Effects of Ammonium Chloride Fertilizer and its Application Stage on Cadmium Concentrations in Wheat (*Triticum aestivum* L.) Grain

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Abstract: Chloride (Cl) in saline soil increases the cadmium (Cd) concentration in crops. Here, we conducted a field experiment to investigate changes in Cd concentrations in wheat grain after the application of the Cl-containing fertilizer ammonium chloride (NH₄Cl), with the aim of reducing its potential health risk. Effects of the application stage of NH₄Cl fertilizer and leaching treatment (i.e., heavy rainfall) were also investigated in field and pot experiments. Both field and pot experiments showed that the Cd concentration of wheat grain was higher with NH₄Cl fertilizer than with ammonium sulfate or urea fertilizers. Grain Cd concentration in wheat fertilized with NH₄Cl at the tillering–jointing and flowering stages in the field experiment was 0.223 mg kg⁻¹, which was about 1.5 times higher than that fertilized with urea. This finding is important because, in Japan, compound fertilizers containing NH₄Cl are commonly used in fields for wheat cultivation. NH₄Cl fertilizer application at the tillering–jointing and flowering stages had nearly equal effects on the Cd concentration in wheat grain. Basal dressing with NH₄Cl fertilizer increased Cd concentrations in wheat grain to a greater extent than topdressing (at the tillering–jointing and flowering stage applications) in a pot experiment that was protected from rain. Leaching treatment (assuming two lots of 100 mm rainfall) negated the effect of NH₄Cl fertilizer application on Cd concentration in wheat grain. We recommend the use of ammonium sulfate or urea preferentially as the nitrogen fertilizer because heavy rainfall rarely occurs during this period in Japan.

Key words: Ammonium chloride, Cadmium, Leaching, Wheat.

Cadmium (Cd) is an element that is highly toxic to humans, and it is mainly acquired from food. In 2005, the maximum permissible Cd concentration in wheat grain was set at 0.2 mg kg⁻¹ globally (Codex Alimentarius Commission, 2005a, 2005b). According to a survey on Cd concentrations performed by the Ministry of Agriculture, Forestry and Fisheries of Japan in 2000 – 2002, the Cd concentration in wheat grain harvested in Japan was 0.072 mg kg⁻¹ on average, with a standard deviation of 0.057 mg kg⁻¹, and a maximum concentration of 0.470 mg kg⁻¹ (WHO and FAO, 2006). The Cd concentration in wheat grain is influenced by certain properties, including soil pH (Singh et al., 1995; Oliver et al., 1998; Adams et al., 2004), nitrogen fertilizer application (Mitchell et al., 2000; Wångstrand et al., 2007; Gao et al., 2010; Perilli et al., 2010; Li et al., 2011), soil Cd concentration (Ibaraki et al., 2005), and wheat cultivars (Kubo et al., 2008). Of these properties, soil salinity, particularly soil chloride (Cl) concentration, is an important factor that influences the accumulation of Cd in various crops, such as sunflower, potato, Swiss chard, wheat, kenaf, and corn (Li et al., 1994; McLaughlin et al., 1994; Smolders and McLaughlin, 1996; McLaughlin et al., 1997; Smolders et al., 1998; Norvell et al., 2000; Weggler-Beaton et al., 2000; Wu et al., 2002; Weggler et al., 2004; Hattori et al., 2006; Ghallab and Usman, 2007). These studies indicated that increasing Cl concentration in the soil promotes the formation of CdCl₂, which increases Cd solubility in the soil and, hence, Cd uptake by plants.

Given that soil Cl increases the uptake of Cd by plants, it is expected that fertilizers containing ammonium chloride (NH₄Cl) influence Cd concentrations in wheat. Matsuyama et al. (2003) conducted a pot experiment and showed that NH₄Cl fertilizer significantly increases Cd concentrations in the shoots of *Brassica napus* L. Perviridis Group and *Spinacia oleracea* L. compared with urea or...
ammonium sulfate fertilizers. Ohtani et al. (2007) showed that NH₄Cl fertilizer significantly increased Cd concentrations in rice and spinach shoots grown in Entisol-filled pots compared with other nitrogen fertilizers, such as ammonium sulfate, ammonium nitrate, and ammonium dihydrogen phosphate. However, there was little difference in the Cd concentration in barley shoots among the four ammonium salts. Further, Cd concentrations in rice, spinach, and barley grown in pots filled with Andisol were similar in all four ammonium salts. Zhao et al. (2010) used a pot experiment to show that the application of NH₄Cl fertilizer increased Cd concentration in wheat more than the application of ammonium sulfate, ammonium nitrate, or urea fertilizers. Moreover, Jiaka et al. (2010) obtained the same results with rice. To the best of our knowledge, field experiments evaluating the effects of NH₄Cl fertilizer application on Cd concentrations in wheat grain have not yet been reported, nor has the influence of the application stage of NH₄Cl fertilizer been investigated.

In Japan, compound fertilizers containing NH₄Cl are widely used on crops, including wheat. Therefore, it is important to assess the effects of NH₄Cl fertilizer application on Cd concentrations in wheat grain, with the aim of reducing any potential health risks. In this study, we compared the concentrations in wheat grain, with the aim of reducing the amount of leaching caused by rainfall, on the Cd concentrations in wheat grain.

Materials and Methods

1. Field experiment

We conducted a field experiment in paddy fields (640 m²) at the NARO Western Region Agricultural Research Center, Japan (N34°30', E133°23', 1 m above sea level). The field soil was a fine-textured (light clay) Gray Lowland soil. At the experimental site, paddy rice was cultivated uniformly before wheat cultivation. Subsequently, the hard red wheat (Triticum aestivum L.) cultivar ‘Setokirara’ was sown by drill seeding at 30-cm row spacing and at a sowing rate of 6 g m⁻² (140 seeds m⁻²) on 25 November 2011. The wheat plants were harvested at crop maturity on 4 June 2012. Temperature and precipitation data during the wheat cultivation period are shown in Table 1. The experimental design was a randomized complete block with four replicates.

All treatments were applied with the same basal dressing: 150 g m⁻² magnesium lime (CaCO₃ + MgCO₃) on 9 November 2011, followed by 6.8 g m⁻² N as ammonium sulfate and ammonium phosphate, 8.6 g m⁻² P₂O₅ as ammonium phosphate, and 6.8 g m⁻² K₂O as potassium chloride at sowing on 25 November 2011. The basal dressing (N, P₂O₅, and K₂O) was applied as NPK compound fertilizer “Aladdin 484.” All treatments were top-dressed with the same amount of N as NH₄Cl or urea. Specifically, 5.5 g m⁻² N was applied at the tillering and jointing stages (termed “tillering–jointing stage” hereafter) (5 February and 14 March 2012) and 6.5 g m⁻² N was applied at the flowering stage (28 April 2012). Five types of topdressing fertilizers were applied: (A) urea at all stages, (B) urea + potassium chloride at all stages, (C) NK compound fertilizer C54 (NKC54) at the tillering–jointing stage and urea at the flowering stage, (D) urea at the tillering–jointing stage and NKC54 at the flowering stage, and (E) NKC54 at all stages. The fertilizer, NKC54, contained 15% N as NH₄Cl, 10% K₂O as potassium chloride, and 4% K₂O as potassium silicate. All fertilizers were surface broadcast. Application details are provided in Table 2.

Soil samples were collected from the rooting zone (0 – 30 cm soil depth) during the grain filling period, nine days after the flowering stage. The samples were air-dried at 40°C, ground, and passed through a 2-mm sieve. At crop maturity on 4 June 2012, the wheat plants were harvested at ground level and air-dried. After the moisture of the grains had decreased to < 13.5%, the plants were threshed and the grains and straw (which comprised the culm, leaf sheath, and rachis) were dried in an oven at 40°C. The straw was ground with a Wiley mill.

2. Pot experiment

Soil was collected from the plow layer (0 – 15 cm depth) of a paddy field at the NARO Western Region Agricultural Research Center after rice harvest in November 2012. The soil was a fine-textured (light clay) Gray Lowland soil. The contents of water-extractable Cl and 0.1 mol L⁻¹ HCl-extractable Cd were 94 and 0.13 mg kg⁻¹, respectively. Approximately 4.2 kg of the wet soil (dry weight of approximately 3.3 kg) was put in each pot, with a 200 cm² soil surface area and 3.2 L soil volume. Water-extractable Cl was 94 mg kg⁻¹ × 3.3 kg pot⁻¹ = 310 mg pot⁻¹, and 0.1 mol L⁻¹ HCl-extractable Cd was 0.15 mg kg⁻¹ × 3.3 kg pot⁻¹ = 0.43 mg pot⁻¹ before sowing. Before put in the pot, the soil was fertilized with 0.38 g pot⁻¹ of P₂O₅ as magnesium

<table>
<thead>
<tr>
<th>Month and year</th>
<th>Average temperature (°C)</th>
<th>Precipitation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>December 2011</td>
<td>6.4</td>
<td>24</td>
</tr>
<tr>
<td>January 2012</td>
<td>4.0</td>
<td>7</td>
</tr>
<tr>
<td>February 2012</td>
<td>3.6</td>
<td>71</td>
</tr>
<tr>
<td>March 2012</td>
<td>8.0</td>
<td>98</td>
</tr>
<tr>
<td>April 2012</td>
<td>14.2</td>
<td>72</td>
</tr>
<tr>
<td>May 2012</td>
<td>18.9</td>
<td>67</td>
</tr>
</tbody>
</table>
No water ran to maintain soil moisture tension at pH 1.8 – 2.4, except for December 2012. The pots were placed outside under a shade. ‘Setokirara’ were seeded in each pot on 3 December 2012. As the topdressing, 2.5 g m$^{-2}$ fertilizer treatments, except for the flowering-stage NH$_4$Cl was not applied, ammonium sulfate was applied. NH$_4$Cl at flowering stage (10 and 21 April). When NH$_4$Cl at flowering stage (28 March) stages. Water ran down through the soil, and excess water (approximately 1.7 L) ran out of the pots within 17 h. The leachate was collected and analyzed. Pots without leaching treatment were irrigated with the same amount of water as that retained by treated pots (0.3 L).

Four different NH$_4$Cl treatments were applied: (1) without NH$_4$Cl at all stages, (2) with NH$_4$Cl at germinating stage (25 December), (3) with NH$_4$Cl at the tillering–jointing stage (9 February and 11 March), and (4) with NH$_4$Cl at flowering stage (10 and 21 April). When NH$_4$Cl was not applied, ammonium sulfate was applied. NH$_4$Cl and ammonium sulfate were of fertilizer grade. At each stage, 0.22 g nitrogen per pot was applied. Each treatment, including the leaching treatments, was replicated 6 – 10 fold (with 6 – 10 pots per each treatment).

The leaching treatments were combined with the fertilizer treatments, except for the flowering-stage NH$_4$Cl treatment. The leaching treatment might drain off both Cl and nitrogen from the soil, with nitrogen deficiency causing retarded wheat growth. To avoid growth retardation, the leaching treatments were conducted just before the subsequent application of fertilizer. At the experimental site, the maximum precipitation in a given rainfall event during wheat cultivation period in 2005 – 2012 was approximately 100 mm, corresponding to 2 L per pot. Thus, the same quantity of water was added to each pot at the pre-tillering (29 January) and pre-flowering (28 March) stages. Water ran down through the soil, and excess water (approximately 1.7 L) ran out of the pots within 17 h. The leachate was collected and analyzed. Pots without leaching treatment were irrigated with the same amount of water as that retained by treated pots (0.3 L).

On 25 May 2013, after the crop had reached maturity, the wheat plants were harvested at ground level and processed as part of the field experiment. Soil samples were collected after the wheat was harvested and were passed through a 2-mm sieve. The moisture of these soil samples was approximately 16% by weight. Analyses were conducted on each pot.

### 3. Analytical procedure

Soil pH was measured using a glass electrode in a settled 1:2.5 (field experiment) or 1:5 (pot experiment) soil/water (w/w) suspensions (dry matter weight basis). Soil electrical conductivity (EC) was measured using a compact conductivity meter (B-771, HORIBA, Ltd., Kyoto, Japan) in a 1:5 soil/water (w/w) extract. Soil Cl was measured using an ion chromatography system (TOSOH IC-2010 equipped with a TSKgel SuperIC-AZ column, Tosoh Corporation, Tokyo, Japan) in a 1:5 soil/water (w/w) extract. Soil Cd extracted with 0.1 mol L$^{-1}$ HCl was determined using inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer ELAN DRC II, PerkinElmer Japan Co., Ltd., Yokohama, Japan). The Cl

### Table 2. Effects of applied fertilizer on soil, wheat straw, and wheat grain properties in a field experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Applied fertilizer</th>
<th>Cl in fertilizer (g m$^{-2}$)</th>
<th>Soil</th>
<th>Straw</th>
<th>Grain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal dressing</td>
<td>Tiller stage – jointing stage</td>
<td>Flowering stage</td>
<td>Total</td>
<td>pH</td>
</tr>
<tr>
<td>A</td>
<td>Urea</td>
<td>5.0</td>
<td>0.0</td>
<td>0.0</td>
<td>5.0</td>
</tr>
<tr>
<td>B</td>
<td>Urea + KCl</td>
<td>5.0</td>
<td>3.9</td>
<td>4.6</td>
<td>13.5</td>
</tr>
<tr>
<td>C</td>
<td>NKC54</td>
<td>5.0</td>
<td>16.7</td>
<td>0.0</td>
<td>21.7</td>
</tr>
<tr>
<td>D</td>
<td>Urea</td>
<td>5.0</td>
<td>0.0</td>
<td>19.7</td>
<td>44.7</td>
</tr>
<tr>
<td>E</td>
<td>NKC54</td>
<td>5.0</td>
<td>16.7</td>
<td>19.7</td>
<td>44.7</td>
</tr>
</tbody>
</table>

ANOVA

KCl: potassium chloride fertilizer.

NKC54 is “NK compound fertilizer C54” containing 15% N as NH$_4$Cl, 10% K$_2$O as KCl, and 4% K$_2$O as potassium silicate.

As the basal dressing, 6.7 g m$^{-2}$ N as ammonium sulfate and ammonium phosphate, 8.6 g m$^{-2}$ P$_2$O$_5$, as ammonium phosphate, and 6.7 g m$^{-2}$ K$_2$O as KCl were applied to all treatments.

As the topdressing, 2.5 g m$^{-2}$ N was applied at the tillering stage (5 February), 3.0 g m$^{-2}$ N was applied at the jointing stage (14 March), and 6.5 g m$^{-2}$ N was applied at the flowering stage (28 April) of all treatments. The amount of K in the KCl applied for treatment B was the same as the amount of K in the NKC54 applied for treatment E.

Soil samples were collected at the grain filling period, nine days after the flowering stage, from 0 – 30 cm soil depth. Straw and grain samples were collected after harvest.

Values followed by the same letters within a column are not significantly different based on Tukey’s multiple comparison at the 5% level.

ns: not significant at $P = 0.05$; ** and ***: significant at $P = 0.01$ and 0.001, respectively.
and Cd concentrations of leachate were determined using the ion chromatography system and ICP-MS (PerkinElmer ELAN 6000, PerkinElmer Japan Co., Ltd.), respectively.

Straw Cl was extracted from the ground straw by autoclaving a 1:100 straw/water (w/w) suspension at 120°C for 20 min, followed by measurement using the ion chromatography system. The Cd concentration in the grains and straw were measured using ICP-MS (Agilent 7500ce, Agilent Technologies Japan, Ltd., Tokyo, Japan) after incinerating and dissolving them in HCl and nitric acid. The protein concentration in the grains was measured with a near infrared spectrometer (Infratec 1241 Grain Analyser, FOSS Japan Ltd., Tokyo, Japan) using a protein conversion factor of 5.70 on a 13.5% moisture basis.

Analyses of variance and correlation were performed using the SAS Add-In 5.1 for Microsoft Office connected with SAS 9.3 (SAS Institute Japan Ltd., Tokyo, Japan).

Results

1. Field experiment

The concentrations of soil Cd extracted with 0.1 mol L⁻¹ HCl were 0.16 and 0.06 mg kg⁻¹ at 0 – 15 cm and 15 – 30 cm soil depth, respectively, using soil mixture of equal parts of 20 treatment plots at the grain filling period, nine days after flowering (the data are not shown in tables and figures).

Soil pH and EC at the grain filling period were not significantly influenced by fertilizer treatments (Table 2). Soil Cl concentration at the grain filling period increased as the amount of Cl in the applied fertilizer increased (Table 2). Straw Cl increased after NH₄Cl-containing fertilizer application (Table 2). Straw Cd concentration, grain yield, and grain protein concentration did not show any significant differences among the treatments (Table 2).

Fig. 1. Relationship between grain Cd concentrations and (a) soil Cl concentration at the grain filling period, (b) straw Cl concentration at maturity in a field experiment.

Grain Cd concentration was not affected by the application of potassium chloride fertilizer (Table 2, treatment A vs. B). Grain Cd concentration in treatment E (NH₄Cl-containing fertilizer NKC54, applied at the tillering–jointing and flowering stages) was 0.223 mg kg⁻¹, which was about 1.5 times higher than that in treatment A (urea, applied at the tillering–jointing and flowering stage) (Table 2). There was no significant difference in grain Cd concentration between treatment C (NKC54, applied at the tillering–jointing stage) and treatment D (NKC54, applied at the flowering stage) (Table 2).

Grain Cd concentration was significantly correlated with soil Cl concentration at the grain filling period, nine days after the flowering stage (r = 0.664**), and straw Cl concentration at maturity (r=0.509*) (Fig. 1).

2. Pot experiment

Soil pH at maturity was not significantly influenced by fertilizer treatments (Table 3). Soil EC at maturity increased slightly after NH₄Cl fertilizer application (Table 3). Soil Cl concentration at maturity markedly increased after NH₄Cl fertilizer application, but was similar at the three application stages (Table 3). Straw Cl concentration increased after NH₄Cl fertilizer application at the germinating and tillering–jointing stages, but not by NH₄Cl application at the flowering stage (Table 3). Straw Cd concentration showed no significant difference among treatments (Table 3). Grain yield increased slightly after NH₄Cl application at the germinating stage (Table 3).
Leaching treatment had no or minimal influence on soil pH (Table 4). Leaching treatment caused a marked decrease in soil EC, soil Cl, and straw Cl concentrations, along with a slight decrease in grain yield (Table 4). Straw Cd and grain protein concentrations were reduced by leaching treatment when NH4Cl was applied (Table 4). Leaching treatment reduced the grain Cd concentration when NH4Cl was applied, almost negating the effects of NH4Cl at increasing grain Cd (Table 4).

Leaching treatment removed relatively large amounts of Cl. In the pots applied with NH4Cl at the germinating stage, straw Cd concentration was 0.131 mg kg\(^{-1}\) (Fig. 2).

Grain protein concentration was not significantly different among treatments (Table 3). Grain Cd concentration applied with NH4Cl was 0.112 – 0.095 mg kg\(^{-1}\), which was 1.4 – 1.2 times higher than that without NH4Cl (Table 3). Grain Cd concentration was higher when NH4Cl was applied at the germinating stage than at the tillering–jointing and flowering stages (Table 3). Grain Cd concentration was not significantly different between the tillering–jointing application and flowering-stage application (Table 3).

Grain Cd concentration was significantly correlated with soil Cl concentration at maturity (r = 0.818***) and straw Cd concentration (r = 0.744***) (Fig. 2).
ammonium sulfate and urea fertilizers. At the experimental site, the concentrations of soil Cd extracted with 0.1 mol L
-1 HCl were 0.16 and 0.06 mg kg
-1 at 0 – 15 cm and 15 – 30 cm soil depth, respectively. These values are common across Japan, with soil Cd concentrations of 109 out of 227 samples collected from wheat fields across Japan being 0.1 – 0.2 mg kg
-1 (Ministry of Agriculture, Forestry, and Fisheries of Japan, 2004). Even in these fields, the grain Cd concentration of wheat fertilized with NH₄Cl at the tillering–jointing and flowering stages was 0.223 mg kg
-1, which exceeded the maximum permissible concentration of 0.2 mg kg
-1 set by Codex Alimentarius Commission (2005a, 2005b). This finding is important because compound fertilizers containing NH₄Cl are commonly applied for wheat cultivation in Japan. For wheat cultivation, fertilizers are usually applied three to five times from the sowing to flowering stages of each crop. NH₄Cl fertilizer applications at the tillering–jointing and flowering stages have nearly equal effects on wheat grain Cd concentration. Basal dressing (applied at the germinating stage in our pot experiment) increased grain Cd concentration more than topdressing (applied at the tillering–jointing or flowering-stage) in pots that were protected from the rain. This result may be explained by the persistence of Cl applied as basal dressing, allowing it to influence Cd mobility throughout the entire crop growth period.

Rainfall might cause the leaching of Cl in the fields; although, we did not examine the effects of leaching in fields. Addiscott et al. (1978) reported that 79.5% of the applied Cl on 9 October moved below 50 cm depth by the application, 400 mg Cl had leached out by the pre-tillering stage leaching treatment, whereas 557 mg Cl had added by NH₄Cl application at the germinating stage (Table 5). Likewise, in the pots applied with NH₄Cl at the tillering–jointing stage, 101 mg Cl had leached out by the pre-flowering stage leaching treatment (Table 5). In contrast, a maximum of just 0.2 μg Cd per pot leached out, which was equivalent to <1/2000th of 0.1 mol L
-1 HCl-extractable Cd (0.43 mg pot
-1) (Table 5).

### Discussion

Both field and pot experiments showed that NH₄Cl fertilizer increased wheat Cd concentration compared to ammonium sulfate and urea fertilizers. At the experimental site, the concentrations of soil Cd extracted with 0.1 mol L
-1 HCl were 0.16 and 0.06 mg kg
-1 at 0 – 15 cm and 15 – 30 cm soil depth, respectively. These values are common across Japan, with soil Cd concentrations of 109 out of 227 samples collected from wheat fields across Japan being 0.1 – 0.2 mg kg
-1 (Ministry of Agriculture, Forestry, and Fisheries of Japan, 2004). Even in these fields, the grain Cd concentration of wheat fertilized with NH₄Cl at the

### Table 4. Effects of the leaching treatment on soil, wheat straw, and wheat grain properties at maturity in a pot experiment.

<table>
<thead>
<tr>
<th>NH₄Cl application</th>
<th>Leaching treatment</th>
<th>Soil</th>
<th>Cl conc.</th>
<th>Straw</th>
<th>Grain</th>
</tr>
</thead>
</table>
|                   | pH | EC (dS m
-1) | HCl-extractable Cd (mg pot
-1) | Cl conc. (g kg
-1) | Cd conc. (mg kg
-1) | Yield (g pot
-1) | Protein (%) | Cd conc. (mg kg
-1) |
| Without NH₄Cl     | No | 6.45 | 0.37 | 50 | 9.3 | 0.103 | 24.4 | ns | 11.2 | 0.080 |
|                   | Yes | 6.45 | 0.31 | 27 | 7.4 | 0.095 | 22.8 | ns | 10.9 | 0.075 |
| Germinating stage | No | 6.40 | 0.42 | 186 | 11.6 | 0.131 | 26.0 | ns | 11.1 | 0.112 |
|                   | Yes | 6.45 | 0.29 | 55 | 8.7 | 0.097 | 23.0 | ns | 10.5 | 0.087 |
| Tillering–jointing stage | No | 6.43 | 0.41 | 187 | 10.8 | 0.131 | 25.9 | ns | 10.9 | 0.099 |
|                   | Yes | 6.39 | 0.32 | 101 | 9.1 | 0.099 | 24.1 | ns | 10.5 | 0.088 |

Grain yield and protein concentrations are shown on a 12.5% and 13.5% moisture basis, respectively. Soil Cl and straw Cl concentrations are shown on a dry-matter basis. Grain Cd and straw Cd concentrations are shown as recorded (grain moisture was about 9%). *, **, and ***: significant at P = 0.05, 0.01, and 0.001, respectively.

### Table 5. Amount of Cl and Cd leached out of the soil in the pots by the leaching treatments.

| Leaching treatment stage | Application at germinating stage | Application at tillering–jointing stage | Volume of leachate (L pot
-1) | Cl in leachate (mg pot
-1) | Cd in leachate (μg pot
-1) |
|--------------------------|----------------------------------|----------------------------------------|-------------------------------|-----------------------------|-----------------------------|
| Pre-tillering stage      | Applied fertilizer | Cl in fertilizer (mg pot
-1) | Applied fertilizer | Cl in fertilizer (mg pot
-1) | 1.715 | 134 | 0.134 |
|                          | Ammonium sulfate | 0 | Ammonium sulfate | 0 | 1.593 | 5 | 0.000 |
|                          | NH₄Cl | 557 | Ammonium sulfate | 0 | 1.556 | 37 | 0.005 |
| Pre-flowering stage      | Ammonium sulfate | 0 | NH₄Cl | 557 | 1.601 | 101 | 0.014 |

All pots were treated twice: once at the pre-tillering stage and once at the pre-flowering stage. Water-extractable Cl and 0.1 mol L
-1 HCl-extractable Cd in the soil before sowing were 310 and 0.43 mg pot
-1, respectively.
end of May in Rothamsted (UK), with 564 mm precipitation during this period. Saso et al. (2012) reported that over 70% of applied Cl was lost to deep drainage (below 80 cm depth) from November to April in Ontario (Canada), with 478 mm precipitation during this period. In our study, 46% (400 mg pot$^{-1}$) of initially existed Cl (initial Cl 310 mg pot$^{-1}$ + applied Cl 557 mg pot$^{-1}$ = 867 mg pot$^{-1}$) was leached by the pre-tillering stage leaching treatment (assuming 100 mm rainfall) in the NH$_4$Cl-applied pots. In contrast, just 0.2 μg pot$^{-1}$ Cd at the maximum leached out of the soil, which was less than 1/2000th of 0.1 mol L$^{-1}$ HCl-extractable Cd (0.43 mg pot$^{-1}$). Thus, the leaching treatment negated the effects of NH$_4$Cl fertilizer application on wheat Cd concentration. Although 100 mm rain rarely occurs at any one time during the wheat cultivation period in Japan, these results indicate that abundant rainfall would decrease the effects of basal dressing with NH$_4$Cl fertilizer, preventing an increase in wheat Cd concentration. Additional studies are required to assess the degree of leaching caused by rainfall. In any case, we recommend the use of ammonium sulfate or urea in place of NH$_4$Cl as the nitrogen fertilizer.

Yet, Cl is essential for plants. From a practical perspective, a certain level of Cl in soil must be maintained for wheat production. Soil Cl concentrations sufficient for optimal wheat yield have been reported to be 43.5 kg ha$^{-1}$ (at 0 – 60 cm soil depth at sowing) by Fixen et al. (1986), 33 kg ha$^{-1}$ (at 0 – 60 cm soil depth at around sowing) by Engel et al. (1998), and 13.2 mg kg$^{-1}$ (at 0 – 20 cm soil depth at sowing) by Díaz-Zorita et al. (2004). In our field experiment, the concentration of Cl in soil (at 0 – 30 cm soil depth at the grain filling period), which had been toppedressed with urea only, was 10.4 mg kg$^{-1}$, corresponding to 31 kg ha$^{-1}$. Because straw Cl concentration was 7.9 g kg$^{-1}$ and plant dry weight (above ground plants excluding grain) was 8000 kg ha$^{-1}$ (the data are not shown in tables and figures), the amount of Cl in the wheat plants was estimated to be 7.9 g kg$^{-1}$ × 8000 kg ha$^{-1}$ = 63 kg ha$^{-1}$. Therefore, the soil Cl concentration in the field experiment at sowing was expected to be sufficient. In our pot experiment, the Cl concentration in the soil before sowing was 94 mg kg$^{-1}$, corresponding to 155 kg ha$^{-1}$, which was expected to be sufficient. Therefore, this study was completed under the conditions where soil Cl was sufficient for wheat production.

Like NH$_4$Cl, potassium chloride is also a commonly used fertilizer containing Cl for wheat cultivation. However, the reported results of experiments investigating the effects of potassium chloride fertilizer on Cd concentration in crops are inconsistent. For instance, Sparrow et al. (1994) reported that potatoes grown using potassium sulfate fertilizer had 20 – 30% lower tuber Cd concentrations than those grown using potassium chloride fertilizer at four out of six sites in northern Tasmania, Australia. In contrast, McLaughlin et al. (1995) reported that changing the potassium fertilizer form from potassium chloride to potassium sulfate had no effect on potato tuber Cd concentrations in an irrigated field in southern Australia. Furthermore, Zhao et al. (2003) reported that Cd uptake by spring wheat plants grown in pots was similar for potassium chloride and sulfate fertilizer applications. Gao et al. (2011) reported that the application of potassium chloride fertilizer did not consistently influence the grain Cd concentration of bread and durum wheat grown in fields in Manitoba, Canada. In our experiment, the effects of potassium chloride fertilizer were not significant. This result was obtained because the Cl content in the applied potassium chloride fertilizer was only one fourth of that in the applied NH$_4$Cl fertilizer. The ratio of Cl to K$_2$O in potassium chloride fertilizer is 0.75, whereas that of Cl to N in NH$_4$Cl fertilizer is 2.53. Moreover, the amount of K$_2$O fertilizer needed for wheat production in Japan is generally less than that of N fertilizer (Oyanagi, 2011). Thus, potassium fertilizer is not as important as nitrogen fertilizer with respect to the concentration of Cd in wheat grain.

López-Chukun et al. (2012) showed that adding sodium chloride to the soil enhances the solubility of Cd and increases the concentration of CdCl$^{-}$ and CdCl$_2$ in soil pore water. Many researchers have suggested that this phenomenon explains the increasing Cd concentration in crops grown in fields rich in Cl. In contrast, Ozkutlu et al. (2007) showed that leaf-applied sodium chloride promotes Cd accumulation in durum wheat grain. In the present study, the application of NH$_4$Cl fertilizer increased Cl concentrations in both the soil and wheat straw, with grain Cd concentration being correlated with both soil and straw Cl concentrations. Thus, it is possible that Cl enhances the transfer of Cd from the root/shoot to the grain, in addition to increasing the solubility of soil Cd. Cd translocation from roots to shoots is considered to be important for the accumulation of Cd in wheat grain (Chan and Hale, 2004; Greger and Löfstedt, 2004; Harris and Taylor, 2004). Further studies are required to elucidate the mechanisms underlying the effects of Cl on grain Cd accumulation.

In this study, we demonstrated that the application of NH$_4$Cl fertilizer increases the Cd concentration in wheat grain, unless massive leaching occurs due to heavy rainfall. It is important to regulate the quantity of Cl in the soil to reduce the health risk to humans and to maintain existing wheat grain yields. In addition to fertilization, the effect of implementing of various field-management techniques, such as irrigation and drainage, should be investigated as a means of controlling soil Cl concentrations and, hence, the Cd concentrations in wheat grain.

References
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* In Chinese with English abstract.
** In Japanese.
*** In Japanese. The title was translated by the authors.