Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Effects of split applications of nitrogen fertilizers on the Cd level and nutritional quality of Chinese cabbage[*#]

Shi-kai FAN, Jun ZHU, Wen-hao TIAN, Mei-yan GUAN, Xian-zhi FANG, Chong-wei JIN[†‡]

(*College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China*)

[†]E-mail: jincw@zju.edu.cn

Received June 13, 2016; Revision accepted Jan. 16, 2017; Crosschecked Sept. 15, 2017

Abstract: Cadmium (Cd) contamination in soil is an increasingly serious problem. Management of plant nutrients has been proposed as a potentially promising strategy for minimizing Cd accumulation in crops grown in contaminated soil. This study investigated the effects of split applications of nitrogen (N) fertilizers on the Cd concentration in Chinese cabbage (*Brassica chinensis* L.) plants grown in Cd-contaminated soil. Compared with single applications, split applications of ammonium or urea resulted in significantly lower Cd concentrations, and higher biomass production and antioxidant-associated nutritional quality in the edible plant parts. However, when nitrate was used as the N fertilizer, there were no significant differences between the split and single applications for the same parameters. We conclude that a split application could be more beneficial than a single application method when ammonium or urea is used as the N fertilizer for vegetable cultivation in Cd-contaminated soil.

Key words: Fertilization method; Cadmium (Cd); Nitrogen (N); Nutritional quality
http://dx.doi.org/10.1631/jzus.B1600272 **CLC number:** X592; S365

1 Introduction

Contamination of soil by heavy metals is an increasingly serious problem due to various anthropogenic activities, such as the application of sewage sludge, mining and smelting of metalliferous ores, and overuse of agrochemicals (Kirkham, 2006; Wei and Yang, 2010). Cadmium (Cd) is one of the most toxic heavy metals. In the human body, it adversely affects kidneys and bones (Clemens *et al.*, 2013). The primary route of Cd entry into the body is through the consumption of crops grown in Cd-contaminated soil (Clemens *et al.*, 2013). A recent survey indicated that vegetables account for about 40% of the total Cd exposure of residents of Shanghai, China's largest city (He *et al.*, 2013). Therefore, measures should be developed to minimize the amount of Cd entering vegetables through contaminated soil. It has been proposed that the management of plant nutrients is potentially a promising strategy for minimizing Cd accumulation in crops grown in contaminated soil (Sarwar *et al.*, 2010). Growers have already applied nutrients, particularly macronutrients, to obtain a higher crop yield. Many nutrients have direct and indirect effects on Cd bioavailability in soil and Cd uptake through the roots of plants. For instance, phosphate favors precipitation to decrease Cd availability in soil (Dheri *et al.*, 2007; Dong *et al.*, 2007) and divalent cations, such as Fe^{2+} and Zn^{2+}, compete with Cd^{2+} for the same membrane transporters thereby reducing Cd entry into plants (Lux *et al.*, 2011). Accordingly, if growers keep in mind the interactions between nutrients and Cd, with the proper management of nutrients, they could have a cost-effective and time-saving strategy for reducing Cd accumulation in crops.

[‡] Corresponding author
[*] Project supported by the National Key Research and Development Project of China (No. 2016YFD0200103), the National Natural Science Foundation of China (Nos. 31622051 and 31670258), and the Zhejiang Provincial Natural Science Foundation of China (No. LR13C130001)
[#] Electronic supplementary materials: The online version of this article (http://dx.doi.org/10.1631/jzus.B1600272) contains supplementary materials, which are available to authorized users
ORCID: Shi-kai FAN, http://orcid.org/0000-0001-5324-3522

Management of nitrogen (N) is one of the most frequent agronomic practices. Nitrogen is taken up by plants mainly in the form of ammonium (NH_4^+) and nitrate (NO_3^-). Physiologically, NO_3^- uptake by roots is accompanied by a simultaneous uptake of protons (H^+), resulting in an increase in rhizosphere pH (Crawford and Glass, 1998). Conversely, when NH_4^+ is taken up, the H^+ is released into the rhizosphere, resulting in acidification of the rhizosphere soil (Hinsinger *et al.*, 2003). The nitrification of NH_4^+ by microorganisms also produces H^+, further increasing the soil acidification caused by the release of H^+ by roots during ammonium uptake (Herman *et al.*, 2006). The soil pH strongly affects Cd availability, which is increased when the pH drops (Kirkham, 2006). Accordingly, it has often been suggested that NH_4^+ fertilizers could result in higher Cd availability in rhizosphere soil than NO_3^- fertilizers (Sarwar *et al.*, 2010). This suggestion has been supported by many studies (Florijn *et al.*, 1992; Tsadilas *et al.*, 2005; Zaccheo *et al.*, 2006), in which N fertilizers were applied to the soil as a single fertilizer treatment. Nevertheless, in practical crop cultivation, a split application of N fertilizers at different growth stages is often recommended by agronomists, since this method of fertilization favors plant growth (López-Bellido *et al.*, 2005). A change in plant growth may affect Cd uptake by roots, thereby altering the Cd accumulation in plant tissues. In addition, NH_4^+ has a high rate of nitrification in soil (Zerulla *et al.*, 2001). Accordingly, in comparison with a single application, split fertilization theoretically decreases the rate of NH_4^+ nitrification in the soil, thereby increasing N uptake by the roots in the form of NH_4^+. Since more protons are released from NH_4^+ nitrification than from NH_4^+ uptake by the root cells, a split application of NH_4^+ might be expected to alleviate the acidification-increased Cd solubility, compared with a single application. Considering the approach described above, the method of N fertilization may dramatically affect Cd accumulation in vegetables and other crops. However, little information is available to support this speculation.

Currently, three forms of N fertilizers, ammonium, nitrate, and amide, are most commonly used in practical crop production. Nitrate is generally the preferred form for most vegetable plants (Chen *et al.*, 2005). However, which forms of N fertilizer are used in practical vegetable cultivation in a region depends largely on the traditional practice of the local growers and the fertilizer provided by local fertilizer dealers. Therefore, it is necessary to investigate the effects of the method of fertilization application (single or split applications) on Cd accumulation in the edible parts of vegetables when using different forms of the N nutrient. In this study, the Chinese cabbage (*Brassica chinensis* L.), one of the most popular vegetables in China, was used to address this issue. The quality of the nutritional characteristics of vegetables, such as the levels of soluble sugar, soluble protein, and antioxidants, is also very important for human health. Previous studies have found that Cd contamination in the growth medium clearly affects the nutritional quality of vegetables (Prince *et al.*, 2002). Therefore, we also investigated the effects of the N fertilization method on the nutritional quality of Chinese cabbage grown in Cd-contaminated soil.

2 Materials and methods

2.1 Soil and plants

The soil used for the pot experiment was collected from Jianggan District, Hangzhou, China (30°16′ N, 120°12′ E). The basic properties of the soil, determined by standard procedures (Bao, 2008), are summarized in Table 1. After the air-dried soil had been ground to pass through a 2-mm sieve, chemical fertilizers were mixed into the soil: K_2SO_4 at 160 mg K/kg soil and KH_2PO_4 at 150 mg P/kg soil. Then, half of the soil was mixed with $CdCl_2$ at 10 mg Cd/kg soil. Plant pots, 2.5 L in volume, were then each filled with 3 kg of soil. The Chinese cabbage vegetable plant *B. chinensis* L. cv. Changfeng was used in this study.

Table 1 Relevant agrochemical properties of the tested soil

Sample	pH	CEC (cmol/kg)	EC (mS/cm)	Clay (%)	Silt (%)	Sand (%)	Organic C (g/kg)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Total Cd (mg/kg)
Pot-culture soil	7.2	7.9	0.7	6.2	28.8	65.0	11.5	2.5	13.4	0.2

CEC: cation-exchange capacity; EC: electrical conductivity. 1 cmol/kg=0.01 mol/kg

2.2 Plant cultivation and nitrogen treatments

The pot experiment was conducted in a greenhouse. The soil in the pots was watered to 60% field water holding capacity (FWHC; 22% water content, w/w). Chinese cabbage seeds were sown at a depth of 1 cm, with ten seeds per pot. The pots were divided into two groups, with one group given a single N application, and the other a split N application. For the single N application, at the sowing stage, the soil was fertilized with either $(NH_4)_2SO_4$, $Ca(NO_3)_2$, or $CO(NH_2)_2$ (urea) at 400 mg N/kg soil. For the split N application, one of the above three N fertilizers was applied to the soil at rates of 120, 120, and 160 mg N/kg soil at the sowing, seedling, and vegetative growth stages, respectively. When the second true leaves were observed, the seedlings in each pot were thinned to three plants of similar size. During the experimental period, water was added to compensate for evaporation and transpiration, and soil moisture content was maintained at about 60% of FWHC. The edible parts of the 8-week-old plants were harvested for further analysis.

2.3 Measurements of the concentrations of soluble sugar, soluble protein, ascorbate, total phenolics, and total flavonoids, and DPPH free radical-scavenging activity

The concentrations of soluble sugar, soluble protein, ascorbate, total phenolics, and total flavonoids, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity were analyzed as described in our previous study (Jin *et al.*, 2013).

2.4 Analysis of cadmium content

Plant tissues were dried at 80 °C for 48 h, and then the dried samples were wet-digested, as previously described (Luo *et al.*, 2012). The digestates were diluted with ultrapure water, and the concentrations of Cd in the digestates were analyzed using an absorption spectrometer (Thermo Scientific AAS, iCE 3300), following a standard procedure. The same procedure without samples was used as a control and three replications were conducted for each sample. Quality assurance and quality control of the analysis included the recovery of spiked Cd (better than 95%) from an independent standard source, the analysis of blank and standard references, and the use of duplicates for every set of ten samples.

2.5 Statistics

All statistical analyses were conducted with IBM SPSS Version 20 (IBM Corp., Armonk, NY, USA). Means were compared using Duncan's multiple range test with a significance level of $P<0.05$ in all cases.

3 Results

3.1 Effects of the N fertilization methods on the yield of the edible parts

The effect of the fertilization method on the biomass production of Chinese cabbage was highly dependent on the form of N fertilizer in both the control (no Cd added) and the Cd-added soils (Fig. 1). When ammonium ($(NH_4)_2SO_4$) or amide (urea) was used as the N source, the biomass of the edible parts of the plants from the split dressing treatment was increased by 46% and 30% in the control soil, respectively, and by 47% and 24% in the Cd-added soil, respectively, compared with that of the plants from the single dressing treatment. However, when nitrate ($Ca(NO_3)_2$) was used as the N source, there was no statistically significant difference in the biomass of the edible parts between the single and split dressings in either the control or Cd-added soils. The above results indicate that the impact of the N fertilization method on the biomass production of Chinese cabbage was independent of Cd contamination in the soil. Note that, within the same fertilization method, the edible-part biomass of the plants fed with nitrate was greater than that of the plants fed with ammonium or amide in either the control or the Cd-added soil.

3.2 Effects of the N fertilization methods on the Cd levels in edible parts

No Cd was detected in the edible parts of the Chinese cabbage grown in the control soil following any treatment (data not shown). In the Cd-added soil, the N fertilization method clearly affected the Cd concentration in the edible parts, but the effect was dependent on the form of the N fertilizer. The Cd concentrations in the edible parts following split dressing treatments of ammonium and amide were about 40% and 60% lower, respectively, than those following single dressing treatments of these two fertilizers (Fig. 2). However, when nitrate was used as the N source, the Cd concentration in the edible parts

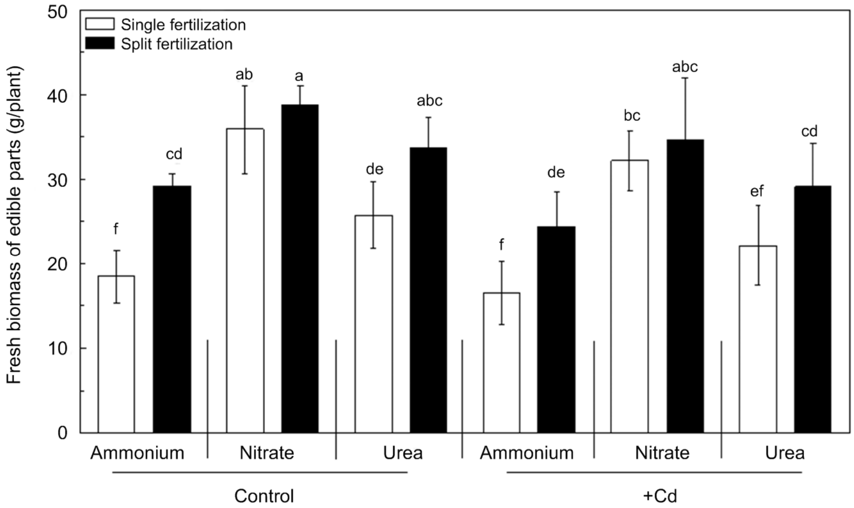

Fig. 1 Effects of the N fertilization methods on the yield of edible parts

The plants were cultured in pots. For single N applications, the soil was fertilized with $(NH_4)_2SO_4$ (ammonium), $Ca(NO_3)_2$ (nitrate), or $CO(NH_2)_2$ (urea) at 400 mg N/kg soil at the sowing stage. For split N applications, each of the above three N fertilizers was applied to the soil at rates of 120, 120, and 160 mg N/kg soil at the sowing, seedling, and vegetative growth stages, respectively. The edible parts of 8-week-old plants were harvested for analysis. Data are expressed as mean±standard deviation (SD) (n=6). Different letters represent significantly different values (Duncan's test, P<0.05)

following the split dressing treatment was similar to that following the single dressing treatment. The N form also significantly affected the Cd concentration in the edible parts when the fertilizers were applied using the same method (Fig. 2). With single applications, the Cd concentration was much lower in the nitrate treatment than in the ammonium or amide treatment. With split applications, the Cd concentrations in the nitrate and amide treatments were similar, but were clearly lower than those in the ammonium treatment.

3.3 Effects of the N fertilization methods on the nutritional quality of the edible parts

The fertilization method had little effect on the level of soluble sugar in the edible parts of Chinese cabbage grown in the control soil for all three N forms of fertilizer. In the Cd-added soil, the soluble sugar levels with the split dressing method were higher in the ammonium treatments but lower in the nitrate and amide treatments, compared with the levels in the single dressing method (Fig. 3a). We also determined the level of soluble protein in the edible plant parts. In the control soil, it was higher following the split dressing than following the single dressing regardless of the N form, but the difference was not statistically significant in the plants fed with nitrate. However, in the Cd-added soil, the split dressing method resulted in a slightly lower level of soluble protein in the plants fed with ammonium or urea, but a significantly higher level (by about 35%) in the plants fed with nitrate, compared with the single dressing (Fig. 3b).

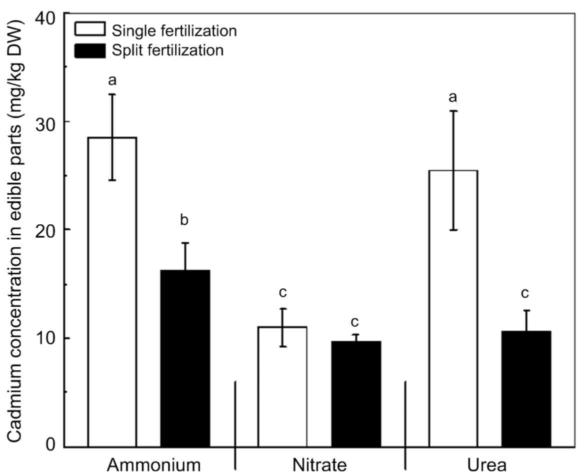

Fig. 2 Effects of the N application methods on Cd levels in the edible parts in the Cd-added soil

Treatments are the same as in Fig. 1. The data are expressed as mean±SD (n=5). Different letters represent significantly different values (Duncan's test, P<0.05)

To evaluate the antioxidant capacity in the edible parts of the Chinese cabbage, we first measured the

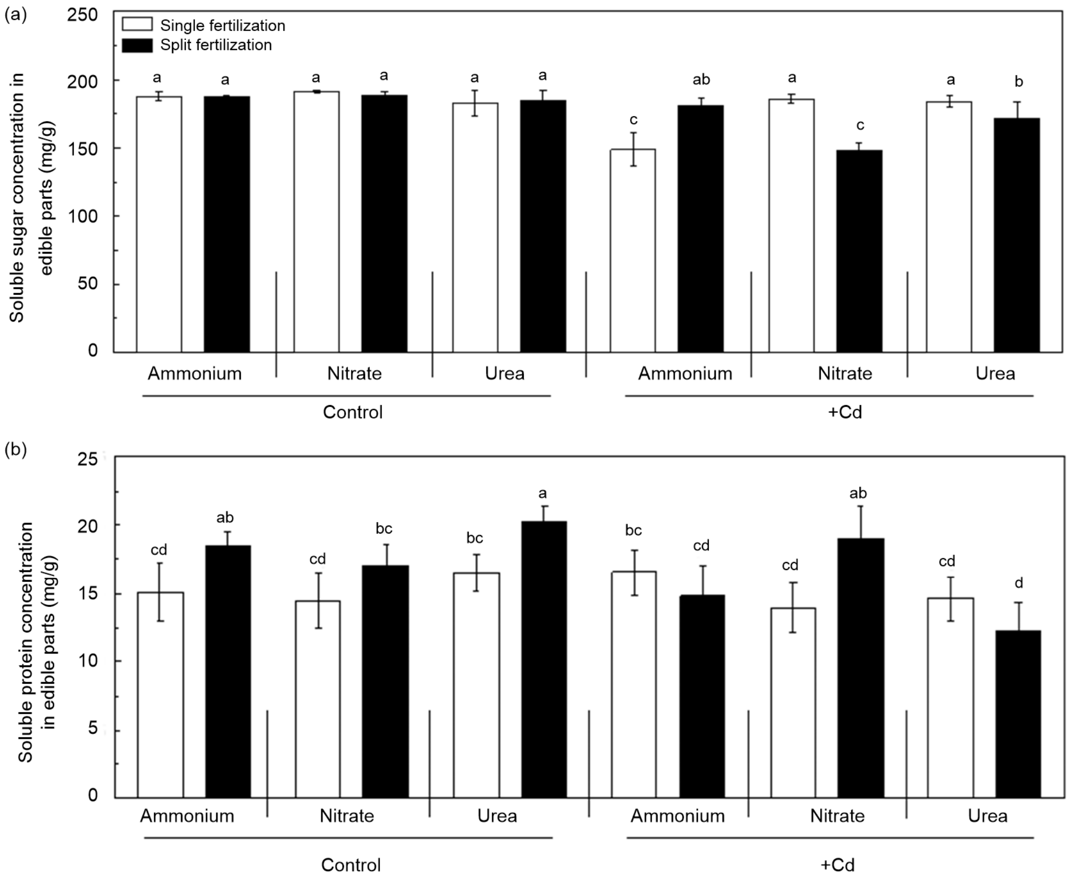

Fig. 3 Effects of the N application methods on soluble sugar and soluble protein levels in the edible plant parts
(a) The levels of soluble sugar in the edible plant parts. (b) The levels of soluble protein in the edible plant parts. Data are expressed as mean±standard deviation (SD) (n=6). Different letters represent significantly different values (Duncan's test, P<0.05)

levels of antioxidation-associated compounds. In both the control and Cd-added soils, the application method barely affected the ascorbate level in the edible plant parts for all three forms of N treatment (Table 2). The application method also had little effect on the levels of total phenolics and flavonoids in the edible parts of the plants grown in the control soil. However, in the Cd-added soil, the total phenolic level in the edible plant parts following the split dressing was significantly higher for all three forms of N treatment, compared with the single dressing. In addition, the split dressing also had a significantly higher level of flavonoids than the single dressing with the nitrate treatment. We then analyzed the total antioxidant capacity. The results show that, in the control soil, the application method had little effect on the DPPH radical-scavenging activity of the edible plant parts for all three forms of N treatment. In the Cd-added soil, the DPPH radical-scavenging activity of the edible plant parts with the ammonium and urea treatments was significantly higher following a split dressing compared with a single dressing, whereas with the nitrate treatment, this activity was not affected (Fig. 4).

4 Discussion

Usually, a split application of N fertilizer gives a greater yield in crop cultivation than a single application, since the split fertilization favors a greater increase in N use efficiency (de Ruijter *et al.*, 2010). However, in China, growers often use single fertilizer applications in the cultivation of short life-cycle crops like Chinese cabbage. In this study, we found that split applications of ammonium and urea clearly resulted in a higher yield and a lower Cd concentration in the edible parts of Chinese cabbage, compared with

Table 2 Effects of the N fertilization methods on levels of total phenolics, ascorbate, and flavonoids in the edible plant parts

Treatment		Total phenolics (mg GAE/g DW)		Ascorbate (μmol/g DW)		Flavonoids (μmol DAE/g DW)	
N form	Application	Control	+Cd	Control	+Cd	Control	+Cd
Ammonium	Single	18.42±0.98^{a}	17.85±0.78^{bc}	14.92±5.30^{a}	14.42±2.54^{a}	3.14±0.27^{a}	2.75±0.05^{b}
	Split	17.10±0.63^{ab}	20.07±0.29^{a}	12.95±1.82^{a}	14.80±1.86^{a}	2.89±0.12^{a}	3.12±0.53^{ab}
Nitrate	Single	16.17±0.85^{b}	17.50±0.09^{c}	14.28±1.18^{a}	11.06±2.43^{a}	2.77±0.18^{a}	2.65±0.17^{b}
	Split	16.86±0.80^{ab}	18.68±0.15^{b}	14.55±4.17^{a}	11.91±1.48^{a}	2.84±0.22^{a}	3.20±0.38^{a}
Urea	Single	17.47±0.88^{ab}	18.76±0.68^{b}	12.89±2.10^{a}	12.22±2.66^{a}	3.11±0.45^{a}	3.13±0.10^{a}
	Split	16.15±1.48^{b}	20.63±1.26^{a}	15.65±2.89^{a}	11.42±2.61^{a}	2.91±0.51^{a}	2.98±0.47^{ab}

The plants were cultured in pots. For single N applications, the soil was fertilized with $(NH_4)_2SO_4$ (ammonium), $Ca(NO_3)_2$ (nitrate), or $CO(NH_2)_2$ (urea) at 400 mg N/kg soil at the sowing stage. For split N applications, each of the above three N fertilizers was applied to the soil at rates of 120, 120, and 160 mg N/kg soil at sowing, seedling, and vegetative growth stages, respectively. The edible parts of 8-week-old plants were harvested for analysis. The data are expressed as mean±SD (n=4). Different letters represent significantly different values in the same column (Duncan's test, P<0.05). GAE: gallic acid equivalent; DAE: 3,4-dihydroxybenzoic acid equivalent; DW: dry weight

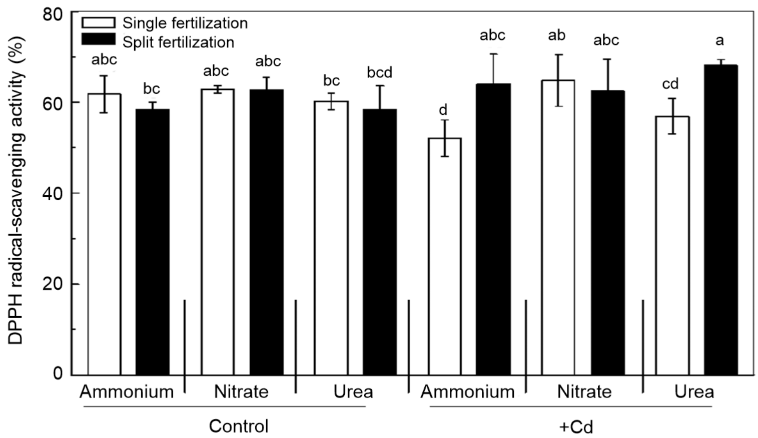

Fig. 4 Effects of the N fertilization methods on DPPH radical-scavenging activity in the edible plant parts
Data are expressed as mean±standard deviation (SD) (n=6). Different letters represent significantly different values (Duncan's test, P<0.05)

single applications of these fertilizers (Figs. 1 and 2). Note that, although the application method for the nitrate treatment did not affect the Cd level or biomass of edible parts (Figs. 1 and 2), the split fertilization did have the potential for higher nitrogen use efficiency and could reduce the emission of greenhouse gases such as nitrous oxide (López-Bellido *et al.*, 2005; Gillam *et al.*, 2008). Therefore, in Cd-contaminated soil, we recommend split fertilization for vegetable cultivation, regardless of the type of N fertilizer. The Cd uptake by the roots of plants can be affected by a number of factors, including soil pH, chelation, plant nutrients, root exudates, plant species, and microorganisms (Sarwar *et al.*, 2010; Clemens *et al.*, 2013). There are two main ways by which these factors affect the Cd uptake in plants: (1) by altering the bioavailability of Cd in the growth media; (2) by modulating the root uptake capacity for Cd. Soil acidification resulting from ammonium nitrification is more pronounced than that caused by ammonium uptake by root cells (Tudoreanu and Phillips, 2004), and thus ammonium nitrification should increase the Cd solubility in the soil more than ammonium uptake. The behavior of urea in soil is similar to that of ammonium as urea can be decomposed into ammonium by urease. However, in our study, we found that the fertilization method had little effect on the pH of the soil (Fig. S1). This may be because the soil used in the present study had a higher buffering capacity against acidification (Fig. S2). Therefore, soil acidification resulting from ammonium nitrification cannot explain why the split applications of ammonium or urea had lower Cd levels in the edible parts of the Chinese cabbage plants compared with the single applications of these fertilizers. Our previous study showed that the presence of nitrate in the growth medium facilitates the uptake of Cd by root cells (Luo *et al.*, 2012; Mao *et al.*, 2014). In this context, the decreased nitrate generation from ammonium and urea due to the decreased nitrification from split fertilization may prevent the effect of nitrate in promoting the uptake of Cd by plant roots. This may explain why split applications of ammonium and urea resulted in lower Cd levels in the edible parts of the Chinese cabbage, compared with single applications of these fertilizers. In addition, because the split applications of ammonium and urea resulted in a higher biomass than the single applications (Fig. 1), the dilution effect may be another key reason for the lower Cd level in the edible parts with the split fertilization method. In recent decades, controlled-release fertilizers (CRFs) have been rapidly developed (Shaviv, 2001). CRFs enable nutrients to be released over an extended period of time, regulating the nutrient release time through the excipients, and therefore are more efficient than conventional fertilizers (Sempeho *et al.*, 2014). Theoretically, application of ammonium- or urea-based CRFs could further decrease the rate of ammonium nitrification in soil. Therefore, CRFs may be a promising fertilizer for use in Cd-contaminated soil. Note that, in single applications, the Cd concentration in the edible plant parts was much lower with the nitrate treatment than with the ammonium or amide treatment. One explanation may be the dilution effect, because the nitrate fertilizer produced a higher biomass than the other two fertilizers (Fig. 1). In addition, the nitrate fertilizer used in the present study was $Ca(NO_3)_2$. The Ca^{2+} and Cd^{2+} would compete for the same Ca^{2+} channels during their uptake by roots cells (Perfus-Barbeoch *et al.*, 2002). Therefore, the antagonism of Ca to Cd may be another reason for the lower Cd level in the edible plant parts following nitrate treatment.

Our results showed that the nutritional quality of Chinese cabbage grown in Cd-added soil was affected by different fertilization methods. Soluble sugar levels are used to evaluate the taste characteristics of vegetables and soluble protein accumulation as an indicator of value for human nutrition (Hurrell, 2003; Jin *et al.*, 2009). The split applications produced a higher level of soluble sugar in the ammonium treatment, and a higher level of soluble protein in the nitrate treatment, compared with the single applications. The level of antioxidants is another important factor for evaluating vegetable nutritional quality (Shyamala *et al.*, 2005). A higher intake of vegetables containing antioxidants including phenolics, flavonoids, and ascorbic acid may contribute to protecting against oxidative damage, thus lowering cancer, cardiovascular disease, and other chronic disease risks. For instance, it is reported that human serum and low-density lipoprotein oxidation can be inhibited by phenolic compounds while ascorbic acid can protect against DNA damage in human sperm and improve the efficacy of antineoplastic drugs such as paclitaxel (Fraga *et al.*, 1991; Frankel *et al.*, 1995; Abu-Amsha *et al.*, 1996; Kurbacher *et al.*, 1996). In addition, flavonoids can increase plasma antioxidant capacity in humans and protect human retinal pigment epithelial cells from oxidative-stress-induced death (Hanneken *et al.*, 2006; Lotito and Frei, 2006). Here, we found that, following any of the three forms of N treatments, the split application resulted in higher levels of phenolics in the edible parts of the plants grown in Cd-added soil, but had little effect on the level of ascorbic acid, compared with the single application. Only the split application of nitrate to Cd-added soil resulted in higher levels of flavonoids in edible plant parts. The above results indicate that the fertilization method differentially affected the production of antioxidants in edible plant parts. Therefore, we further investigated the effect of the fertilization method on the total antioxidant capacity in the edible parts of Chinese cabbage. The DPPH antioxidant assay is used widely to estimate total antioxidant capacity in vegetable extracts (Sanchez-Moreno, 2002). We showed that, in the Cd-added soil, the DPPH radical-scavenging activity in the edible plant parts following the split application was higher than that following the single application for the ammonium or urea treatments. However, there was no statistical difference between the two application methods when nitrate was used as the sole N source. Interestingly, the effects of the application methods on elevating DPPH radical-scavenging activity correlated well with their effects in either decreasing Cd levels or increasing the biomass of edible parts, indicating that an appropriate fertilization method not only reduces the human health risk of Cd exposure from crops grown in Cd-contaminated soil, but also improves the nutritional quality and yield of the crops.

5 Conclusions

In summary, this study provided evidence that in Cd-added soil, the split application of ammonium or amide resulted in a significantly lower Cd concentration, higher biomass, and improved nutritional quality in the edible parts of Chinese cabbage compared with a single application. Therefore, in Cd-contaminated soil, we recommend a split application method for vegetable cultivation to increase food safety when ammonium or urea is used as the N fertilizer.

Compliance with ethics guidelines

Shi-kai FAN, Jun ZHU, Wen-hao TIAN, Mei-yan GUAN, Xian-zhi FANG, and Chong-wei JIN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Abu-Amsha, R., Croft, K.D., Puddey, I.B., *et al*., 1996. Phenolic content of various beverages determines the extent of inhibition of human serum and low-density lipoprotein oxidation in vitro: identification and mechanism of action of some cinnamic acid derivatives from red wine. *Clin. Sci*., **91**(4):449-458. http://dx.doi.org/10.1042/cs0910449

Bao, S.D., 2008. Soil Agro-chemistrical Analysis. China Agriculture Publishing House, Beijing, China, p.14-69 (in Chinese).

Chen, W., Luo, J.K., Shen, Q.R., 2005. Effect of NH_4^+-N/NO_3^--N ratios on growth and some physiological parameters of Chinese cabbage cultivars. *Pedosphere*, **15**(3):310-318.

Clemens, S., Aarts, M.G.M., Thomine, S., *et al*., 2013. Plant science: the key to preventing slow cadmium poisoning. *Trends Plant Sci*., **18**(2):92-99. http://dx.doi.org/10.1016/j.tplants.2012.08.003

Crawford, N.M., Glass, A.D.M., 1998. Molecular and physiological aspects of nitrate uptake in plants. *Trends Plant Sci*., **3**(10):389-395. http://dx.doi.org/10.1016/S1360-1385(98)01311-9

de Ruijter, F.J., ten Berge, H.F.M., Smit, A.L., 2010. Strategies to increase nitrogen use efficiency and reduce nitrate leaching in vegetable production in the Netherlands. *Acta Hortic*., **852**:107-114. http://dx.doi.org/10.17660/ActaHortic.2010.852.11

Dheri, G.S., Brar, M.S., Malhi, S.S., 2007. Influence of phosphorus application on growth and cadmium uptake of spinach in two cadmium-contaminated soils. *J. Plant Nutr. Soil Sci*., **170**(4):495-499. http://dx.doi.org/10.1002/jpln.200625051

Dong, J., Mao, W.H., Zhang, G.P., *et al*., 2007. Root excretion and plant tolerance to cadmium toxicity—a review. *Plant Soil Environ*., **53**(5):193-200.

Florijn, P.J., Nelemans, J.A., van Beusichem, M.L., 1992. The influence of the form of nitrogen nutrition on uptake and distribution of cadmium in lettuce varieties. *J. Plant Nutr*., **15**(11):2405-2416. http://dx.doi.org/10.1080/01904169209364483

Fraga, C.G., Motchnik, P.A., Shigenaga, M.K., *et al*., 1991. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proc. Natl. Acad. Sci. USA*, **88**(24):11003-11006. http://dx.doi.org/10.1073/pnas.88.24.11003

Frankel, E.N., Waterhouse, A.L., Teissedre, P.L., 1995. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem*., **43**(4):890-894. http://dx.doi.org/10.1021/jf00052a008

Gillam, K.M., Zebarth, B.J., Burton, D.L., 2008. Nitrous oxide emissions from denitrification and the partitioning of gaseous losses as affected by nitrate and carbon addition and soil aeration. *Can. J. Soil Sci*., **88**(2):133-143. http://dx.doi.org/10.4141/CJSS06005

Hanneken, A., Lin, F.F., Johnson, J., *et al*., 2006. Flavonoids protect human retinal pigment epithelial cells from oxidative-stress-induced death. *Invest. Ophth. Vis. Sci*., **47**(7):3164-3177. http://dx.doi.org/10.1167/iovs.04-1369

He, P., Lu, Y.H., Liang, Y.H., *et al*., 2013. Exposure assessment of dietary cadmium: findings from Shanghainese over 40 years, China. *BMC Public Health*, **13**:590. http://dx.doi.org/10.1186/1471-2458-13-590

Herman, D.J., Johnson, K.K., Jaeger, C.H., *et al*., 2006. Root influence on nitrogen mineralization and nitrification in *Avena barbata* rhizosphere soil. *Soil Sci. Soc. Am. J*., **70**(5):1504-1511. http://dx.doi.org/10.2136/sssaj2005.0113

Hinsinger, P., Plassard, C., Tang, C.X., *et al*., 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. *Plant Soil*, **248**(1):43-59. http://dx.doi.org/10.1023/A:1022371130939

Hurrell, R.F., 2003. Influence of vegetable protein sources on trace element and mineral bioavailability. *J. Nutr*., **133**(9):2973s-2977s.

Jin, C.W., Du, S.T., Wang, Y., *et al*., 2009. Carbon dioxide enrichment by composting in greenhouses and its effect on vegetable production. *J. Plant Nutr. Soil Sci*., **172**(3):418-424. http://dx.doi.org/10.1002/jpln.200700220

Jin, C.W., Liu, Y., Mao, Q.Q., *et al*., 2013. Mild Fe-deficiency improves biomass production and quality of hydroponic-cultivated spinach plants (*Spinacia oleracea* L.). *Food Chem*., **138**(4):2188-2194. http://dx.doi.org/10.1016/j.foodchem.2012.12.025

Kirkham, M.B., 2006. Cadmium in plants on polluted soils: effects of soil factors, hyperaccumulation, and amendments. *Geoderma*, **137**(1-2):19-32. http://dx.doi.org/10.1016/j.geoderma.2006.08.024

Kurbacher, C.M., Wagner, U., Kolster, B., *et al.*, 1996. Ascorbic acid (vitamin C) improves the antineoplastic activity of doxorubicin, cisplatin, and paclitaxel in human breast carcinoma cells in vitro. *Cancer Lett.*, **103**(2):183-189. http://dx.doi.org/10.1016/0304-3835(96)04212-7

López-Bellido, L., López-Bellido, R.J., Redondo, R., 2005. Nitrogen efficiency in wheat under rainfed Mediterranean conditions as affected by split nitrogen application. *Field Crop. Res.*, **94**(1):86-97. http://dx.doi.org/10.1016/j.fcr.2004.11.004

Lotito, S.B., Frei, B., 2006. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? *Free Radical Biol. Med.*, **41**(12):1727-1746. http://dx.doi.org/10.1016/j.freeradbiomed.2006.04.033

Luo, B.F., Du, S.T., Lu, K.X., *et al.*, 2012. Iron uptake system mediates nitrate-facilitated cadmium accumulation in tomato (*Solanum lycopersicum*) plants. *J. Exp. Bot.*, **63**(8): 3127-3136. http://dx.doi.org/10.1093/jxb/ers036

Lux, A., Martinka, M., Vaculik, M., *et al.*, 2011. Root responses to cadmium in the rhizosphere: a review. *J. Exp. Bot.*, **62**(1):21-37. http://dx.doi.org/10.1093/jxb/erq281

Mao, Q.Q., Guan, M.Y., Lu, K.X., *et al.*, 2014. Inhibition of nitrate transporter 1.1-controlled nitrate uptake reduces cadmium uptake in *Arabidopsis*. *Plant Physiol.*, **166**(2): 934-944. http://dx.doi.org/10.1104/pp.114.243766

Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A., *et al.*, 2002. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. *Plant J.*, **32**(4):539-548. http://dx.doi.org/10.1046/j.1365-313X.2002.01442.x

Prince, W.S., Kumar, P.S., Doberschutz, K.D., *et al.*, 2002. Cadmium toxicity in mulberry plants with special reference to the nutritional quality of leaves. *J. Plant Nutr.*, **25**(4):689-700. http://dx.doi.org/10.1081/PLN-120002952

Sanchez-Moreno, C., 2002. Review: methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci. Technol. Int.*, **8**(3):121-137. http://dx.doi.org/10.1177/1082013202008003770

Sarwar, N., Saifullah, Malhi, S.S., *et al.*, 2010. Role of mineral nutrition in minimizing cadmium accumulation by plants. *J. Sci. Food Agric.*, **90**(6):925-937. http://dx.doi.org/10.1002/jsfa.3916

Sempeho, S.I., Kim, H.T., Mubofu, E., *et al.*, 2014. Meticulous overview on the controlled release fertilizers. *Adv. Chem.*, **2014**:1-16. http://dx.doi.org/10.1155/2014/363071

Shaviv, A., 2001. Advances in controlled-release fertilizers. *Adv. Agron.*, **71**:1-49. http://dx.doi.org/10.1016/S0065-2113(01)71011-5

Shyamala, B.N., Gupta, S., Lakshmi, A.J., *et al.*, 2005. Leafy vegetable extracts—antioxidant activity and effect on storage stability of heated oils. *Innov. Food Sci. Emerg.*, **6**(2):239-245. http://dx.doi.org/10.1016/j.ifset.2004.12.002

Tsadilas, C.D., Karaivazoglou, N.A., Tsotsolis, N.C., *et al.*, 2005. Cadmium uptake by tobacco as affected by liming, N form, and year of cultivation. *Environ. Pollut.*, **134**(2): 239-246. http://dx.doi.org/10.1016/j.envpol.2004.08.008

Tudoreanu, L., Phillips, C.J.C., 2004. Modeling cadmium uptake and accumulation in plants. *Adv. Agron.*, **84**:121-157. http://dx.doi.org/10.1016/S0065-2113(04)84003-3

Wei, B.G., Yang, L.S., 2010. A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from china. *Microchem. J.*, **94**(2):99-107. http://dx.doi.org/10.1016/j.microc.2009.09.014

Zaccheo, P., Crippa, L., Pasta, V.D.M., 2006. Ammonium nutrition as a strategy for cadmium mobilisation in the rhizosphere of sunflower. *Plant Soil*, **283**(1):43-56. http://dx.doi.org/10.1007/s11104-005-4791-x

Zerulla, W., Barth, T., Dressel, J., *et al.*, 2001. 3,4-Dimethylpyrazole phosphate (DMPP)—a new nitrification inhibitor for agriculture and horticulture. *Biol. Fert. Soils*, **34**(2):79-84. http://dx.doi.org/10.1007/s003740100380

List of electronic supplementary materials

Fig. S1 Effects of the N fertilization methods on soil pH
Fig. S2 pH buffer capacity of the soil

中文概要

题　目：氮肥分次施用对小白菜镉积累及品质的作用

目　的：研究不同形态氮肥的分次施用对小白菜镉积累、产量及品质的影响，并初步分析了其作用机制。

创新点：首次提出尿素、铵态氮和硝态氮肥料的分次施用方式可提升镉污染土壤上的作物安全生产。

方　法：选用尿素、硫酸铵和硝酸钙等 3 种常用氮肥，通过盆栽试验，比较了这 3 种肥料的分次施用方法和一次性施用方法对小白菜的镉含量、生物量以及营养品质的影响。

结　论：在镉污染的条件下，与一次性施肥相比，尿素和硫酸铵的分次施肥方法显著降低了小白菜可食部位的镉含量，增加了可食部位的生物量并改善了抗氧化相关的营养品质指标；然而，当硝酸钙作为氮肥时，小白菜可食部位的镉含量、生物量以及抗氧化相关的营养品质指标在两种不同的施肥方法之间并无统计学上的显著差异。因此，在镉污染的土壤上，当硫酸铵或尿素作为氮肥时，分次施肥方法比一次性施肥方法更加有利于保障蔬菜的安全生产与品质改善。

关键词：施肥方法；镉污染；氮肥；营养品质