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Cadmium uptake by onions, lettuce and spinach in New Zealand: Implications for management to meet regulatory limits

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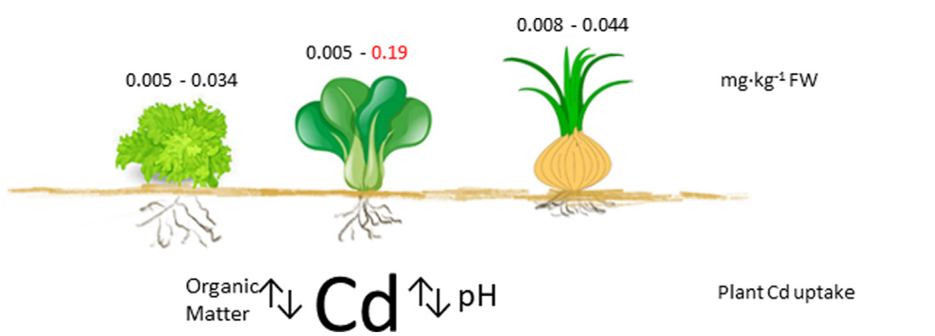
HIGHLIGHTS

- Variation of Cd in lettuce types or onion cultivars was inconsistent across sites.
- Soil Cd concentration, pH and region significantly predicted Cd in onions.
- Soil Cd concentration and carbon significantly predicted Cd in bunching spinach.
- The soil Cd concentration range was limited, typically between 0.07 and 0.8 mg kg^{-1}.
- Soil-plant relationships explained only a low-moderate proportion of Cd in plants.

GRAPHICAL ABSTRACT



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ABSTRACT

Paired soil and plant samples collected from the main commercial growing areas for onions (*Allium cepa*), lettuce (*Lactuca sativa*) and spinach (*Spinacia olearacea*) in New Zealand were used to assess the influence of plant and soil factors on cadmium (Cd) uptake in these crops. Differences in Cd concentration between eight lettuce sub-types were not consistent across sites, nor were differences in Cd concentrations in three crisphead cultivars assessed at two sites. Similarly, differences in Cd concentrations between four onion cultivars were inconsistent across sites. Mean lettuce Cd concentrations in eight lettuce varieties (range 0.005–0.034 mg·kg^{-1} (fresh weight, FW) were markedly lower than those in baby leaf and bunching spinach, (range 0.005–0.19 mg·kg^{-1} FW). Significant regional variation was observed in Cd concentrations in one onion cultivar (mean range 0.007–0.05 mg·kg^{-1} FW). Soil Cd concentration, pH and region were statistically significant predictors of onion Cd concentration, explaining low (38% for soil Cd and pH) to moderate (50% for all three parameters) percentage of the variation. Soil Cd concentration and exchangeable magnesium or total carbon were statistically significant predictors of Cd concentration in baby leaf and bunching spinach, respectively, explaining a moderate percentage (49% and 42%) of the variation in Cd concentration. Increasing pH and soil carbon may assist in minimising Cd uptake in onion and bunching spinach, respectively. The low to moderate proportion of explained variation is partly attributable to the narrow range in some measured soil properties and indicates factors other than those assessed are influencing plant uptake. This highlights a challenge in using these relationships to develop risk-based soil guideline values to support compliance with food standards. Similarly, the inconsistency in Cd concentrations in different cultivars across sites highlights the need for multi-site assessments to confirm the low Cd accumulation status of different cultivars.

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1. Introduction

Plant uptake of cadmium (Cd) from soil is a key factor affecting food chain transfer. The uptake of Cd by plants is influenced by a number of factors, including crop species and cultivar (e.g. Alexander et al., 2006; Guttieri et al., 2015; Sghayar et al., 2015), and the use of low-Cd-accumulating cultivars provides one means to manage Cd in food crops (e.g. Grant et al., 2008; Zhang et al., 2013). Soil properties, including pH, organic matter, salinity, cation-exchange capacity (CEC), clay content, availability of macronutrients, and micronutrients such as zinc (Zn), also influence Cd uptake in plants (e.g. Chaney, 2012; de Meeus et al., 2002; Golia et al., 2008; Grant and Bailey, 1998; McBride, 2002). Identified relationships between soil properties and plant uptake can be used as the basis for setting soil guideline values intended to ensure that human health is protected when home-grown produce is consumed, and intended to support food standards being met (de Vries et al., 2007; de Vries and McLaughlin, 2013; Romkens et al., 2009, 2011; Smolders et al., 2008; Swartjes, 2007; Yang et al., 2016). Further, these relationships can indicate the likely efficacy of potential management options for reducing plant uptake of Cd; so far pH management (through lime addition) and the addition of organic matter (as compost) are most common (e.g. Bolan and Duraisamy, 2003; Kumarpandit et al., 2017).

There is concern worldwide regarding the accumulation of Cd in agricultural soils, particularly in relation to the potential adverse effect Cd can have on food quality, leading to dietary or trade risks where food standards are not met (EFSA, 2011; Lin et al., 2015; Rizwan et al., 2017; Toth et al., 2016). In New Zealand a strategy for managing the risks of Cd to agriculture and horticulture has been in place since 2011 (MAF, 2011). The strategy aims to "ensure that cadmium in rural production poses minimal risks to health, trade, land use flexibility and the environment over the next 100 years". A key component of the strategy is the Tiered Fertiliser Management System (TFMS), which comprises five tiers and four trigger concentrations to minimise Cd accumulation in soil by imposing increasingly stringent fertiliser management practices as Cd concentrations increase.

However, the trigger values are interim given a lack of New Zealand-specific data on soil Cd concentrations that may pose a risk for local agricultural systems and how these risks might be managed (MAF, 2011). Soil Cd concentrations to support compliance with food standards may often be lower than soil Cd concentrations causing detrimental effects on ecological receptors, including plants and soil biota (Cavanagh, 2013; de Vries et al., 2007; MPI, 2012). Furthermore, food standards have previously been reported to have been occasionally exceeded in potatoes (Kim, 2005) and wheat grown in New Zealand (Gray et al., 2001), and our other papers discuss soil and plant factors influencing Cd uptake in these crops (Gray et al., accepted-a, accepted-b).

Onions (*Allium cepa*) are one of the highest-value export crops for New Zealand, valued at $112 million in 2017 (Plant and Food Research, 2017). There are few studies on Cd uptake by onions, possibly because Cd concentrations in onions are relatively low and they represent a small contribution to dietary intake (e.g. EFSA, 2012; Pearson et al., 2018). However, regulatory maximum levels (MLs) established for onions are also low (e.g. 0.05 $mg \cdot kg^{-1}$ FW, Codex, 2018, EC, 2006), arguably leading to a greater potential for these to be exceeded. For example, in 2016 five shipments of US onions were rejected at the Taiwanese border due to non-compliance with Taiwan's food safety standards for Cd of 0.05 $mg \cdot kg^{-1}$ FW.[2]

Leafy green vegetables such as lettuce (*Lactuca sativa*) and spinach (*Spinacia olearacea*), which typically accumulate significantly more Cd in their edible parts than other vegetables (Alexander et al., 2006; Baldantoni et al., 2016; He and Singh, 1994), potentially provide a sensitive indicator for assessing the risk posed by soil Cd in relation to compliance with food standards. Numerous studies have assessed Cd uptake in lettuce, with some also assessing variations between cultivars (Alexander et al., 2006; Li et al., 2014; Zhang et al., 2013). Fewer studies report on the variation in Cd uptake by different lettuce 'types', with lettuce generally grouped into crisphead, buttterhead or buttercrunch, romaine/cos, and loose-leaf types. Crews and Davies (1985) identified that crisphead lettuce, which comprises the bulk of New Zealand lettuce production, had lower Cd concentrations compared with three other lettuce types. Spinach, as baby-leaf (immature spinach) or bunching (mature) spinach, is a widely consumed leafy green vegetable, globally as well as in New Zealand, and it is frequently identified as one of the highest Cd accumulating vegetables (e.g. Lin et al., 2015).

The objectives of this study were to (i) determine the baseline concentrations of Cd in onions, lettuce and spinach grown in the main commercial growing areas in New Zealand, and (ii) assess the influence of plant and soil factors on Cd uptake in these crops to inform management strategies, including the development of risk-based soil guideline values, to support compliance with regulatory food quality standards.

2. Methods

2.1. Sites and sampling

Cultivars selected for sampling were those identified by industry grower groups as commonly grown in New Zealand. Onions were all sampled from commercial crops. Cadmium concentrations were determined in four onion cultivars (Rhinestone [BV1], Plutonis [BV2], RLK-X1 [BV3], and Red [RV1]) grown across three sites in 2015, and in one onion cultivar (Rhinestone) grown across 25 additional sites over 2016/17. Leafy greens were sampled from commercial crops, where possible, with seed for specific cultivars provided to other growers and grown as field trials at additional commercial sites. For lettuce, Cd concentrations were determined in three crisphead (Iceberg) cultivars (Constanza [V1], Icegreen [V2] and Vegas [V3]), grown at two sites in 2015, and in three lettuce types (eight lettuce sub-types) (loose-leaf: Red Frill [FR], Green Frill [GF]; romaine/cos: Green Cos [GC], Red Cos [CR), Baby Cos [BC]; crisphead: Iceberg [IB]), grown across five sites in 2016/17. Cadmium concentrations were determined in one spinach cultivar (Jedi) sampled across 18 sites in 2016/17, with two additional spinach cultivars (Black Glove, Nightfall) sampled from one site on separate occasions. Sampling details are summarised in Table 1, and general locations are shown in Fig. A.1.

2.2. Soil and plant analysis

Soils were oven-dried (35 °C) until a constant weight obtained and sieved (<2 mm) before analysis. Soil pH was determined in a 1:2 soil: water solution by potentiometric analysis (Blakemore et al., 1987). Exchangeable potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) were measured in a 1 M neutral ammonium acetate extract (Blakemore et al., 1987), and analysed by Inductively-Coupled Plasma Optical Emission Spectrometry (ICP-OES; Varian 720 ES- USA). Cation Exchange Capacity (CEC) was calculated by summing concentrations of extractable cations and extractable acidity. Bioavailable phosphorous (P as Olsen P) was determined by bicarbonate extraction (Olsen, 1954). Total carbon (C) and nitrogen (N) were determined by combustion using an Elementar Vario-Max CN elemental analyser. Extractable chloride concentrations were measured in a filtered 1:5 soil:water extract by ion chromatography. (Dionex ICS-2100, Thermo Fisher Scientific Inc.). Total extractable concentrations of Cd, Zn, P, aluminium (Al) and iron (Fe) were determined by microwave digestion (MARSXPRESS, CEM Corp.), using nitric acid and hydrogen peroxide as described by Simmler et al. (2013). The digests were analysed by Inductively-Coupled Plasma Mass Spectrometry (ICP-MS; Agilent 7500cx) for Zn, P, Al and Fe. Particle size analysis was measured using the pipette method (Claydon, 1989).

[2] https://www.wga.com/blog/exported-us-onions-violate-taiwanese-food-standards-requirements.

Table 1
Summary of sites and details of soil and plant sampling.

Crop	Year	Sites	Location	No. of cultivars	Sampling details
Onion (*Allium cepa*)	2015–Cultivar study	3 commercial field sites	Pukekohe Waikato Canterbury	2 4 2	For each cultivar present at each site, 4 plots (approx. 600 × 600 mm) were established randomly across the field. Sampling was undertaken just subsequent to lifting. Within each plot, 5 onions and 5 soil cores (25 × 150 mm depth) were collected and combined to form a single composite plant or soil sample, respectively. In addition, a composite soil sample (25 × 150 mm) was taken along a 50 m transect.
	2016/17–field survey	25 commercial field sites	Canterbury Hawke's Bay Pukekohe Waikato	1	At each site 3 plots were established randomly across the field and sampled as described above.
Lettuce (*Lactuca sativa*)	2015–cultivar study, crisphead lettuce	Field trial at 2 commercial field sites	Pukekohe Gisborne	3 3	Four replicate plots containing 6 plants for each variety were arranged in a randomised design. Sampling was undertaken at the time of commercial harvest, with 4 lettuces and 5 soil cores (25 × 150 mm depth) collected and combined to form a single composite plant or soil sample. In addition, a composite soil sample (25 × 150 mm) was taken along a 50 m transect.
	2016/17–lettuce type study	3 commercial regions, 5 sites	Pukekohe (2 sites) Gisborne (2 sites) Canterbury	5 5 4	Three replicate plots (approx. 1 m × bed width) were established randomly along the rows of each cultivar. Sampling was undertaken just prior to harvest, as described above, but with 5 lettuces and 5 soil cores (25 × 150 mm depth).
Spinach (*Spinacia oleracea)*	2016/17–field survey	18 commercial field sites	Gisborne, Pukekohe, Canterbury, Hawke's Bay	1 cultivar sampled as baby leaf; site $n = 10$	Commercial growers: field trial or commercial crop. Three plots (approx. 1 m × bed width) were established randomly over field trial area/along the row. Within each plot, spinach leaves (c. 300 g) and 5 soil cores (25 × 150 mm depth) were collected and combined to form a single composite plant or soil sample. In addition, a composite soil sample (25 × 150 mm) was taken along a 50 m transect.
			Auckland, Pukekohe, Tasman, Manawatū, Waikato	1 cultivar sampled as bunching spinach*; site $n = 11$	As described above

Onion roots, leaves and outer skin were removed, and the remaining bulb weighed, dried at 60 °C to a constant weight, and weighed again to enable reporting of data on a fresh weight (FW) basis. Lettuce and spinach leaves were washed and excess water removed, weighed, dried at 60 °C to a constant weight, and weighed again to enable reporting of data on a fresh weight (FW) basis. Plant samples were ground and digested by microwave digestion in nitric acid and hydrogen peroxide using the method described by Cindric et al. (2015) prior to analysis of the digests by ICP-MS. Plant concentration data are expressed as fresh weight concentrations unless otherwise specified.

2.3. Quality control/assurance

Soil and plant samples were digested in batches of up to 40. Each batch included at least one digestion (procedural) blank. One out of every 20 samples was analysed in duplicate to confirm repeatability of the analysis. Cadmium concentrations in procedural blanks were used to estimate the method detection limit and duplicate analysis of samples were within 5% of each other. The accuracy of soil and plant analysis was assessed using several internal and external certified reference materials including for soil (NIST Montana 2711; Interlab internal WEPAL soil 921; Interlab internal WEPAL soil 981), and plant (NIST 1573a, tomato leaves; ASPAC internal clover; ASPAC internal beetroot). Analytical results were within 5% of the certified values. The method detection limits were 0.020 mg kg^{-1} and 0.005 mg kg^{-1} in soil and plant material on a dry weight basis, respectively.

2.4. Soil–plant relationships

We assessed the relationship between plant Cd uptake and soil properties using two approaches. Firstly, we tested Freundlich-type regression relationships (Eq. (1)), with significant soil properties identified through multiple linear regression (see 'Statistical analysis').

$$\text{Log10}(\text{Cd}_{\text{plant}}) = \text{a} + \text{b.pH} + \text{c. log10}(\text{Cd}_{\text{soil}}) + \text{d. log10}(\text{C}) + \ldots \quad (1)$$

where Cd_{plant} is the plant Cd concentration (mg kg^{-1} DW), Cd_{soil} is the soil Cd concentration (mg kg^{-1}) and C is the soil carbon content (%). We also calculated the plant uptake factors (PUF) (Eq. (2)) a commonly used, simple measure of plant uptake of inorganic contaminants.

$$\text{PUF} = \frac{\text{Cd}_{\text{plant}}\ (\text{mg/kg (DW)})}{\text{Cd}_{\text{soil}}\ (\text{mg/kg })} \quad (2)$$

Soil Cd concentrations at which specific concentrations occur in a given cultivar can be back-calculated from Eq. (1) (e.g. de Vries et al., 2007, Romkens et al., 2009, 2011) or by rearrangement of Eq. (2) yielding Eq. (3), assuming conditions such as soil properties do not change. Using the ML as the target plant concentration ($\text{Cd}_{\text{plant limit}}$), an estimate can be made of the soil concentration at which the food standard may be reached (nCdFS). The food standard is expressed as fresh weight, which requires conversion to a Cd concentration based on dry weight. The dry weight was 10% of the fresh weight for onions, 9% for spinach, 5% for non-iceberg lettuce, and 3% for iceberg lettuce, based on the mean dry matter content for our samples. These values are not intended as threshold limits, but rather provide an insight into soil properties influencing plant uptake and the Cd concentrations at which management to mitigate the risk of exceeding food standards might be considered.

$$nCdFS\ (mg/kg\ ) = \frac{Cd_{plant\ limit}\ (mg/kg\ (DW))}{PUF} \quad (3)$$

2.5. Statistical analysis

Statistical analyses were performed using R version 3.4.3 (R Core Team, 2016). Multiple linear regression analyses were a primary focus for assessing the relationship between edible plant Cd concentrations (expressed as dry weight) and soil variables using the MASS and leaps packages (Lumley, 2017; Venables and Ripley, 2002). Soil properties varied within sites, so analyses were undertaken using individual plot data (where they were available). Where soil properties were measured only once at a given site (e.g. particle size, extractable chloride concentrations), site average data (i.e. the average of the plots sampled on each site) were used to investigate the relationship of those properties with plant concentration.

Regional and cultivar differences in plant Cd concentrations (fresh weight basis) were assessed using ANOVA, or using *t*-tests where only two data sets were compared. Effects were considered significant if they differed at the probability level of 5%, based on Tukey's honest significance difference test (HSD).

3. Results and discussion

3.1. Lettuce – cultivar and type

Mean lettuce Cd concentrations[2] in three Iceberg cultivars ranged between 0.005 and 0.019 mg·kg^{-1} ($n = 24$) with significantly higher Cd concentrations observed in the Iceberg V1 cultivar compared with that in V2 and V3 cultivars for lettuce grown in Gisborne (Fig. A.2). Given the low Cd concentrations observed in the Iceberg cultivars, a wider range of lettuce types were subsequently sampled. Mean Cd concentrations in all lettuce types ranged between 0.005 and 0.034 mg·kg^{-1}, with an overall mean of 0.02 ($n = 47$) (Fig. 1). Significant differences in Cd concentrations in different lettuce varieties grown at the same site in Pukekohe and Canterbury were not consistent across the sites (Fig. 1A, C) and no significant differences in Cd concentrations were observed for lettuce varieties grown at the same site in Gisborne (Fig. 1B). Cadmium concentrations in Iceberg lettuce grown at different sites in Pukekohe and Gisborne were similarly low or lower than concentrations in other lettuce types from those regions (Fig. 1). Similarly, Iceberg lettuce grown in Canterbury had lower concentrations than other lettuces at this site. However, the lower concentrations in Iceberg lettuce may be more attributable to differences in dry matter content (typically 3% for Iceberg lettuce and 5% for other lettuce types), as no significant difference was observed in Cd concentrations expressed on a dry weight basis.

Green Cos and Iceberg were the only lettuce grown in all three regions, with significantly higher Cd concentrations observed in lettuce grown in Gisborne compared to those grown in Pukekohe and Canterbury (Fig. 1). Soil Cd concentrations were similarly low in Canterbury (site mean 0.12 mg·kg^{-1}) and Gisborne (0.14–0.23 mg·kg^{-1}), with Pukekohe having the highest concentrations (0.52–0.63 mg·kg^{-1}) (Fig. A.3). Aggregating Cd concentrations from all lettuce samples shows plant uptake of Cd was highest in Gisborne (median PUF: 3.6), followed by Canterbury (median PUF: 2.1), with the lowest uptake occurring in Pukekohe (median PUF: 0.45) (Fig. A.3).

General soil properties for the lettuce sites are shown in Table 2. The mean soil pH across all the sites was close to 7, with all sites except one (IB in Gisborne) having pH > 6. Lettuce (Iceberg) grown at this site had elevated Cd compared to Iceberg lettuce grown at a different site in Gisborne (IB2) with a pH of 6.7 and slightly lower soil Cd (0.15 vs 0.20 mg·kg^{-1}).

Mean lettuce Cd concentrations were comparable to concentrations in lettuce from a recent total diet survey (0.4 mg·kg^{-1} [DW] (Pearson et al., 2018) or 0.02 mg·kg^{-1} [FW]), assuming 5% dry matter;) and Iceberg lettuce sold commercially in the Waikato region (mean: 0.019 mg·kg^{-1}; $n = 11$, range 0.004–0.042 mg·kg^{-1}) (Kim, 2005), and lower than in lettuce from Pukekohe market gardens (mean: 0.04 mg·kg^{-1}; $n = 30$, range 0.01–0.14 mg·kg^{-1}; (Roberts et al., 1995). Cd concentrations in all lettuce types were at the lower end of the range (0.001–0.24 mg·kg^{-1}) reported in international studies (Baldantoni et al., 2016; Grybauskait et al., 2014; Lin et al., 2015; Tang et al., 2016), and markedly below the Food Standard of Australia and New Zealand (FSANZ) ML for Cd in leafy greens of 0.1 mg·kg^{-1}, and the Codex (2018) and European Commission (2006) MLs of 0.2 mg·kg^{-1}.

3.2. Onions – effect of cultivar

Mean onion Cd concentration in the four cultivars ranged between 0.008 mg·kg^{-1} and 0.044 mg·kg^{-1} ($n = 32$), with an overall mean concentration of 0.018 mg·kg^{-1} (Fig. 2). This mean concentration is comparable to that in onions from a recent New Zealand total diet survey (0.2 mg·kg^{-1} [DW] Pearson et al., 2018; 0.02 mg·kg^{-1} [FW], assuming 10% dry matter), those sold commercially in the Waikato region (mean 0.016 mg·kg^{-1}, range 0.003–0.068 mg·kg^{-1}, $n = 36$; Kim, 2005), and Pukekohe (0.02 mg·kg^{-1}, range 0.01–0.11 mg·kg^{-1}, $n = 30$; Roberts et al., 1995). New Zealand onion concentrations were at the lower end of the range reported in international studies (0.003 to 0.41 mg·kg^{-1}) (Alexander et al., 2006; Bester et al., 2013; Bystricka et al., 2016; Grybauskait et al., 2014; Lin et al., 2015; Weeks et al., 2007), which were largely from residential gardens or pot trials. No ML is specified for bulb vegetables by FSANZ, although the mean concentration of Cd in the red onion from Pukekohe (RV1) was approaching the Codex and European MLs of 0.05 mg·kg^{-1} (FW) (Codex, 2018; EC, 2006).

There were significant differences in onion Cd concentrations between cultivars, although these were not consistent across sites (Fig. 2). Bystricka et al. (2016) found significant differences in onion Cd concentrations in six onion cultivars, with mean concentrations ranging between 0.022 and 0.04 mg·kg^{-1} (FW), while Alexander et al. (2006) reported no difference in Cd uptake by six onion cultivars. The higher Cd concentration in the red onion from Pukekohe (RV1) may be attributable to the lower soil pH observed at this site compared to the other sites; soil carbon was also lower than most other sites (data not shown). Bester et al. (2013) found that higher pH and organic matter reduced onion Cd concentrations. A more extensive comparison between Cd uptake and soil properties for this study is reported in the next section.

3.3. Field survey – soil properties

Mean concentrations of selected soil properties from the survey sites for onion and spinach are given in Table 2. Total soil Cd concentrations for the onion sites ranged between 0.08 and 1.35 mg·kg^{-1}. The overall mean soil Cd concentration for the onion sites was 0.42 mg·kg^{-1} (median 0.29 mg·kg^{-1}), which is higher than the mean and median Cd for cropping soil of 0.28 mg·kg^{-1} and 0.21 mg·kg^{-1}, respectively (Cavanagh, 2014). Cadmium concentrations in Waikato and Pukekohe are higher than those in Canterbury and Hawke's Bay (Fig. 3A), as observed in larger soil surveys (Cavanagh, 2014). The highest Cd concentrations in the Pukekohe region occurred in an organic soil, which had a low volume weight (surrogate for bulk density). Total soil Cd concentrations for the spinach sites ranged between 0.07 and 0.46 mg·kg^{-1} (Fig. 3B), with mean soil concentration of 0.25 mg·kg^{-1} (median 0.26 mg·kg^{-1}). Most onion samples (73%) and all spinach samples had soil Cd concentrations below the TFMS tier 1 trigger of 0.6 mg·kg^{-1}. Other soil properties recognised as affecting plant uptake of Cd also varied, with soil pH ranging from low to high, while total C and CEC were low to medium (Blakemore et al., 1987). Soil type ranged from clay to silt loams.

3.4. Field survey – cadmium concentrations in onions and spinach

Mean onion Cd concentration ranged between 0.007 and 0.05 mg·kg^{-1} ($n = 88$), with an overall mean concentration of 0.016 mg·kg^{-1} (Fig. 3C), similar to the cultivar study (Fig. 2). There was significant regional variation in onion Cd concentrations (Fig. 3C), with onions from Pukekohe, Waikato and Canterbury having significantly higher concentrations than those from Hawke's Bay ($P < 0.05$). Cadmium concentrations in onions from Pukekohe were significantly higher than those from Canterbury ($P < 0.05$), although there was no significant difference between Cd in onions from Canterbury and Waikato ($P = 0.093$). Plant uptake also varied between regions (Fig. 3E), and was highest in Canterbury and similar across the other regions. One site (three replicate plots) in Pukekohe showed elevated plant uptake; this site had an unusually low mean pH of 5.4 when compared to the other sites in the region (mean pH ranging between 6.0 and 6.6), which may explain the discrepancy.

Mean spinach Cd concentrations ranged between 0.005 and 0.11 mg·kg^{-1} for baby leaf spinach ($n = 31$), with an overall mean of 0.06 mg·kg^{-1} (Fig. 3D). Concentrations were generally higher in bunching spinach, and site mean concentrations ranged from 0.07 mg·kg^{-1} to 0.19 mg·kg^{-1}, with an overall mean concentration of 0.1 mg·kg^{-1} ($n = 33$). The overall mean for both spinach stages was 0.08 mg·kg^{-1}. Cadmium concentrations in spinach were at the lower end of the range (0.015 to 4.14 mg·kg^{-1}) reported in international studies (Grybauskait et al., 2014; Lin et al., 2015; Tack, 2017). The mean concentrations at a number of sites exceeded the FSANZ ML for Cd in leafy greens of 0.1 mg·kg^{-1}, although they were less than the Codex (2018) and European Commission MLs of 0.2 mg·kg^{-1} (EC, 2006).

The influence of harvest stage on Cd concentrations was assessed at two sites where baby leaf spinach, and subsequently bunching spinach from the same crop, was sampled. Bunching spinach had significantly higher concentrations than baby spinach, suggesting Cd continues to be accumulated over time and is not diluted with growth (Fig. A.3). Limited assessment of variation between cultivars was undertaken, with three cultivars tested at one site: two were grown at the same time, while a third was grown over winter and thus for a longer period. This cultivar had significantly elevated Cd compared to the other cultivars (Fig. A.3), which may be attributable to a longer growth period and/or cultivar variation.

A second baby-leaf spinach crop (P1b2) grown at P1 had significantly higher Cd compared to the first baby spinach crop (P1b1), although the reason for this difference is unclear. Tack (2017) observed significantly higher concentrations in spinach subjected to limited water supply during periods of high demand, which may suggest water stress is a reason for the observed difference. However, further investigation, including of other crop management factors that have been shown to influence Cd uptake, such as fertiliser (N, micronutrients), is required (Jonsson and Asp, 2011, Paul and Chaney, 2017).

3.5. Relationship between soil properties and cadmium uptake by onions and spinach

Soil Cd and pH were significant predictors of onion Cd concentrations, explaining 38% of the variation, while inclusion of region as an additional variable explained 50% of the variation (Table 3). Assessing the influence of a wider set of soil properties (and excluding region) also improved prediction, with soil Cd, CEC, and exchangeable Mg and Ca concentration explaining approximately 48% of the variability in onion Cd concentrations (Table 3), which may suggest it is differences in these variables that are leading to the observed influence of region.

Clay content and chloride were not significant predictors of Cd concentration. The low proportion of variation explained may be attributable to the relatively small range in Cd concentration (0.08 to 1.35 mg·kg^{-1}) and other soil properties. For example, Bester et al. (2013) found pH, soil Cd and organic C explained 85% of the variation in onion Cd concentrations. However, in their study the range of soil Cd concentrations (0.2 to 40 mg·kg^{-1}) and organic matter (3.3% to 13.8%, or 1.9% C to 8% C), was much greater than in our study; moreover the Bester et al. (2013) study was undertaken over a much more limited geographical area, which we would expect to also constrain differences in plant uptake mainly to soil properties. Onion Cd concentrations predicted using the identified relationships (Eqs. (3) and (4) in Table 3) show that the models tend to under-predict onions with high Cd, compared with observed concentrations (Fig. A4).

Soil Cd and exchangeable Mg were significant predictors of Cd concentration in baby spinach, explaining 49% of the variation in spinach

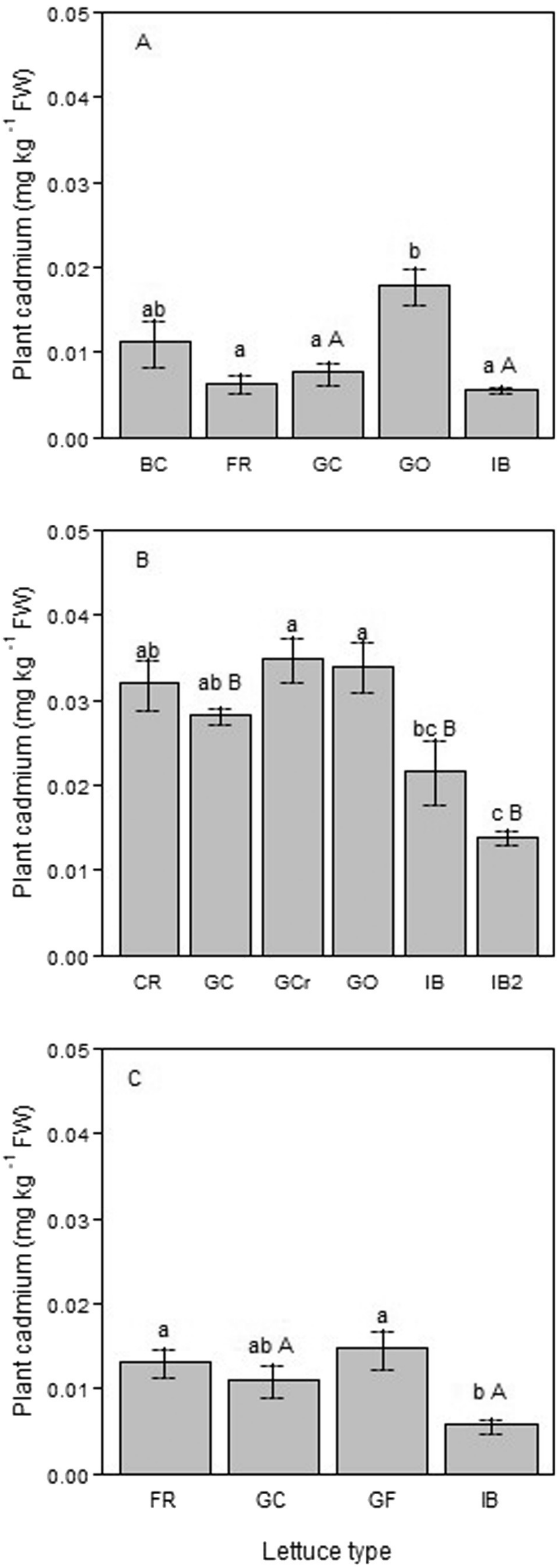

Fig. 1. Cadmium concentration (mg·kg^{-1}) of different lettuce varieties grown in (A) Pukekohe, (B) Gisborne and (C) Canterbury. Mean and standard error ($n = 3$) indicated by the same lower case letter are not significantly different ($P < 0.05$) within a given region. Note: IB and IB2 grown in Pukekohe and Gisborne were grown at different locations from the other lettuce types. Mean and standard error ($n = 3$) indicated by the same uppercase letter are not significantly different ($P < 0.05$) between regions for the same lettuce type.

Table 2
Summary of properties of sampled soils.

Soil property	Lettuce soils			Onion soils			Spinach soils		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
Total Cd (mg·kg^{-1})	0.3	0.16	0.10–0.78	0.42	0.29	0.08–1.35	0.25	0.27	0.07–0.47
pH	6.7	6.7	5.4–7.7	6.2	6.2	5.9–7.8	6.6	6.6	5.6–7.6
Olsen P (mg·kg^{-1})	108	113	44–178	41	41	20–70	90	81	30–205
Total P (mg·kg^{-1})	1530	1160	951–2690	1990	1660	694–4390	1645	1703	664–3376
Total C (%)	1.8	1.9	1.1–2.33	4.2	4.3	2.1–6.67	2.47	2.26	1.08–4.87
Total N (%)	1.3	1.01	0.55–2.55	0.39	0.42	0.17–0.64	0.24	0.23	0.08–0.51
K (cmolc·kg^{-1})	14.9	11.1	7.6–27.4	0.86	0.79	0.29–1.56	1.1	0.9	0.3–3.22
Ca (cmolc·kg^{-1})	2.3	1.6	0.78–3.89	9.91	9.6	7.4–12.8	12.8	11.9	5.1–22.9
Mg (cmolc·kg^{-1})	0.3	0.23	0.1–0.63	1.53	1.36	0.63–2.85	1.8	1.49	0.64–6.45
Na (cmolc·kg^{-1})	21.5	20	11–35	0.16	0.16	0.06–0.24	0.14	0.11	0.05–0.94
CEC (cmolc·kg^{-1})	0.2	0.15	0.08–0.23	20	20	14–26	18	19	9–27
Total Zn (mg·kg^{-1})	92	86	68–138	108	115	73–141	92	92	53–188
Total Cl (mg·kg^{-1})[a]	22.3	21	9.1–50	28.5	30.2	17.7–36.1	13.5	10.1	2.0–47.6
Sand (%)[a]	12	12	3–27	17	14	2–35	18	11	0–43
Silt (%)[a]	44	47	28–61	52	50	43–63	49	49	25–71
Clay (%)[a]	43	50	12–60	27	28	23–29	31	29	12–66

[a] Total chloride and soil texture determined from a single composite transect taken across the site.

Cd concentrations (Table 3). The inclusion of Zn increased the percentage of variation explained to 55%. While the influence of Zn on Cd uptake by plants is widely reported (e.g. Oliver et al., 1994; Sarwar et al., 2010), there are few studies that have examined the relationship between Cd and Mg, with studies generally reporting increased soil Mg concentrations lead to reduced Cd concentrations (Borisev et al., 2016; Kudo et al., 2015), whereas we observed the opposite. Further investigation is required to determine the significance of this finding. In bunching spinach, soil Cd and C were significant predictors of Cd concentrations, explaining 42% of the variation in spinach Cd concentrations (Table 3). Comparison of predicted Cd concentrations with observed concentrations showed no bias toward under- or over-prediction of either baby or bunching spinach Cd concentrations (Fig. A4).

The low to moderate proportion of the variation in plant Cd explained by the derived soil–plant relationships suggests that factors other than those assessed (e.g. water management, fertiliser input, climate) are also influencing plant uptake. The poor explanatory power is also partly attributable to the relatively narrow soil Cd concentration range, particularly for spinach, and other soil properties (e.g. pH) that are typically managed to meet agronomic optimums for individual crops. As sampling was undertaken in the main growing areas of each

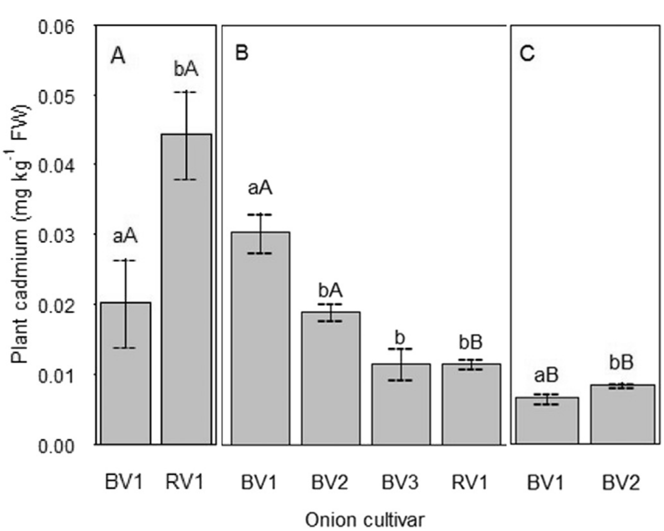

Fig. 2. Cadmium concentration in onion cultivars (mg·kg^{-1} FW) from (A) Pukekohe, (B) Waikato and (C) Canterbury. Mean ($n = 4$) and standard error indicated by the same lower case letter are not significantly different ($P < 0.05$) *within* a given site, or *between* sites (capital letters) for cultivars present at more than one site.

crop, soil Cd concentrations are considered to be representative of the concentrations in which these crops are currently grown, and further sampling within these regions is unlikely to extend the concentration range.

3.6. Implications for managing Cd uptake in leafy greens and onions to meet regulatory maximum levels

Cadmium concentrations in all lettuce types assessed in this study were markedly below the FSANZ ML. The inclusion of regions where plant uptake of Cd was comparatively high, thereby posing greater risk of elevated plant Cd, suggests there is minimal risk of Cd concentrations in lettuce exceeding regulatory standards. Therefore the requirement for active management of Cd in lettuce crops is low. This is also reflected in the estimates of soil Cd concentrations above which food standards might be exceeded (nCdFS) for individual sites, which were markedly above current soil Cd concentrations measured in this study for the individual regions (Tables 4 & 5).

A small number of onion samples had Cd concentrations close to or above the EC (2006) ML of 0.05 mg·kg^{-1}, and sites with elevated onion Cd concentrations often had a soil pH below 6. However, the nCdFS derived on the basis of the identified soil–plant relationships (Eqs. (3) and (4)), including at a pH of 5.5, were markedly above current soil Cd concentrations. This suggests caution in the use of these values, and relationships, as triggers for managing compliance with regulatory food standards. The nCdFS, derived from the PUF perhaps provides a better reflection of the Cd concentrations to indicate the risk of non-compliance and highlights the variability that can occur across individual sites.

From a practical perspective, maintaining soil pH at or above 6 may be sufficient to ensure that Cd concentrations in the onion cultivar assessed in this study comply with relevant MLs. However, further testing of onions and soil, including additional cultivars, is required to determine the wider applicability of this observation. Similarly, further research could be undertaken to determine if plant management practices (such as irrigation, fertiliser type and rates, and amendment addition) affect Cd uptake, and could explain a greater proportion of the variation in onion Cd concentrations.

The comparatively high Cd concentrations in baby and bunching spinach (close to or above the FSANZ ML of 0.1 mg·kg^{-1} [FW]) suggest that management actions should be undertaken to reduce Cd uptake. The nCd_{FS} derived using soil–plant relationships and PUF similarly identify that current soil Cd concentrations present a risk of non-compliance. As soil pH is typically managed to around 7 for spinach crops, adding lime offers limited value for reducing Cd uptake in spinach. Further,

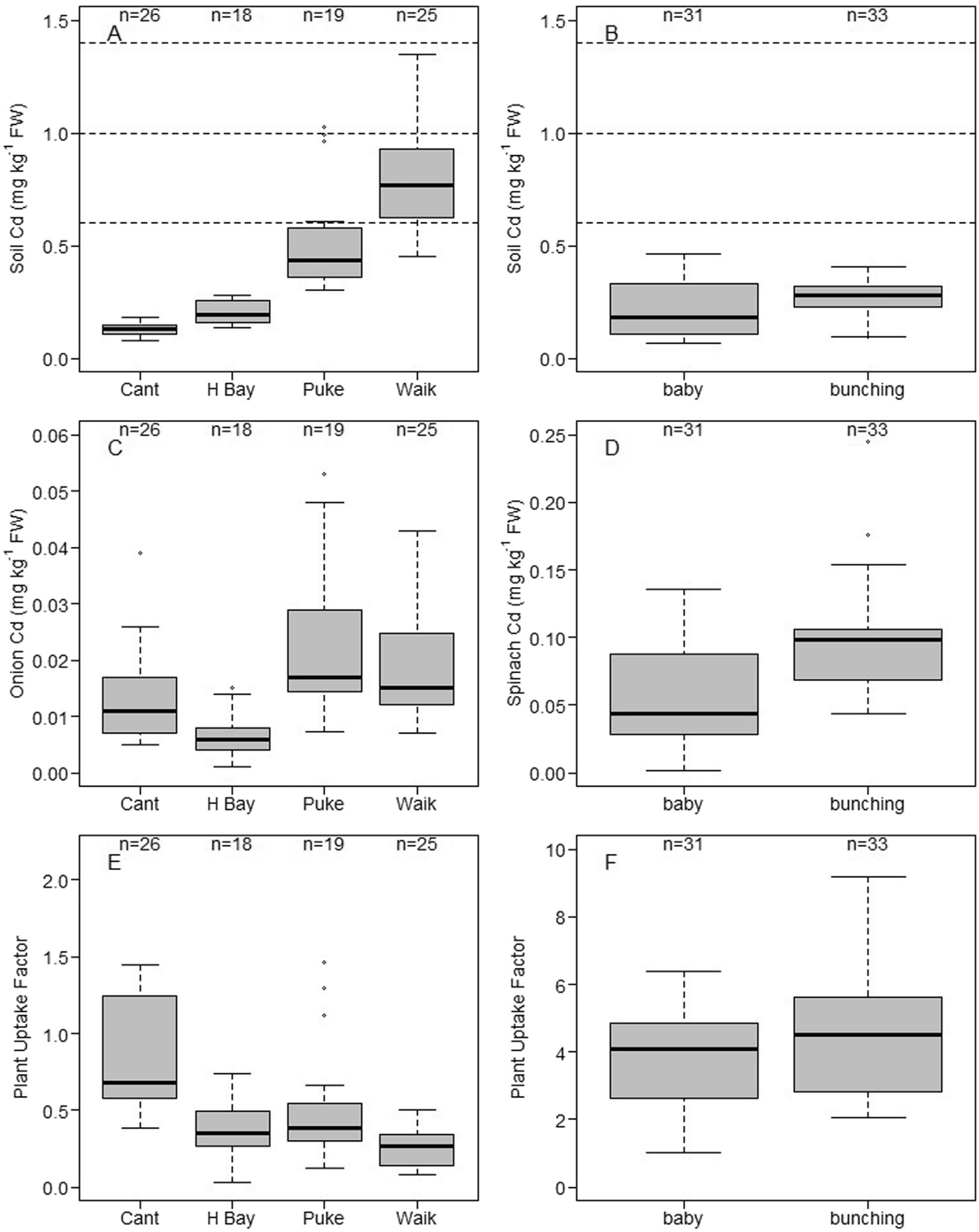

Fig. 3. Total soil cadmium concentration (mg·kg^{-1}) in each region (Cant = Canterbury, H Bay = Hawkes Bay, Puke = Pukekohe, Waik = Waikato) sampled for onions (A) or in soils sampled for baby and bunching spinach (B). The dashed line is the TFMS trigger value for Tiers 1, 2, and 3. Mean Cd concentrations (mg·kg^{-1} FW) in onions from each region (C) and baby and bunching spinach (D). Plant Uptake Factor for each region for onions (E) or for baby and bunching spinach (F). Boxes depict the 25th and 75th percentile values, with horizontal lines plotted within boxes representing the median value. Whiskers show the 10th and 90th percentile values and outliers are represented by circles and are values that are >1.5 times the interquartile range. n = individual plot samples, with three or four plots sampled per site.

spinach from a number of sites with a pH close to 7 were close to or exceeded the ML.

Given the significant relationship between Cd concentrations in bunching spinach and soil C, compost addition may help to reduce Cd uptake. However, field trials are required to establish the extent to which Cd concentration in bunching spinach is reduced. Some greenhouse trials have shown that adding municipal compost or manure to soil can reduce Cd uptake by spinach (Al Mamun et al., 2016; Kumarpandit et al., 2017), while others found that the reduction in pH following biosolids compost addition negated the Cd binding by the compost (Paul and Chaney, 2017). The latter study highlighted the importance of pH management, along with Zn supplementation, to

Table 3
Soil–plant transfer relationships derived for onion and spinach.

Crop	Equation	Adj-R^2
Onion	3) $\log Cd_{onion} = 1.079 + 0.572 \log(Cd_{soil}) - 0.271$ pH	0.38
	4) $\log Cd_{onion}$ = Region $+0.50519 \log(Cd_{soil}) - 0.2422$ pH	0.5
	Region	
	Canterbury = 0.933	
	Hawke's Bay = 0.652	
	Pukekohe = 0.947	
	Waikato = 0.888	
	5) $\log Cd_{onion} = -1.365 + 0.481 \log(Cd_{soil}) + 1.60 \log(CEC) - 0.511 \log(Mg_{ex}) - 1.252 \log(Ca_{ex})$	0.48
Baby spinach	6) $\log Cd_{spin} = -0.138 + 0.2562 \log(Cd_{soil}) + 0.3875 \log(Mg_{ex})$	0.49
	7) $\log Cd_{spin} = 1.37 + 0.405 \log(Cd_{soil}) + 0.36 \log(Mg_{ex}) - 0.732 \log(Zn)$	0.55
Bunching spinach	8) $\log Cd_{spin} = 0.8022 + 0.8988 \log(Cd_{soil}) - 0.7958 \log(C)$	0.42

minimise Cd uptake by spinach. Other management factors such as the watering regime (Tack, 2017), and nitrogenous fertilisers may also influence Cd uptake (e.g. Jonsson and Asp 2011). Further research on the influence of crop management practices, in particular water management but also N fertiliser and trace element application (e.g. Zn), on Cd uptake by spinach is required to determine the extent to which these practices can also be implemented to lower Cd uptake.

Finally, we note that the EU ML for spinach is double that of the FSANZ ML (0.2 mg·kg^{-1} [FW] vs 0.1 mg·kg^{-1} [FW]). As the standards are established on the basis of being as low as reasonably achievable while ensuring protection of human health, it may be relevant to review the FSANZ ML for spinach to determine whether this principle is being met.

Soil Cd concentrations are often the primary focus for managing Cd uptake in food crops with soil–plant transfer models indicating key soil variables that influence Cd uptake and used to develop risk-based soil guideline values as a function of soil properties (e.g. de Vries and McLaughlin, 2013; Lu et al., 2017). Such values have successfully been used by farmers in the border area of the Netherlands and Belgium, resulting in trust between supermarkets, food companies, and regulators (Smolders et al., 2008). Our derived values are similar to those derived by Smolders et al., 2008 for onions and spinach, although are much higher for lettuce. However, the relatively low proportion of variation in plant Cd explained by our derived soil–plant relationships limits their application for this purpose. The wide range of nCdFS values derived using PUF highlights the variation that can exist between sites, and the challenge in applying generic Cd soil guideline values without considering other soil properties, even if developed for a specific crop and region. Equal weight should be placed on managing other soil properties, in particular pH and C, to ensure these are also at an optimum for minimising Cd uptake. Further, we suggest that crop management factors, in particular water management, may play a greater role in determining Cd uptake than previously thought, especially where relatively high plant Cd concentrations are observed in crops grown in soils with comparatively low soil Cd concentrations.

4. Conclusions

Cadmium concentrations in a range of lettuce types and cultivars were more than tenfold lower than the FSANZ ML of 0.1 mg·kg^{-1} (FW), with the lowest Cd concentrations consistently occurring in Iceberg lettuce. Cadmium concentrations in onions varied between sites, and mean concentrations (0.016 mg.kg^{-1} FW) were well below the Codex and EC MLs of 0.05 mg·kg^{-1}(FW). The highest Cd concentrations were observed in spinach, with concentrations in baby leaf and bunching spinach approaching or exceeding the FSANZ ML of 0.1 mg·kg^{-1} (FW) at a number of sites, although they were below the Codex and EU MLs of 0.2 mg·kg^{-1} (FW).

Table 4
Soil cadmium concentrations at which EU maximum levels for onions (0.05 mg·kg^{-1} FW) and FSANZ maximum levels for leafy greens (0.1 mg·kg^{-1} FW) are predicted to be met (nCdFS), as a function of soil properties.

Onions						Spinach	
Soil pH	nCdFS (mg kg^{-1})					soil C	nCdFS (mg kg^{-1})
	National average	Canterbury	Hawkes Bay	Pukekohe	Waikato		
5.5	1.7	1.6	5.6	1.5	1.9	2	0.24
6	2	2.7	9.8	2.5	3.3	3.5	0.36
6.5	2.2	4.7	17.0	4.4	5.8	5	0.48

In addition to soil Cd concentrations, pH was a significant factor influencing Cd uptake in onions, and exchangeable Mg or C significantly influenced Cd concentrations in baby leaf and bunching spinach, respectively. The identification of pH and C as significant factors influencing Cd uptake in onion and bunching spinach, respectively, indicates management of these properties may help to minimise Cd uptake in those crops.

Soil–plant relationships identified for onions and spinach explained a low to moderate proportion of the variation, creating a low confidence in risk-based soil guideline values determined from these relationships. This is particularly the case for onions, for which the soil–plant relationship under-predicted elevated onion Cd concentrations. This low to moderate proportion of explained variation is partly attributable to the narrow range in some measured soil properties, but also suggests that factors other than those assessed are influencing plant uptake. More comprehensive research is required to determine the extent to which plant management practices such as water management or fertiliser type (especially micronutrients) and rates affect Cd uptake in these crops.

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Competing interests

The authors declare no competing interests.

Table 5
Soil Cd concentrations (mean and 95th percentile range) at which EU maximum levels for onions (0.05 mg·kg^{-1} FW) and FSANZ maximum levels for leafy greens (0.1 mg·kg^{-1} FW) are predicted to be met (nCdFS), based on observed plant uptake in different regions.

Region	nCdFS (mg kg^{-1})		
	Onion	Lettuce	Spinach (all regions[a])
Pukekohe	1.43 (0.38–2.94)	5.6 (2.7–8.2)	0.35 baby leaf (0.14–0.7)
Waikato	2.54 (1.05–5.0)		
Gisborne		0.7 (0.45–1.1)	0.29 bunching spinach 0.2–0.44)
Hawke's Bay	2.32 (0.80–5.7)		
Canterbury	0.72 (0.35–1.24)	1.2 (0.65–1.9)	

[a] Regional estimates were not made as samples were from a limited number of sites within each region.

Author contributions

JC conceptualised the project, acquired the funding undertook the investigation data analysis and developed the original draft of the manuscript. ZY assisted with investigation under the supervision of NL, JC and BR. KM assisted with investigation, data curation and review of the manuscript. CG, NL, BR supported conceptualisation of the project and undertook review and editing of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.03.010.

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