Development of a rational sampling method for evaluation of Cd concentration in wheat (*Triticum aestivum*) grain and soil

Naomi Aoyama[A], Genki Kobori[B], Masanori Okazaki[B], Takashi Motobayashi[C] and Masaharu Murakami[D]

[A]Tokyo University of Agriculture and Technology, 3-5-8,Saiwai-cho, Fuchu, Tokyo 183-8509,Japan,50006153003@st.tuat.ac.jp
[B]Graduate School of Bio-Applications and System Engineering, Tokyo University of Agriculture and technology, 2-24-16, Naka-cho, Koganei, Tokyo 184-8588, Japan
[C]Field Science Center for Education and Resarch, Tokyo University of Agriculture and Techonology, 3-7-1, Hommachi, Fuchu, Tokyo 183-0027, Japan
[D]National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki 305-8604 Japan

Abstract
The rational method of sampling for the evaluation of Cd concentrations in wheat (*Triticum aestivum)* (Norin No. 61 and Ayahikari cultivar) grain and soil was investigated. Wheat grain and soil samples were collected from Hommachi Farm and Saiwaicho Farm, Tokyo University of Agriculture and Technology (TUAT). Soil of Hommachi is Gray Lowland Soils (Haplaquept) and soil of Saiwaicho is Cumulic Andosols (Melanudand). Hommachi Farm was polluted by cadmium in 1970's and contained higher cadmium concentration than Saiwaicho Farm. The intersections of the 5 meter mesh of the field were used for sampling sites. Sampling sites of 5, 9 and 32 or 120 for wheat grain and soil were prepared. The concentration of Cadmium was determined by ICP-MS. There was no significant difference of the mean values by the sampling numbers and sampling methods of wheat grain and soil, although increasing number of sampling sites decrease in the 95% confidence interval. The variation of the Cd concentration in Hommachi Farm was larger than that in Saiwaicho Farm. The error (percent) (sampling error / mean value x 100) at the probability level of 95 % decreased with increasing the number of wheat grain and soil sampling. For wheat grain, more than 30 sampling at Hommachi Farm and more than 100 sampling at Saiwaicho Farm provided the acceptable error value of less than 10 %. Meanwhile, for soil sample, 5 sites sampling at Hommachi Farm and 9 sites sampling at Saiwaicho Farm gave the acceptable error value of less than 10 %. It can be recommended that more than 30 wheat panicles at the place with high risk of cadmium pollution are collected from whole field and soil sampling is done at up to 5 sites.

Key Words
Cadmium, wheat, sampling method.

Introduction
Cadmium (Cd) is one of the most toxic and carcinogenic elements. Long term exposure to Cd even at low concentration affects human body, as it is accumulated in the kidney. Cadmium intake source for human is mainly from foods. Humans eat crops and vegetables which take up and accumulate Cd from soil. It is important to control Cd concentration in cereals. The Codex Committee (Codex, 2005) adopted 0.2 mg kg $^{-1}$ FW (Fresh Weight) of cadmium concentration in wheat grain in 2005, based on 7 µg per kg weight per week of Provisional Tolerable Weekly Intake (PTWI) after long discussion in Joint FAO/WHO Expert Committee on Food Additives (JECFA). Wheat, a major cereal for many areas, can absorb more Cd than rice when cultivated in the same field (Kikuchi *et al.* 2007). There were several reports on prediction of Cd concentration in wheat by total/extractable soil Cd concentration and pH using a regression analysis (Kuboi *et al.* 1986; Kuboi *et al.* 1987; Eriksson 1990; Singh *et al.* 1995; Robert *et al.* 1998; Gray *et al.* 2001; Ibaraki 2003; Mench and Baize 2003; Adams *et al.* 2004; Kusa *et al.* 2005; Spiegel *et al.* 2006; Matsumoto *et al.* 2007; Römkens *et al.* 2009). The objectives of this study are to show the relationship between Cd concentration in wheat grain and soil at two different fields of Hommachi Farm (Haplaqept) and Saiwaicho Farm (Melanudand) which hold different Cd levels and to propose the rational sampling method for evaluation of Cd concentration in wheat grain and soil.

Methods
Site description
This study was conducted at two Farms, Field Science Center for Education and Research of TUAT. Hommachi Farm is located on an alluvial lowland plain of Tama river in Fuchu City, Tokyo (35°39'N, 139°25'E) and consists of 13 rice paddy plots with a total area of 2.4 ha. It was reported in 1970's that the irrigation water to this farm derived from the Fuchu Irrigation Canal had been polluted by Cd from industrial

effluents (Fuchu City 1978).Since then, the ground water from a 150-m depth has been used for irrigation (Saito and Shimoda 1984; Okazaki and Saito 1989). Saiwaicho Farm, also is located in Fuchu City, has soil derived from volcanic ash.

Cultivation practices of winter wheat

In 2009, 32 sites of intersections 5m×5m at Hommachi Farm and 120 sites at Saiwaicho Farm were used for sampling (Figure 1). The fertilizer rate of N: P_2O_5: K_2O = 40: 40: 40 kg/ha and 14: 14: 14 kg/ha were applied at Hommachi Farm and Saiwaicho Farm on the 18th of November, 2008. The application rate of fertilizer was 50% of the standard fertilization amount in Tokyo. *Triticum aestivum L.* cv.Norin No.61and *Triticum aestivum L.* cv.Ayahikari ware sown at Hommachi on the 1st of December and Saiwaicho Farm on the 2nd of October, 2008.

Sampling and analysis

The soil samples were taken from 120, 32, 9 and 5 sites in the field by an auger. After air-drying soil samples were passed through a 2mm nylon sieve. Five gram of soil sample was treated with 25 mL of 0.1 mol L^{-1} hydrochloric acid solution and the mixture was shaken by a mechanical end-over-end shaker (Daiki, DIK 2102) at 25ºC. The extract was filtered with a Whatman No.5 C filter paper and cadmium concentration in the filtrate was determined by an atomic absorption spectrophotometer (Hitachi Z-5010) with a flame at 228.8 nm. The grain samples of wheat obtained from the selected sampling at 120, 32, 9 and 5 sites were washed with tap water and deionized water, dried at 70 ºC for 48 hours. These were ground with a ball mill (MM301, Retsch, Haan, Germany) for subsequent Cd analysis. The ground samples were digested using concentrated HNO_3, H_2O_2 and H_2O by a microwave apparatus, and the Cd concentration in the digests was determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS/Thermo X-SERIES II, Japan). Statistical analysis by Student's t-test using JMP 8 program (SAS Institute Inc., Cary, NC, USA)

Results

Suggestion of sampling methods

The yields of wheat were 3.0 t ha^{-1} in Hommachi Farm (Norin No.61) and 4.2 t ha^{-1} in Saiwaicho Farm (Ayahikari). The moisture content of wheat grain was 13.5 % and 12.5 %, respectively.
The mean, median, standard deviation, coefficient of variation, standard error and variation of 95% confidence interval of Cd concentrations in wheat grain and soil at two Farms are shown in Table.1and 2. The increasing number of sampling plots of wheat grain and soil showed no difference in mean value of soil Cd concentration. To increase the confidence level (decrease in standard error), the increase in number of sampling was necessary (Table 1and 2).

Relationship between Cd concentration in wheat grain and extractable Cd concentrations in soil

The relationship between Cd concentration in grain of two cultivars and extractable Cd concentration in soil is shown in Figure 2. Cd concentration in wheat grain increased with increasing Cd concentration in soil, even though cultivars and soils were different. This coincided with the results that there were positive correlations between Cd concentration in wheat grain and soil (0.025 mol/L HCl as an extraction solution) (Ibaraki, 2003). Yoshida and Sugito (2007) proposed that 0.01 mol/L HCl extraction gave a good variable to predict Cd concentration in wheat grain. While Eriksson (1990) reported that 2 mol/L HNO_3 extractable Cd concentration in soil should be recommended to predict Cd concentration in edible part using multiple regression analysis.

Sampling number and reliability of data

The relationship between the number of sampling and sampling error (D / mean value x 100) was calculated, based on Table 1 and 2 according to Yanai *et al.* (2008). The variance (s^2) had been given by standard deviation (s). The equation using confidence interval (L), mean (M), number of sampling (N) and probability of 95 % (t_α) (N-1 degree of freedom) was introduced from t-test for estimation of interval of mean value.

$$L = M \pm t_\alpha s / N^{0.5} \quad (1)$$

The precision for estimating error (D) of sampling number was calculated according to the following equation

$$D = t_\alpha s / N^{0.5} \quad (2)$$

The relationship between the sampling number and the error from equation (1) and (2) is shown in Figure 3, indicating that increasing sampling number made the error small. For wheat grain sampling number should be required to be more than 30 at Hommachi Farm and more than 100 at Saiwaicho Farm. In case of the soil, the error at the probability level of 95% was 7.98 % for sampling number of 5, 3.92 % for sampling number of 9 and 2.55 % for sampling number of 32. It suggested that less than 10% of the error was often found in the mean value of Cd concentration in soil when more than 9 sampling of soil was carried out. Considering that the error in the number of sampling was within 5 %, soil sampling was acceptable to be less than 5 in Hommachi Farm and 9 sites in Saiwaicho Farm.

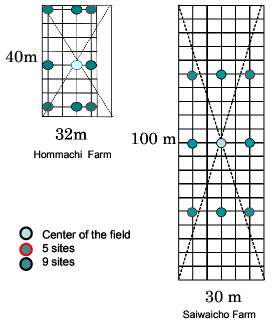

Figure 1. The arrangement of the sites in the experiment field (Hommachi Farm and Saiwaicho Farm).

Table 1. Cd concentration in wheat grain and its variation at Hommachi and Saiwaicho Farm.

Number of sites	Hommachi Farm			Saiwaicho Farm		
	32	9	5	120	9	5
Mean	0.18	0.18	0.20	0.011	0.013	0.012
Median	0.18	0.18	0.18	0.010	0.010	0.010
SD.	0.037	0.064	0.074	0.0048	0.0075	0.0065
CV (%)	20.3	36.0	37.4	43.6	57.7	54.2
Standard Error	0.0064	0.021	0.033	0.00044	0.0025	0.0029
95% Confidence Interval	0.18±0.0131	0.18±0.0480	0.20±0.0850	0.011±0.000867	0.013±0.00566	0.012±0.00748

Table 2. Cd concentration in soil and its variation at Hommachi and Saiwaicho Farm.

	Hommachi Farm			Saiwaicho Farm		
Number of sites	32	9	5	120	9	5
Mean	0.99	0.98	0.98	0.10	0.10	0.11
Median	0.99	0.99	0.99	0.098		
SD.	0.07	0.05	0.063	0.014	0.012	0.013
CV (%)	7.00	5.10	6.40	14.4	11.8	12.8
Standard Error	0.0124	0.0167	0.0282	0.0013	0.0040	0.0058
95% Confidence Interval	0.99±0.025	0.98±0.039	0.98±0.078	0.10±0.0025	0.10±0.0095	0.11±0.016

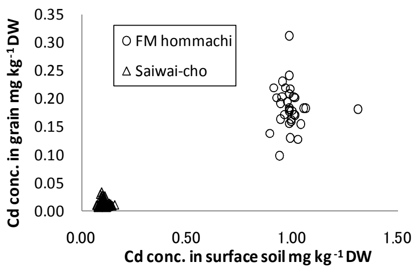

Figure 2. The relationship between Cd concentration in wheat grain and soil.

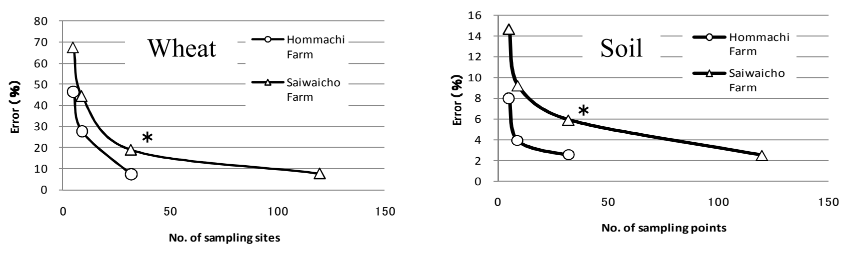

Figure 3. The relationship between the estimation error and number of sampling sites in soil and wheat ∗32 sites were randomly selected in Saiwaicho Farm.

Conclusion

The error at probability level of 95 % remarkably decreased with increasing number of sampling. For wheat grain the panicle sampling of more than 30 at the field with high risk of Cd pollution is performed from the whole filed by walking along the row. Five sampling sites for soil in the field are prepared to evaluate high risk of Cd pollution.

Reference

Adams ML, Zhao FJ, McGrath SP, Nicholson FA, Chambers BJ (2004) Predicting cadmium concentrations in wheat and barley grain using soil properties. *Journal of Environmental Quality* **33**, 532-541.

Codex (2005) Report of the 37th session of the Codex Committee on Food Additives and Contaminants. Codex Alimentarius Commission 25, Alinorm.

Eriksson JE (1990) A field study on factors influencing Cd levels in soils and in grain of oats and winter wheat. *Water, Air, and Soil Pollution* **53,** 69-81.

Fuchu City (1978) Environmental Pollution in Fuchu City, Musashi-Fuchu Sosho No.6, p.75-80,Fuchu City.

Gray CW, McLaren RG, Roberts AHC, Condron LM (1999) Cadmium phytoavailability in some New Zealand soils. *Australian Journal of Soil Research* **37,** 461-477.

Gray CW, MaLaren RG, Roberts AHC (2001) Cadmium concentrations in some New Zealand wheat grain. *New Zealand J. Crop and Horticultural Science* **29**, 125-136.

Ibaraki T (2003) Evaluation of bio-available cadmium for wheat. *Promising Agricultural Practices Technologies for Reducing Heavy Contamination in Relevant Staple crops With a Special Emphasis on Cadmium Contamination* **23**, 98-99.

Kikuchi T, Okazaki M,Toyota K, Motobayashi T, Kato M (2007) The input–output balance of cadmium in a paddy field of Tokyo. *Chemosphere* **68**, 920-927.

Kikuchi T, Okazaki M, Motobayashi T (2009) Suppressive effect of magnesium oxide materials on cadmium accumulation in winter wheat grain cultivated in a cadmium-contaminated paddy field under annual rice–wheat rotational cultivation. *Journal of Hazardous Materials* **168**, 89-93.

Kuboi T, Noguchi A, Yazaki J (1986) Family-dependant cadmium accumulation characteristics in higher plants. *Plant and Soil* **92**, 405-415.

Kuboi T, Noguchi A. Yazaki J (1987) Relationship between tolerance and accumulation characteristics of cadmium in higher plants. *Plant and Soil* **104**, 275-280.

Kusa K, Hatta K, Hara Y, Tsuchiya K (2005) Variental difference in concentration and location of cadmium in wheat and barley grain. Report of Kyushu Agriculture **67**, 46.

Matsumoto T, Kara H, Higasa Y (2007) Evaluation of pollution risk of cadmium in crops by chemical characteristics of arable soils as an indicator. Report Sophisticated Project of Ministry of Agriculture, Forestry and Fisheries, pp. 3. (MAFF:Tsukuba).

Mench M, Baize D (2003) Trace element distribution in relevant arable soils and staple crops in France. *Promising Agricultural Practices Technologies for Reducing Heavy Contamination in Relevant Staple crops With a Special Emphasis on Cadmium Contamination* **23**, 8-12.

Garrett RG, MacLaurin AI, Gawalko EJ, Tkachuk R, Hall GEM (1998) A prediction model for estimating the cadmium content of duram wheat from soil chemistry. *Journal of Geochemical Exploration* **64**, 101-110.

Römkens PFAM, Guo HY, Chu CL, Liu TS, Chiang CF, Koopmans GF (2009) Prediction of Cadmium uptake by brown rice and derivation of soil–plant transfer models to improve soil protection guidelines. *Environmental Pollution* **157**, 2435-2444.

Singh BR, Narwal RP, Jeng AS, Almas A (1995) Crop uptake and extractability of cadmium in soils naturally high in metals at different pH levels. Commun. *Soil Science and Plant Analysis* **26**, 213-2142.

Spiegel H, Dersch G, Oberforster M, Mechtier K, Baumgarten A (2006) Cadmium in Austrian wheat and potatoes - an inventory of sites and varieties. Abstract of 18th World Congress of Soil Science, pp. 168-17, July 9-15, 2006, Philadelphia, USA

Yanai J, Matsubara T, Lee CK, Moritsuka N, Shinjo H, Kosaki T (2008) Establishment of Rational Sampling Science for the Paddy Field-Relationship between Sampling frequency and Data Reliability- , Japanese Society of Soil Science and Plant Nutrition **79**, 61-67 (in Japanese).

Yoshida K, Sugito T (2007) Determination method of cadmium highly correlated to crop uptake. Report Sophisticated Project of Ministry of Agriculture, Forestry and Fisheries, pp. 3. (MAFF:Tsukuba).