxidation of paddy soils following drainage results in the formation of Fe and Mn oxides and potential co-precipitation of Cd (Alloway 1997). If reducing conditions reoccur, co-precipitated Cd becomes exchangeable. Therefore, extractable Fe was also determined on field moist and air-dry samples as an indicator of soil redox and availability of Cd.

Concentrations of Cd, Zn, Fe and soil pH as determined on field moist and air dried samples using varying CaCl_2 concentrations are presented in Table 3. With increasing concentration of CaCl_2 , mean values of Cd and Zn extracted increased consistently regardless of whether the samples were field moist or air dry when extracted (Table 3). This is to be expected since increases in the concentration of extractants will enhance the efficiency of extraction. Significantly ($p > 0.001$) greater Cd (0.149 ± 0.016 versus $0.089 \pm 0.020 \text{ mg Cd kg}^{-1}$) was extracted from field moist when compared to air dried samples using 0.01 M CaCl_2 , whilst there were no significant differences in Zn concentrations between the two samples (Table 3). In contrast, significantly ($p > 0.001$) higher Cd was extracted on air dried samples when compared to field moist samples for both the 0.05 and 0.1 M CaCl_2 whilst this trend was reversed with respect to Zn with higher levels of extraction being attained in field moist samples (Table 3).

Table 2 Mean, standard error of the mean and range of selected soil properties determined on air dried soil samples collected during grain fill from 20 fields within a Zn/Cd co-contaminated site in Thailand

Total-Cd (mg kg^{-1})	Total-Zn (mg kg^{-1})	Org-C (g kg^{-1})	pH _w (1:5)	Sand (%)	Silt (%)	Clay (%)
36.6 ± 5.28 (2.38–168)	1704 ± 194 (254–6030)	19.2 ± 0.29 (12.8–24.2)	6.97 ± 0.068 (5.68–7.61)	40.2 ± 0.876 (28.8–66.1)	29.2 ± 0.526 (18.4–38.1)	30.6 ± 0.744 (15.5–46.8)

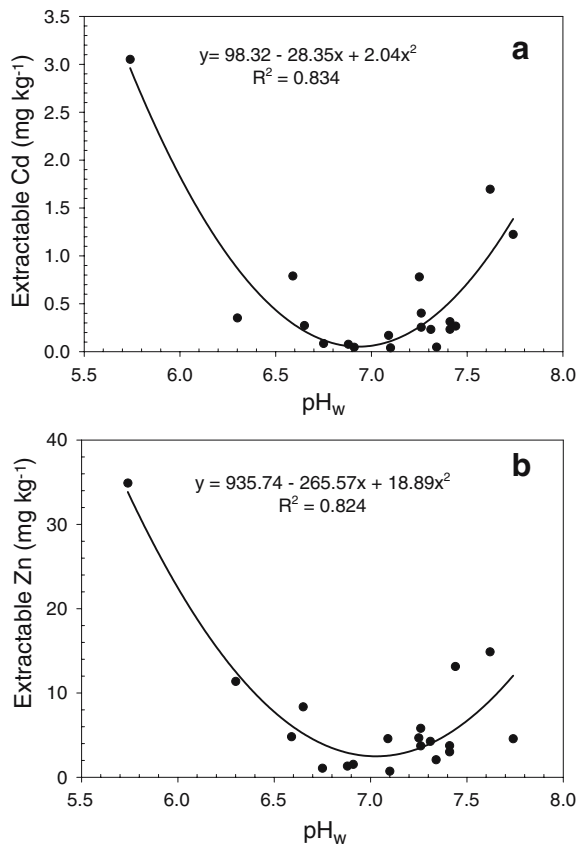


Fig. 4 Relationship between 0.1M CaCl_2 -extractable Cd **a** and Zn **b** and soil pH (1:5w) at field moist condition

For both 0.05 and 0.10 M CaCl_2 Fe concentrations were significantly higher ($p > 0.001$) in field moist conditions as compared with air-dry samples with values of 0.417 (± 0.06) and 0.104 (± 0.008) and 1.04 (± 0.195) and 0.072 (± 0.008), respectively (Table 3). This effectively reflects the higher availability of Fe in soils under reducing conditions. In direct contrast 1 M NH_4NO_3 (at either 2 or 4 h) extractable Fe was significantly higher ($p > 0.05$) under air-dry conditions as compared with field moist samples demonstrating the inappropriateness of utilizing 1 M NH_4NO_3 as a means of accessing Fe availability on soil samples at field moisture condition. No significant differences in Cd and Zn extracted were observed with 1 M NH_4NO_3 at either 2 or 4 h regardless of whether the sample was air dried or field moist (Table 3). This would suggest that extraction time and sample preparation do not influence the efficiency of extraction with respect to 1 M NH_4NO_3 . Similarly there were no significant differences in pH between air dried or field moist samples (Table 3).

The Cd and Zn concentrations in the leaf, stem and unpolished rice grain are presented in Table 4. Stem material contained the highest mean concentrations of Cd and Zn as well as the widest range in measured values (Table 4). This is in line with the findings of Simmons et al. 2003. There was little difference in Cd concentrations in the leaf and unpolished grain with mean concentrations of 0.94 ± 0.22 and 1.05 ± 0.33 mg Cd kg^{-1} respectively (Table 4). Mean Zn concentration in leaf material was higher than in the unpolished rice grain.

The relatively high concentrations of Cd and Zn within the leaf and stem materials, are potential bioavailable sources as these plant components are often used as animal fodder and bedding by farmers in the area. In addition, rice straw is collected from fields and is extensively used in mushroom production by farmers and could therefore contribute to the over all human dietary Cd intake. The concentration of Cd in unpolished rice exceeded the Codex rice

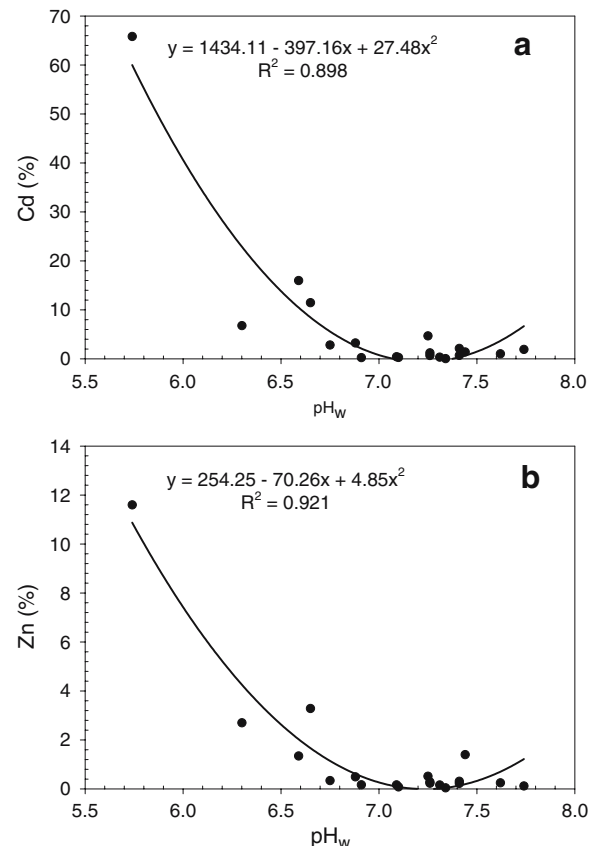


Fig. 5 Relationship between field moist 0.1M CaCl_2 -extractable Cd **a** and Zn **b** as a percentage (%) of aqua regia digestible soil Cd and Zn and soil pH (1:5w)

Table 3 Mean, standard error of the mean and range of Cd, Zn and pH determined on air dried and field moist soil samples collected during grain fill from 20 fields within a Zn/Cd co-contaminated site in Thailand

Soil attribute	Air dry	Field moist	Student <i>t</i> test
<i>0.01 M CaCl₂</i>			
Cd (mg kg ⁻¹)	0.089±0.020 (0.026–0.421)	0.149±0.016 (0.097–0.415)	a
Zn (mg kg ⁻¹)	0.932±0.399 (0.167–7.16)	0.911±0.421 (0.153–8.11)	ns
Fe (mg kg ⁻¹)	Nd	nd	–
<i>0.05 M CaCl₂</i>			
Cd (mg kg ⁻¹)	0.548±0.101 (0.143–2.01)	0.355±0.122 (0.008–2.16)	a
Zn (mg kg ⁻¹)	2.54±1.045 (0.463–17.9)	4.56±1.883 (0.548–35.9)	b
Fe (mg kg ⁻¹)	0.104±0.008 (0.056–0.173)	0.417±0.06 (0.548–1.21)	a
<i>0.10 M CaCl₂</i>			
Cd (mg kg ⁻¹)	1.13±0.150 (0.293–2.91)	0.543±0.171 (0.040–3.05)	a
Zn (mg kg ⁻¹)	3.76±1.371 (0.842–22.9)	6.76±1.815 (0.717–34.9)	b
Fe (mg kg ⁻¹)	0.072±0.008 (0.008–0.136)	1.04±0.195 (0.190–4.12)	a
<i>1 M NH₄NO₃ 2 h</i>			
Cd (mg kg ⁻¹)	0.179±0.019 (0.076–0.424)	0.192±0.026 (0.035–0.571)	ns
Zn (mg kg ⁻¹)	1.73±0.810 (0.012–14.3)	2.19±0.767 (0.038–15.2)	ns
Fe (mg kg ⁻¹)	7.07±2.783 (0.531–38.26)	2.19±0.776 (0.035–9.95)	b
<i>1 M NH₄NO₃ 4 h</i>			
Cd (mg kg ⁻¹)	0.160±0.022 (0.032–0.475)	0.143±0.009 (0.076–0.265)	ns
Zn (mg kg ⁻¹)	1.91±0.817 (0.135–14.9)	2.03±1.032 (0.139–15.9)	ns
Fe (mg kg ⁻¹)	6.02±2.486 (0.225–34.3)	1.81±0.670 (0.010–8.57)	b
pH	6.93±0.12 (5.68–7.59)	7.05±0.11 (5.73–7.74)	ns

nd, non-detectable

^a Student *t* test comparisons between air dry and field moist samples presented as ns (non-significant), or significant at the 5% level of confidence

^b Student *t* test comparisons between air dry and field moist samples presented as ns (non-significant), or significant at the 1% level of confidence

grain Cd concentration of 0.4 mg Cd kg⁻¹ in 14 of the 20 fields sampled, clearly representing a potential threat to human health.

In an effort to assess factors governing the uptake of Cd to stem, leaf and unpolished rice grain, regression analysis was undertaken on extractable Cd, Zn and Fe and soil pH_w for each of the extractants and for both field moist and air dry samples. Due to the strong

collinearity between the variables assessed under a general linear regression model as indicated by variance inflation factors for Cd/Zn, Cd/pH and Zn/pH of 78,494, 2,500 and 13,941, respectively, Stepwise Linear Regression was undertaken (SAS 2005). The Stepwise Linear Regression Model utilized for all extraction methods and soil test portions under both air-dry and field moist condition was as follows

Table 4 Mean concentrations, standard error of the mean and range of Cd and Zn in the stem, leaf and unpolished rice from samples collected at physiological maturity from 20 fields co-contaminated with Zn/Cd in Thailand

Plant component	Cd (mg kg ⁻¹)	Zn (mg kg ⁻¹)
Stem	2.31±1.06 (0.04–16.2)	77.9±10.2 (39.4–199)
Leaf	0.94±0.22 (0.29–4.55)	38.3±2.41 (23.7–67.0)
Unpolished rice grain	1.05±0.33 (0.29–5.83)	23.0±0.46 (19.5–28.5)

Independent variable : Org – C(%) + Clay Content(%)

+ pH_w + Extractable – Cd(mgkg⁻¹)

+ Extractable Zn(mgkg⁻¹)

+ Extractable Fe(mgkg⁻¹).

Where

The independent variable was either Cd concentration (mg kg⁻¹) in rice stem, leaf or unpolished rice grain.

The results indicate that all extractions undertaken on air dried samples were ineffectual in describing the uptake of Cd to rice stem, leaf and un-polished grain (Table 5). For all extractions undertaken on air dried samples no variable met the 0.050 significance level for entry into the model.

In contrast, extractions undertaken on field moist samples were able to describe the uptake of Cd to rice stem, leaf and un-polished grain (Table 5). The results indicate that extractable soil Cd on its own was a poor predictor of Cd concentrations in crop components (Table 5) suggesting that other soil or plant factors are influencing overall uptake of Cd. However, the inclusion of soil pH_w to the model results in substantial increases in the percentage variance accounted for by the model (Table 5). For rice stem the effectiveness of the extractants tested to predict Cd uptake in association with pH_w (1:5) was in the order 0.1 M CaCl₂>

0.05 M CaCl₂>1 M NH₄NO₃ 2 h>1 M NH₄NO₃ 4 h>0.01 M CaCl₂ with *R*² values of 0.670, 0.646, 0.593, 0.498 and 0.481, respectively (Table 5). Similarly, the effectiveness of the extractants tested to predict Cd accumulated in rice leaf in association with pH_w (1:5) was in the order 0.05 M CaCl₂>1 M NH₄NO₃ 4 h>0.1 M CaCl₂>1 M NH₄NO₃ 2 h with *R*² values of 0.540, 0.538, 0.529 and 0.492, respectively (Table 5).

The results demonstrate the comparative effectiveness of the CaCl₂-extractions to predict Cd uptake by rice crops when compared to 1 M NH₄NO₃ for field moist soils.

With regard to un-polished rice grain, only the 0.1 M CaCl₂ extraction in association with pH_w (1:5) was able to predict accumulation of Cd in rice grain with an *R*² value of 0.638. Cadmium accumulation in rice stem and leaf tissue at grain fill reflects Cd uptake over a prolonged growth period under submerged

Table 5 Percentage variance accounted for between varying strengths of CaCl₂ and NH₄NO₃ soil extractible Cd, Zn and pH and the Cd concentration in the leaf, stem and grain of rice using calcium chloride extractions

Dependent Variable	Plant Component								
	Leaf			Stem			Grain		
	Partial <i>R</i> ²	Model <i>R</i> ²	<i>p</i> value	Partial <i>R</i> ²	Model <i>R</i> ²	<i>p</i> value	Partial <i>R</i> ²	Model <i>R</i> ²	<i>p</i> value
<i>0.01 M CaCl₂</i>									
Dry soil	–	–	–	–	–	–	–	–	–
Moist soil									
pH _w	0.2524	0.2524	0.0028	0.2586	0.2586	0.0262	–	–	–
Cd	–	–	–	0.2224	0.4811	0.0186	–	–	–
<i>0.05 M CaCl₂</i>									
Dry soil	–	–	–	–	–	–	–	–	–
Moist soil									
pH _w	0.2524	0.2524	0.0284	0.2586	0.2586	0.0262	–	–	–
Cd	0.2881	0.5405	0.0060	0.3881	0.6468	0.0007	–	–	–
<i>0.1 M CaCl₂</i>									
Dry soil	–	–	–	–	–	–	–	–	–
Moist soil									
pH _w	0.2524	0.2524	0.0284	0.2586	0.2586	0.0262	0.4148	0.4148	0.0006
Cd	0.2769	0.5293	0.0074	0.4113	0.6700	0.0004	0.2232	0.6380	0.0411
<i>1 M NH₄NO₃ 2 h</i>									
Dry soil	–	–	–	–	–	–	–	–	–
Moist soil									
pH _w	0.2524	0.2524	0.0284	0.2586	0.2586	0.0262	–	–	–
Cd	0.2403	0.4927	0.0141	0.3351	0.5937	0.0022	–	–	–
<i>1 M NH₄NO₃ 4 h</i>									
Dry soil	–	–	–	–	–	–	–	–	–
Moist soil									
pH _w	0.2524	0.2524	0.0284	0.2586	0.2586	0.0262	–	–	–
Cd	0.2857	0.5382	0.0062	0.2403	0.4989	0.0237	–	–	–

conditions. In contrast, Cd accumulation in rice grain reflects a much shorter phyto-availability-uptake window that is associated with dynamic shifts in soil pH and redox associated with drainage. The results indicate that 0.1 M CaCl₂ extractable soil Cd in association with pH_w (1:5) accurately reflects Cd phyto-availability at the critical grain fill stage. Strong correlations between stem and rice grain Cd ($R^2=0.861$) and leaf and grain Cd ($R^2=0.812$) may allow the future prediction of grain Cd at earlier growth stages and facilitate the timely application of management options. Further research is required to validate this assumption.

The Stepwise Regression equation that best describes the uptake of Cd to un-polished rice grain is as follows:

$$\begin{aligned} &\text{Unpolished rice grain: Field moist samples; 0.1 M} \\ &\text{CaCl}_2 \\ &\text{Cd grain (mg kg}^{-1}\text{)} = -14.129(\pm 3.439) \\ &\text{Intercept} + 2.044(\pm 0.4775)\text{pH}_w + \\ &1.393(\pm 0.312)\text{ Extr - Cd} \end{aligned}$$

It is of note that although there is a general trend to under-predict grain Cd particularly at high grain Cd concentrations, predicted grain Cd concentrations closely comply with a 1:1 relationship (Fig. 6). The point of intersection between observed and predicted grain Cd occurs at 1.0 mg Cd kg⁻¹. This is comparable to the mean rice grain Cd concentration for the 308 fields sampled during 2001–2002 of 1.15 mg Cd kg⁻¹. Further 85% rice grain samples had

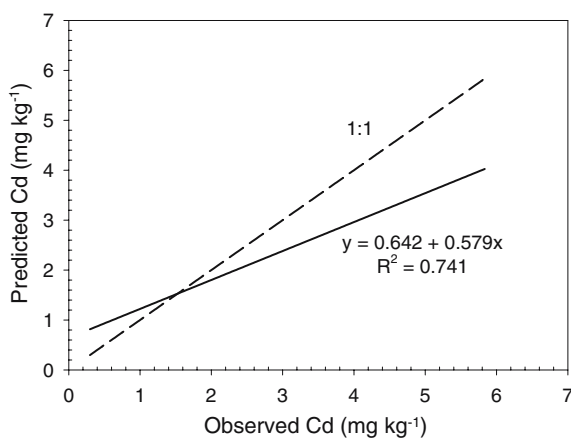


Fig. 6 Relationship between observed and predicted polished rice grain Cd (mg Cd kg⁻¹)

a Cd concentration ranging from 0.02 to <2.0 at which range the prediction is most accurate.

The translocation of Cd and Zn within the rice plant, although as yet not fully understood, is also critical to the accumulation of Cd and Zn in rice during the grain fill stage. Ogawa et al. (1979) detected Zn in phytin globoids at the early ripening stage of rice grain fill indicating a possible metabolic requirement of Zn at this growth stage. Similar observations were made by Welch (1986) and Marschner (1995). Further, Kitagishi and Obata (1981) investigated the accumulation process of ⁶⁵Zn at the early ripening stage and suggested that significant accumulation of ⁶⁵Zn occurred in the endosperm during the period 4 to 14 days after flowering. After this period, the capacity of the rice plant to accumulate ⁶⁵Zn in the endosperm decreased rapidly. Chino (1973) suggests that a direct transport pathway of Cd and Zn from roots to grains is operative at least at the early rice grain ripening stage when accumulation of Cd and Zn in grain is highest. Further, Kitagishi and Obata (1979) demonstrated that the mechanisms operating in rice plants to assimilate trace amounts of Zn from the transpiration stream and transport them actively to sink regions, cannot discriminate Cd ions from Zn ions, resulting in the accumulation of non-essential Cd in sink regions. Chino and Baba (1981) further elucidated the Cd/Zn transport pathway and suggest that Cd and Zn in rice are translocated as organo-metallic complexes in the xylem.

The results indicate that the Stepwise Regression model incorporating 0.1 M CaCl₂ extractable Cd in conjunction with soil pH_w determined on field moist samples accounts for 63.8% of the variability in rice grain Cd (Table 5). In comparison, air-dried soil samples subjected to conventional preparation procedures were not effective in predicting the uptake of Cd by rice plant components, regardless of extractant, and the aforementioned soil variables (Table 5). Intuitively, this is to be expected, as the air-dry sample does not reflect the complex redox/pH interactions associated with the flooded soils at grainfill. Further, this demonstrates the importance of the timing of sample collection and post sample preparation procedures when predicting soil crop interactions. The results confirm that soil pH at field condition during grain filling is a significant factor controlling Cd ‘bioavailability’ in paddy soils.

Conclusions

It is important to note that the elevated rice grain Cd concentrations presented in this paper are in no way indicative of Thai rice as a whole and should be viewed as an isolated case of contamination. The uptake of Cd by rice based production systems in an isolated co-contaminated Zn/Cd rice based production system in the Mae Sot region of Thailand poses a significant health threat to communities that are dependent on these crops for food and income generation. The results from the study clearly demonstrate that when assessing phyto-available Cd, total digestible Cd, which is used to assess the degree of soil contamination, and DTPA extractable Cd which is commonly used to delineate bio-available Cd are ineffective in explaining Cd uptake by rice grain.

From previous studies there is evidence that Cd accumulation in rice grain occurs over a relatively short period during grain fill. Traditionally, farmers often resort to draining their fields during this period in an effort to ensure ease of access during the harvesting stage and to prevent post-harvest grain storage problems. Consequently the soils move from a reduced to an oxidized state with a concomitant change in soil pH, redox and Cd and Zn phyto-availability. It therefore is imperative that soil samples should be collected during this period in order to assess Cd availability for uptake to the grain. In the current study, soil samples were collected during this critical stage and extractions undertaken using neutral salts on both air dried and field moist samples. The inclusion of soil extractable-Cd and pH into a Stepwise Regression model accounted for 63.8% of the variability in rice grain Cd. It is therefore recommended that in order to assess phyto-available Cd and its potential uptake by rice grain in co-contaminated Zn/Cd production systems, the following should be undertaken: Field moist samples should be collected during the grain filling growth stage of the crop; 0.10 M CaCl₂ should be used as the extractant to assess Cd uptake by rice; soil extractable Cd should be determined on the field moist sample along with soil pH; a Stepwise Regression that includes the aforementioned soil attributes should be used to assess the potential risk of rice grain Cd contamination and form the basis for site specific management decisions.

Actively and historically mined Zn and other base metal deposits occur in Vietnam, Myanmar, Lao PDR, Thailand and China. Rice and associated rotation crops are the staple food of the aforementioned countries. It is envisaged that following further cross-regional validation that the 0.10 M CaCl₂ soil extractable Cd soil pH Stepwise Regression model for field moist soils outlined in this paper will form the basis of a cost effective decision support tool to protect public health and export security.

References

- Alloway BJ (1997) Cadmium. In: Alloway BJ (ed) Heavy metals in soils. Blackie Academic and Professional, London, pp 122–151
- Babich H, Strotzky G (1978) Effects of cadmium on the biota: influence of environmental factors. *Adv Appl Microbiol* 23:55
- Bingham FT, Page AL, Strong JE (1980) Yield and cadmium content of rice grain in relation to addition rates of cadmium, copper, nickel with sewage sludge and liming. *Soil Sci* 130:32
- Cai S, Yue L, Shang Q, Nordberg G (1995) Cadmium exposure among residents in an area contaminated by irrigation water in China. *Bull World Health Org* 73(3):359–367
- Canadian Council of Ministers of the Environment (2006) Canadian soil quality guidelines for the protection of environmental and human health: summary tables. (Updated November, 2006). In Canadian environmental quality guidelines, 1999 Canadian Council of Ministers of the Environment, Winnipeg
- Chaney RL, Ryan JA, Li YM, Welch RM, Reeves PG, Brown SL, Green CE (1996) Phyto-availability and bio-availability in risk assessment for cadmium in agricultural environments. In: Sources of cadmium in the environment. OECD, Paris, France, pp 49–78
- Chino M (1973) The distribution of heavy metals in rice plants influenced by the time and path of supply. In: Kitagishi K, Yamane I (eds) Heavy metal pollution in soils of Japan. Japan Scientific Societies Press, Tokyo, pp 95–104
- Chino M, Baba A (1981) The effects of some environmental factors on the partitioning of zinc and cadmium between roots and tops of rice plants. *J Plant Nutr* 3:203–214
- Cui YJ, Zhu YG, Zhai RH, Chen DY, Huang YZ, Qiu Y, Liang JZ (2004) Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. *Environ Int* 30(6):785–791
- European Economic Commission (1986) Council Directive 86/278/EEC on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture, 12 June 1986
- Grant CA, Bailey LD, Harapiak JT, Flore NA (2002a) Effects of phosphorous and Zn fertilizer management on Cd accumulation in flax seed. *J Sci Food Agr* 82:301–308
- Grant CA, Bailey LD, Harapiak JT, Flore NA (2002b) Effect of phosphate source, rate and cadmium content and use of

- Penicillium Bilaii on phosphorous, zinc and cadmium concentration in durum wheat grain. *J Sci Food Agr* 73: 307–314
- Hall GEM, MacLaurin AI, Garrett RG (1998) Assessment of the 1 M NH₄NO₃ extraction protocol to identify mobile forms of Cd in soils. *J Geochem Explor* 64(1–3):153–159
- Houba VJG, Temminghof EJM, Gaikhorst GA, van Vark W (2000) Soil analysis procedure's using 0.01M Calcium Chloride as extraction reagent. *Commun Soil Sci Plan* 31:1299–1396
- Iimura K (1981) Chemical forms and behaviour of heavy metals in soils. In: Kitagishi K, Yamane I (eds) Heavy metal pollution in soils of Japan. Japan Scientific Societies Press, Tokyo, pp 27–35
- JECFA (2005) Report of the 64th Meeting of the JECFA Rome, 8–17 February 2005
- Jin T, Nordberg M, Frech W, Dumont X, Bernard A, Ye TT, Kong Q, Wang Z, Li P, Lundstrom NG, Li Y, Nordberg GF (2002) Cadmium bio-monitoring and renal dysfunction among a population environmentally exposed to cadmium from smelting in China (ChinaCad). *Biometals* 15(4): 397–410
- Kido T, Honda R, Tsuritani I, Yamaya H, Ishizaki M, Yamada Y, Nogawa K (1988) Progress of renal dysfunction in inhabitants environmentally exposed to cadmium. *Arch Environ Health* 43:213–217
- Kitagishi K, Obata H (1979) Distribution of 115mCd in rice plants absorbed by roots at vegetative stage. *Rep Environ Sci Mie Univ* 4:59–65
- Kitagishi K, Obata H (1981) Accumulation of heavy metals in rice grains. In: Kitagishi K, Yamane I (eds) Heavy metal pollution in soils of Japan. Japan Scientific Societies Press, Tokyo, pp 27–35
- Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Sci Soc Amer J* 42:421–428
- Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London
- McGrath SP, Cunliffe CH (1985) A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Cd, Pb, Cr, Co and Mn from soils and sewage sludges. *J Sci Food Agr* 36: 794–798
- Nakashima K, Kobayashi E, Nogawa K, Kido T, Honda R (1997) Concentration of cadmium in rice and urinary indicators of renal dysfunction. *Occup Environ Med* 54:750–755
- Nelson DW, Sommers LE (1996) Carbon and organic matter. In: Sparks DL (ed) Methods of soil analysis part 3, chemical methods. Soil Sci Soc Am and Am Soc Agron. Madison, Wisconsin, pp 961–1010
- NEPC (1999) National environment protection (assessment of site contamination) measure schedule B (1) guideline on the investigations levels for soil and groundwater. National Environment Protection Council, Australia
- Nogawa K, Kido T (1993) Biological monitoring of cadmium exposure in Itai-itai disease epidemiology. *Int Arch Occ Env Hea* 63:43–46
- Nogawa K, Yamada Y, Honda R, Ishizaki M, Tsuritani I, Kawano S, Kato T (1983) The relationship between Itai-itai disease among inhabitants of the Jinzu River basin and cadmium in rice. *Toxicol Lett* 17:263–266
- Nordberg G (2003) Cadmium and human health: a perspective based on recent studies in China. *J Trace Elem Exp Med* 16(4):307–319
- Norvell WA (1984) Comparison of chelating agents for metals in diverse soil materials. *Soil Sci Soc Am J* 48:1285–1292
- Ogawa M, Tanaka K, Kasai Z (1979) Energy-dispersive X-ray analysis of phytin globoids in aleurone particles of developing rice grains. *Soil Sci Plant Nutr* 25:437–448
- Page AL, Bingham FT, Chang AC (1981). In: Lepp NW (ed) Effect of heavy metal pollution on plants, vol. 1. Applied Science, London, pp 72–109
- Pongsakul P, Attajarusit S (1999) Assessment of heavy metals in soils. *Thai J Soil Fert* 21:71–82. (Thai)
- Rayment GE, Higginson FR (1992) Electrical conductivity and soil pH. In: Rayment GE, Higginson FR (eds) Australian laboratory handbook of soil and water chemical analysis. Inkata, Melbourne, pp 15–23
- Saito H, Shioji R, Hurukawa Y, Nagia K, Arikawa T, Saito T, Sasaki Y, Furuyama T, Yoshinaga K (1977) Cadmium-induced proximal tubular dysfunction in a cadmium-polluted area. *Contrib Nephrol* 6:112
- SAS (2005) version 9.1.3. SAS Institute Inc. Cary NC, USA
- Simmons RW, Pongsakul P (2004) Preliminary stepwise multiple linear regression method to predict cadmium and zinc uptake in soybean. *Comm Soil Sci Plan* 35(13&14):1815–1828
- Simmons RW, Pongsakul P, Chaney RL, Saiyisitpanich D, Klinphoklap S, Nobuntou W (2003) The relative exclusion of zinc and iron from rice grain in relation to rice grain cadmium as compared to soybean: Implications for human health. *Plant Soil* 257:163–170
- Simmons RW, Pongsakul P, Saiyisitpanich D, Klinphoklap S (2005) Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice grain downstream of a zinc mineralized area in Thailand: implications for public health. *Environ Geochem Health* 27(5–6):501–511
- Tsuritani I, Honda R, Ishizaki M, Yamada Y, Kido T, Nogawa K (1992) Impairment of vitamin D metabolism due to environmental cadmium exposure, and possible relevance to sex-related differences in vulnerability to bone damage. *Jpn J Tox Env Health* 37:519–533
- Walkley A (1947) A critical examination of a rapid method for determining organic carbon in soils: effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sci* 63:251–263
- Welch RM (1986) Effects of nutrient deficiencies on seed production and quality. *Adv Plant Nutr* 2:205–247
- Wu W, Jin T, Wang Z, Ye T, Kong Q, Nordberg G (2001) Urinary calcium as a biomarker of renal dysfunction in a general population exposed to cadmium. *J Occup Environ Med* 43(10):898–904
- Zachara JM, Smith SC, Resch CT, Cowan CE (1992) Cadmium sorption on silicates and oxides. *Soil Sci Soc Am J* 56: 1074–1084
- Zarcinas BA, Pongsakul P, McLaughlin MJ, Cozens G (2003) Heavy metals in soils and crops in South East Asia. 2. Thailand. *Environ Geochem Health* 162–02:1–13