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Research article

# Watering regime influences Cd concentrations in cultivated spinach

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ABSTRACT

In washed spinach, a maximum Cd concentration of 0.20 mg/kg fresh weight (FW) is allowed according to European regulations. Producers experience that this concentration can sometimes be exceeded even on soils with baseline Cd concentrations. There is a growing need to quantify the factors determining Cd uptake in the crop in order to anticipate the risk of exceedance when selecting a field for cultivation. Interseasonal variation in precipitation may be one of the factors influencing Cd uptake by crops. A pot experiment was set up where spinach plants were subject to different watering regimes. Treatment with more limited water supply during periods of high demand resulted in significantly higher accumulated Cd concentrations (0.25–0.31 versus 0.17–0.23 mg/kg FW). Concentrations at or above the maximum allowed limit were of concern, considering that the soil used in the experiment originated from a typical field in an agricultural region without any specific contamination. Probabilities to exceed maximum concentrations in the different watering regimes were estimated using Monte Carlo simulation. Results suggested that the watering regimes significantly determine the effective risk of exceeding the maximum concentrations. Their effects may be of high practical importance in the field.



## 1. Introduction

Covering 27% (890 000 ton) of the total production in Europe, Belgium is the leading producer of frozen vegetables in Europe followed at a distance by Spain (490 000 ton), Poland (435 000 ton) and France (430 000 ton) (Meulemeester, 2013). As a leaf vegetable, spinach accumulates relatively high concentrations of trace metals, including Cd (Alexander et al., 2006; Nawrot et al., 2010). Data on Cd concentrations in spinach reported world wide are compiled in Table 1. Average spinach Cd concentrations are in the range of 0.05–0.09 mg/kg FW. An analysis of 59 samples of spinach from the food market in Belgium revealed Cd concentrations between 0.079 and 0.290 mg/kg FW with a median of 0.079 mg/kg FW (Vromman et al., 2010).

Three main groups of factors affect Cd uptake in plants. Factors related to the soil environment include soil pH, texture, organic matter contents (Liang et al., 2013; Nan et al., 2002), Cd concentrations and chemical binding forms (McLaughlin et al., 1997; McLaughlin and Singh, 1999), interactions between elements (Grant et al., 1998; Khoshgoftar, 2004; Nan et al., 2002), and effects of soil amendments and soil management practices (Bolan et al., 2003a, 2003b, 2003c). The higher the total concentration of Cd in the soil, the higher the likelihood that Cd levels in the crop may be exceeded, although total contents in soil explain metal levels in plants only to a limited extent. In field data, soil pH and organic matter tend to be significant factors in addition to total soil Cd in explaining observed metal concentrations in spinach plants (del Castilho and Chardon, 1995; Liang et al., 2013). Both factors are negatively correlated with plant concentrations. Agronomic amendments may change the uptake. When adding lime, the pH effect usually dominates, although the increase of Ca and carbonate complexes in the solution are factors that may enhance Cd availability (Bolan et al., 2003c; Tan et al., 2011).

Second, plant physiology related factors will play a very decisive role. Plants have developed complex homeostatic mechanism to keep trace element concentrations within their tissues within narrow physiological limits (Clemens, 2001; Clemens et al., 2002). Different plant species, and even cultivars, may exhibit a significantly different uptake of elements (Alexander et al., 2006; Grant et al., 1998). Uptake of Cd by five cultivars of spinach growing in identical conditions was significantly different by a factor of 2.5 (Alexander et al., 2006).

A third group of factors are these related to season specific growing factors, including sunshine, rain pattern, wind, and temperature. Apart from limited work that has been done on the influence of temperature (Almas and Singh, 2001; Ekvall and Greger, 2003; Fritioff et al., 2005), no research appears to be available about

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**Table 1**
Cadmium contents in marketed spinach (mg/kg FW) reported worldwide.

| Region | n[a] | Mean | Lower | Upper | Reference |
|---|---|---|---|---|---|
| Belgium | 59 | 0.079 | 0.079[c] | 0.290[c] | Vromman et al. (2010) |
| USA | 104 | 0.070[b] | 0.015[c] | 0.710[c] | Page et al. (1987) |
| Spain, Tarragona Province, north (industrial) | – | 0.089 | 0.026[d] | 0.152[d] | Bosque et al. (1990) |
| Spain, Tarragona Province, north (agricultural) | – | 0.042 | 0.018[d] | 0.066[d] | Bosque et al. (1990) |
| Pakistan, Sindh province | 10 | 0.087 | 0.096[d] | 0.078[d] | Abbas et al. (2010) |
| Greece, general | 10 | 0.052 | 0.036[c] | 0.079[c] | Karavoltsos et al. (2002) |
| Greece, organic farming | 5 | 0.037 | – | 0.046[e] | Karavoltsos et al. (2008) |
| Japan | 3 | 0.065 | 0.028[c] | 0.128[c] | Kikuchi et al. (2002) |

[a] Number of observations.
[b] Reported data were converted to fresh weight assuming a dry matter content of 9%.
[c] Minimum and maximum.
[d] Mean and standard deviation were reported. Lower and upper were reported as average minus/plus 3 × standard deviation, respectively.
[e] 90% percentile value.

the specific influences of weather related factors on plant metal uptake. Cadmium uptake by the plant roots from soil is related to the water uptake, which depends on the plant demand and the soil supply (Rao and Mathur, 1994). The distribution of precipitation during the growing season is therefore a potential factor influencing the Cd concentration in spinach at harvest time. To our knowledge, no studies are available where the importance of the watering regime on uptake of metals in crops has been systematically assessed.

Cadmium contents in foods are increasingly being regulated to limit the exposure of the general population to this potentially toxic metal. According to European regulations, for leaf vegetables a maximum concentration of 0.20 mg/kg washed, edible product is allowed (European Commission, 2015, 2006). These standards are rather strict, as they might be exceeded even when cultivating spinach on soils with Cd contents within normal baseline concentration values (Smolders, 2001). As the production sector is at real risk of being confronted with harvests where Cd concentrations exceed the maximum allowed concentration, selection of land and cultivation practices increasingly will need to account for the risk on Cd uptake. There is a growing need to be able to estimate the risk that Cd in crops exceeds maximum allowed concentrations when growing a crop on selected plots of land.

Distribution of precipitation during a growing season may not be uniform and will vary from one season to the next. It is hypothesised that this has effects on the uptake of Cd and the Cd concentration in the harvested crop. A pot experiment was set up to investigate the importance of the moisture regime during the growth of spinach on the uptake of Cd by the crop. Spinach plants were subjected to different water regimes, however within limits that still allowed for a healthy development of the plant. This provides a first look into the specific effect of this weather related factor on Cd uptake in spinach.

## 2. Materials and methods

### 2.1. Pot experiment

A loamy soil from an agricultural field in the region of Tournai, Belgium, with a moderate total Cd content (0.45 mg/kg dry weight) was selected for the study. The soil was fertilized according to a standard advice with commercial fertilizer, i.e., at rates corresponding to 200 kg N per hectare as ammonium nitrate (33-00-00) and 150 kg K per hectare as Patentkali (00-30-00). No P fertilization was recommended.

Plants were cultivated in pots in a growing chamber under artificial light. The temperature and relative air moisture during the growing period were 22 ± 1 °C and ±70%, respectively. Plants were lighted using artificial lights (OSRAM Fluora L 36 W/77, OSRAM GmbH, Germany) with an illuminance of about 3500 lux during 12 h every day. Pots were filled with 850 g of soil and wetted with 150 ml of deionized water. Six seeds of spinach were distributed over the surface, after which the remaining 150 g of soil was used to cover the seeds. During germination, pots were covered with perforated plastic foil to minimize drying. After 10 days, the foils were removed and plants were thinned to 2–3 plants per pot. Then, the experimental treatments were started.

During the subsequent 7 days, no water was added. Different watering regimes were implemented from day 9 onwards. Six treatments in 4 replicates were implemented to investigate different aspects of a watering regime, (1) “Daily high”, (2) “Daily low”, (3) “3 x week”, (4) “1 x week”, (5) “Increasing” and (6) “Decreasing”. (1) and (2) allowed to assess the influence of amount of added water over the growing period, and were implemented by “daily” watering, i.e. 5 days per week, of 50 ml per pot for “Daily high” and 30 ml per pot for “Daily low”. Treatments (2), (3) and (4) served to assess the effect of watering frequency. Total water administered was the same, but while water was added daily in treatment (2), it was administered only a few times a week in Treatment (3) and once a week or less in Treatment (4). Finally, effects of differences in distribution of the water supply throughout the growing period were assessed through treatments (1) (same rates of water addition throughout), (5) (increasing rates with time, i.e. 20 ml daily during week 1 and 2) and (6) (decreasing rates with time). To avoid excessive stress to the plants, some additional watering was sometimes performed in an attempt to produce healthy plants with a similar biomass yield in all treatments. The watering regimes applied are depicted in Fig. 1.

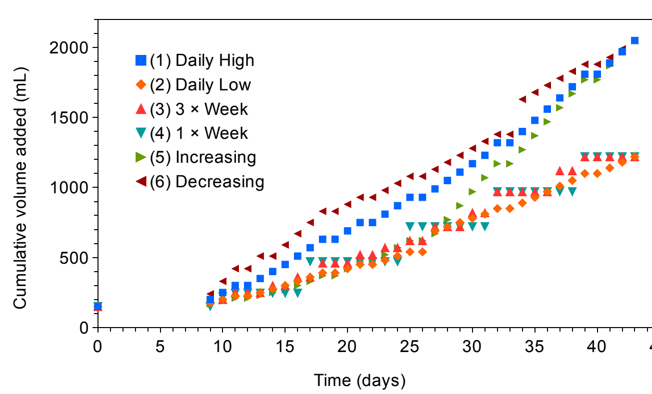


**Fig. 1.** Different watering regimes applied during the cultivation of spinach expressed as cumulative amounts of water added (mL per pot containing 1 kg soil).