Ecotoxicology and Environmental Safety 123 (2016) 89–94

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Cadmium accumulation in leaves of leafy vegetables

Daniela Baldantoni [a,*], Luigi Morra [b], Massimo Zaccardelli [c], Anna Alfani [a]

[a] Dipartimento di Chimica e Biologia, Università degli Studi di Salerno, Via Giovanni Paolo II, 132-84084 Fisciano, SA, Italy
[b] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Unità di Ricerca per la Futticoltura (CRA-FRC), Via Torrino, 2-81100 Caserta, Italy
[c] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di Ricerca per l'Orticoltura (CRA-ORT), Via Cavalleggeri, 25-84098 Pontecagnano, SA, Italy

ARTICLE INFO

Article history:
Received 3 February 2015
Received in revised form
11 May 2015
Accepted 13 May 2015
Available online 23 May 2015

Keywords:
Cichorium endivia
Lactuca sativa
Cd regulation thresholds
external and internal leaves
soil fertilisation.

ABSTRACT

Leafy vegetables have a relatively high potential for Cd uptake and translocation, and are thus considered Cd accumulators. For this reason, leaves and roots of lettuce (*Lactuca sativa* L.) and endive (*Cichorium endivia* L.) plants, grown on different agricultural soils in Campania region (southern Italy), subjected to different fertilisation treatments (unfertilisation, compost amendment and mineral fertilisation), were analysed for Cd concentrations. Moreover, to clarify if the highest concentrations found are linked to older and inedible or to younger and edible leaves, external and internal endive leaves were separately analysed.

All the leafy vegetables analysed showed on average 2-fold higher Cd concentrations in leaves than in roots. Leaf Cd concentrations in both lettuce and endive plants significantly differed among fertilisation treatments, with values highest in the plants grown on mineral fertilised soils. Apart from the soil fertilisation treatments, however, Cd leaf concentrations were often higher (up to 4-fold) than the threshold deduced by the EU 420/2011 Regulation, although the plants grew on unpolluted soils. Anyway, external leaves of endive plants showed significantly higher concentrations than internal leaves (in some cases the values were 3-fold higher), partly reassuring on the consumption of the younger leaves. Moreover, this study points out two major drawbacks in the Italian and European regulatory frameworks: (1) metal concentration (as total and/or available fraction) limits in agricultural soils are lacking; (2) metal concentration thresholds (currently existing only for Cd and Pb in crops) reported in the EU 420/2011 Regulation, expressed on the fresh weight basis rather than on the dry weight basis, appear not suitable.

1. Introduction

All heavy metals are persistent chemical elements and can not be deleted from the environment. Thus, a problem arises when their bioavailability is high, either in relation to high background levels or to anthropogenic activities (Greger, 2004). Cadmium (Cd), in particular, is a toxic element for all living beings, even at low concentrations (Garate et al., 1993). Anthropogenic activities, such as soil fertilisation, have increased both the total amount of Cd in soils (Huang et al., 2004) and the fraction of this element available to plants (Garate et al., 1993). Consequently, Cd uptake and accumulation in edible plants and its possible effects on human health have received great attention in the last decades (Garate et al., 1993; Akoumianakis et al., 2008), considering also that food is the main source of Cd intake in non-smoking people (Järup and Akesson, 2009). Cd is carcinogenic to human beings, according to the International Agency for Research on Cancer (IARC, 2012), and affects reproductive processes and the embryonic development (Thompson and Bannigan, 2008). Moreover, it is involved in human bone disease, lung edema, liver damage, anaemia and hypertension (Nordberg, 1974; Staessen et al., 1999) and it is the cause of Itai-Itai disease (renal damage and osteomalacia) in individuals chronically exposed to high concentrations through the diet (Stayner et al., 1992). Hence, Cd is one of the metals for which the Food and Agricultural Organization and the World Health Organization have set limits (FAO-WHO, 1978), with a maximum permitted human intake of 70 μg/d.

The process of Cd uptake by plant roots can be either active or passive, depending on Cd concentration in the nutrient solution (Cataldo et al., 1983), and its extent depends on temperature, pH, salinity, organic matter content and nutrient concentrations in the

Abbreviations: CRA, Agricultural Research Council and Analysis of the Agrarian Economy; UNF, unfertilised soil; CMP, compost amended soil; MIN, mineral fertilised soil; BF, bioavailability factor; DTPA, diethylenetriamine-pentaacetic acid

* Corresponding author. Fax: +39 089969603.
E-mail addresses: dbaldantoni@unisa.it (D. Baldantoni), luigi.morra@entecra.it (L. Morra), massimo.zaccardelli@entecra.it (M. Zaccardelli), analfani@unisa.it (A. Alfani).

http://dx.doi.org/10.1016/j.ecoenv.2015.05.019
0147-6513/

soil (Bingham et al., 1983; Jackson and Alloway, 1991; McBride, 2002). Anyway, Cd has generally a high soil bioavailability (Baldantoni et al., 2010) and has higher mobility in plants compared to other heavy metals (Greger, 2004; Akoumianakis et al., 2008; Zhang et al., 2013), being easily transported by roots to shoots (Baldantoni et al., 2014).

Plant species, but also genotypes, vary in their capacity for Cd uptake, transport and accumulation (Grant et al., 1999; Greger, 2004; Zhang et al., 2013). In addition, heavy metal accumulation in plants differs greatly not only among species and cultivars, but also among organs of the same plant (Oliver, 1997). As a general rule, metal concentrations are normally higher in roots than in shoots (Garate et al., 1993; Kabata-Pendias, 2011). However, in several plants (Baldantoni et al., 2014), and also in leafy vegetables grown on unpolluted soils (Kabata-Pendias, 2011), Cd is primarily allocated in leaves. Since leafy vegetables, such as lettuces, endives and similar horticultural crops have a relatively high potential for Cd uptake and translocation (Peijnenburg et al., 2000), they are considered Cd accumulators (FAO, 1983). This, coupled with the importance of leafy vegetables in human diet, makes them an important source of Cd intake for people.

It was estimated that vegetable consumption can contribute to Cd exposure from 70% (Sarwar et al. 2010) up to more than 90% (Swartjes et al. 2007) of total Cd intake by human beings. Lettuce (*Lactuca sativa* L.) and endive (*Cichorium endivia* L.) are two important salad crops of the Mediterranean diet and are available worldwide, so their consumption may represent an effective risk for human health. The present study aims to evaluate Cd concentrations in leafy vegetables grown on different agricultural soils underwent to different fertilisation practices, and to clarify if the highest concentrations occur in the external (older and inedible) or the internal (younger and edible) leaves. To this end, the research was carried out in two years: the first targeting Cd accumulation in leaves and roots, and the second focusing on Cd partitioning between edible and inedible leaves.

2. Materials and methods

2.1. Experimental design

Leafy vegetables were cultivated in three experimental stations of the Agricultural Research Council and Analysis of the Agrarian Economy (CRA), namely the Research Units of Pontecagnano (40°38' N, 14°53' E, 48 ma.s.l.), Battipaglia (40°34' N, 14°58' E, 65 ma.s.l.) and Scafati (40°44' N, 14°30' E, 9 ma.s.l.), in southern Italy (Campania Region), all characterized by a Mediterranean climate (Baldantoni et al., 2010; Baldantoni et al., 2015). During 2005, lettuce (*L. sativa* cv Arcadia) at Pontecagnano and endive (*C. endivia* cv Crispum Hegi) at Battipaglia, were cultivated in greenhouse and in open-field, respectively. During 2009, endive (*C. endivia* cv Cuartana) was cultivated in open-field, side by side at Scafati and Battipaglia. Soils at the three sites (Table 1) differed in their physical and chemical properties (Pagano et al., 2008; Baldantoni et al., 2015).

In both years, the study was carried out in plots (three and four replicated for each soil treatment in 2005 and 2009, respectively) subjected to different soil treatments, performed according to a randomized-block design. The treatments were (1) unfertilised soil (UNF), (2) compost amended soil (CMP), and (3) mineral fertilised soil (MIN). High quality compost (Legislative Decree 75, 29 April 2010), obtained from the organic fraction of municipal solid waste and the urban yard trimmings (1:1 = w:w.), was homogeneously spread on the soil surface at the dose of 30 t/ha on dry weight basis and then incorporated by rotovating to a depth of about 20 cm. Mineral fertilizers (N, P, K) were applied two times during growing season, based on soil nutrient availabilities.

Table 1
Physical and chemical properties of the soils (0–20 cm) of Pontecagnano and Battipaglia (from Pagano et al., 2008) as well as Scafati (from Baldantoni et al., 2015) experimental fields; n.a.: not available datum.

	Pontecagnano	Battipaglia	Scafati
Classification (WRB-FAO, 2014)	Sandy Loam Calcaric Cambisol	Clay Gleyc Luvisol	Vitric Calcaric Andosol
Sand (0.02-2.00 mm) (%)	43.0	31.0	45.0
Silt (0.002-0.020 mm) (%)	39.0	29.2	50.0
clay (< 0.002 mm) (%)	18.0	39.8	5.0
pH $_{H2O\ (1:2.5)}$	7.9	7.6	8.4
Organic matter (% d.w.)	4.4	1.3	2.2
Total carbonates (%)	59.1	Traces	3.4
Electrical conductivity (dS/m)	0.22	0.12	0.34
Cation exchange capacity (cmol$_{(+)}$/kg)	22.7	16.7	n.a.
Total nitrogen (g/kg d.w.)	2.25	0.80	1.50
Bioavailable phosphorus (mg/kg d.w.)	45.0	45.0	71.0
Exchangeable potassium (mg/kg d.w.)	458.0	394.0	726.0

2.2. Sampling

Soil samples from each plot were collected in the 0–20 cm layer. At each sampling, six sub-samples were collected from each plot and mixed to obtain one representative sample per plot for the laboratory analyses. From the same plots, three–four lettuce or endive plants were picked up and divided in leaves and roots. Endive cv Cuartana leaves were separated in external (older) and internal (younger) ones. Care was taken in all sampling and in following analyses to avoid metal contamination.

2.3. Laboratory analyses

Soil, sieved through a 2.0-mm mesh screen, and plants were dried at 75 °C up to constant weight. For the determination of total Cd concentration in soil and plants, samples were pulverized in a planetary ball mill (PM4, Retsch, Haan, Germany) and digested by an acid mixture (65% HNO_3 and 50% HF, 2:1 = v:v) in a microwave oven (Ethos, Milestone, Shelton, CT, USA). The Cd bioavailable fraction was extracted from the dried soil samples with a DTPA (diethylenetriamine-pentaacetic acid) solution (0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA, pH 7.3) at room temperature in continuous agitation for two hours (Lindsay and Norvell, 1978). Further details on these methods are reported in Baldantoni et al. (2009). Cadmium concentrations were determined by an atomic absorption spectrophotometer (AAnalyst 100, PerkinElmer, Wellesley, MA, USA), via graphite atomizer. Standard reference materials, namely calcareous loam soil BCR CRM 141R (European Commission, 1996) and *Olea europea* leaves BCR 62 (Commission of the European Communities, 1982) were also analyzed in order to verify the accuracy of soil total and leaf Cd determinations.

2.4. Data processing

Cadmium bioavailability factors (BFs) in the analysed soils were calculated as the percentage of Cd available fractions compared to the total Cd concentrations.

For each experimental field, on the normalised data set through logarithmic transformation, (1) one-way ANOVA was performed in order to check for differences in soil Cd bioavailable concentrations as well as in BFs among soil treatments, and (2) two-way ANOVA, with the plant organ and the soil treatment as fixed factors, was performed in order to evaluate differences in leaf and root Cd concentrations and among soil soil treatments. The ANOVA tests were followed by the *post hoc* tests of Tukey. Normality was