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Partitioning of nutrients and non-essential elements in Swiss chards cultivated in open-air plots

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ABSTRACT

The uptake of metals by plants and their partitioning between different organs is an important issue in fields like plant ecology, phytochemistry, phytoremediation and, in the case of plants with edible parts, food quality. In this work a five-month field experiment was carried out to investigate the uptake and partitioning of 20 elements (Mg, K, Ca, Na, Mn, Fe, Co, Cu, Zn, Mo, Sr, Ba, Al, Ti, V, Cr, As, Cd, Tl and Pb) by Swiss chards (*Beta vulgaris*). The effect of the harvesting time on the accumulation of these elements in roots, stalks and leaf blades was studied using plants cultivated in two different but adjacent air-open plots subjected to conventional or organic farming techniques. Plants were collected at six growth stages during their production cycle and samples were analysed by ICP-MS after microwave assisted acid digestion. Toxic elements (except Cd) were mainly immobilised in roots, whereas essential elements were translocated to aboveground organs. In general, the element concentrations in plant organs decreased with time. The use of organic fertilisers promoted the presence of nutrients in blades and stalks. Pb and Cd concentrations in the edible parts were always below the maximum levels set by the European Union.

1. Introduction

Global food security has become a vital issue to ensure human health and proper nutrition. During the Green Revolution (20th century) synthetic fertilisers (man-made inorganic compounds), insecticides and pesticides started to be used for intensive agriculture, enhancing crops yield and food production (Phillips, 2014). Although world's food needs were fulfilled, several damages to the environment were caused, such as soil degradation (Giller et al., 1997), loss of crop genetics diversity (Tilman et al., 2002), release of greenhouse gases, eutrophication of aquatic ecosystems, ground water depletion (Matson et al., 1997) and increased concentrations of pesticides in foods (Carvalho, 2006). These issues required to look for another production system with less negative environmental impact, such as the organic farming, aiming at being an environmentally-friendly agricultural practice.

The percentage of agricultural land used for organic farming around the world has greatly increased during the last decade. During the period 2004–2013, about 43 million of hectares were used for organic purposes, being Oceania and Europe the outstanding continents, with 40 % and 27 % of the global organic agricultural land, respectively (FIBL, 2015).

A recent clinical report claimed that current evidences do not support any significant nutritional benefit or deficit from eating organic compared to conventionally grown foods, not exiting human studies that directly reveal health benefits or disease protection from consuming organic products (Forman and Silverstein, 2012). However, although the use of synthetic fertilisers is a convenient way to increase fruits production and crops yield, they are usually formed by adulterated products containing raw materials from unknown or questionable sources being, thus, a possible source of toxic elements, such as Pb and Cd (as an example, man-made P fertilisers can increase the solubility of Cd due to changes in the pH and/or ionic strength of the soil solution) (Jiao et al., 2012; Otero et al., 2005). In this way, a recent survey of

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the literature reported that organic crops have lower Cd content than those obtained by conventional practices but for As and Pb, no significant differences were evidenced (Barański et al., 2014).

Generally, plant roots take up both essential (macro and micronutrients) and non-essential trace elements from soil. These elements can be immobilised in roots vacuoles or translocated to the aerial organs of the plants in variable proportions (Clemens et al., 2002; Peralta-Videa et al., 2009). The amount of trace elements taken up by plant roots mostly depends on the bioavailability of these elements in soil, which itself depends on the chemical nature of the elements and the physic-chemical characteristics of the soil (Barber, 1995). High bioavailability results in an important uptake, translocation and accumulation of metals in the aboveground organs of the plant. This process is mediated by several transporters, such as NRAMP (natural resistance-associated macrophage proteins) and ZIP (zinc-regulated transporter, iron-regulated transporter proteins) (Johnson et al., 2011). This can result in an increased nutritive value of the plant if the accumulation of nutrients is below the plant toxicity levels. However, some non-essential elements can also be significantly accumulated in aerial organs via some non-specific transporters. If this accumulation is important in edible parts of the plant, their consumption might result in an unacceptable risk for human health (Kabata-Pendias, 2004).

Swiss chard (*Beta vulgaris* subsp. *cicla*) is a popular vegetable largely consumed all over the world. Its nutritional composition includes polyphenol compounds, flavonoids such as syringic acid and many betalain pigments, which have been shown to provide antioxidant, anti-inflammatory and detoxification support, and blood sugar control (Bozokalfa et al., 2011; Kugler et al., 2004).

Some investigations have been carried out to investigate the uptake and translocation of elements in Swiss chards. They seem to have a high potential to transfer elements from the soil to the edible foliage. As an example, Peris et al. (2007) reported that leafy vegetables (such as Swiss chards) present relatively high concentrations of metals. They also pointed out that the farming technique used in their cultivation has a clear influence on the accumulation of beneficial and toxic trace elements in plant tissues. Madejón et al. (2011), on the other hand, reported low levels of Zn, As and Pb in leaves of Swiss chards which had been grown up in highly contaminated soils using traditional smallholder management practices (annual liming and application of animal manures).

It is to be highlighted that most of the studies concerned with this topic have been carried out at a single phenological stage. The elemental composition of plant tissues, however, fluctuates over the plant life, and highly depends on the age of the organs and the nature and extent of the mechanisms involved in the storage and remobilisation of elements inside the plant (Marschner and Marschner, 2012). Additionally, organic and synthetic fertilisers influence the bioavailability of both nutrients and non-essential elements in soil and, therefore, the nutritional value of the plant and the risk associated to their consumption by humans.

In this work, a five-month open-air field experiment was conducted to investigate the uptake, translocation and accumulation of several nutrients and non-essential elements in Swiss chards (*Beta vulgaris* subsp. *cicla*), during their whole production cycle. Plants were grown in two different but adjacent plots using either organic or conventional practices. We aimed to i) investigate the partitioning of elements between different plant organs, paying special attention to the edible parts, ii) study the influence of the growth stage on the elemental composition of plants, and iii) examine if the agricultural practice (organic *vs.* conventional) has any significant influence on the nutritional value of the plants and on the risk derived from their consumption by humans.

2. Materials and methods

2.1. Implementation of the plots and agricultural treatments

The 5-month experiment was carried out in an open-air garden with no greenhouse protection located in Beotegi, a rural area of the Basque Country (43° 5.370′N, 3° 4.590′W), at 370 m over the sea level. Two different but adjacent plots of 10 m^2, separated each other 35 m, were selected in the garden for conventional and organic practices, respectively.

Four months prior to the plantation, four soil samples were collected at each plot for characterization. The samples were randomly selected and collected from 15 to 30 cm depth, using a garden spade and removing stones and other remains.

In each plot, twenty-five Swiss chard seedlings (*Beta vulgaris* subsp. *cicla*, var. *sima*) with 2–4 leaves were planted the 5th June 2013. Seedlings were obtained from a local producer (Camino Sociedad Civil, Llodio, Spain).

Different certified agricultural products were applied in the garden. The plot intended for conventional practice was supplied with 0.25 kg m^{-2} of a chemical fertiliser [NPK 15.15.15(15); Fertiberia, S.A., Madrid, Spain], applied once 25 days before the plantation. This corresponds to a dose of 375 g of total N, P_2O_5 and K_2O. Phytosanitary treatment included a liquid mixture of an insecticide (Epik 20 SG; Sipcam Jardín S.L., Valencia, Spain) and a fungicide (Galben M.; Sipcam Jardín S.L., Valencia, Spain) applied twice (200 mL m^{-2} in total), 7 and 36 days after plantation, so as to minimise pest attacks. In the plot intended for the organic practice, two applications of an organic fertiliser (natural horse manure, Abonos Naturales Hermanos Aguado, S.L., Toledo, Spain; product approved and certified by CAAE as ecological product; C qualification) were carried out, 10 and 2 days before the plantation, using a total of 6.48 kg m^{-2}, which corresponded to supplied doses of 2981 g of total N, 259 g of P_2O_5 and 583 g of K_2O. Protective plants (*Tagetes patula*) were also planted in the periphery of the organic plot as natural repellent to avoid pests and insects attacks. Both plots were maintained until October 2013.

2.2. Soil characterization

Soil samples were air-dried in a laminar flow hood and sieved to obtain a particle size under 2 mm prior to physic-chemical analyses.

The content in organic matter (OM), organic carbon (OC), total calcium carbonate ($CaCO_3$), and total nitrogen (N), as well as the pH, the cation exchange capacity (CEC) and the soil texture, were determined in each soil sample following the ISO reference analytical methods described by Cools and De Vos (2013).

The OC, $CaCO_3$ and N were measured after dry combustion of the sample. The pH was determined in a dried-soil sample suspended in water at 1:5 ratio (v/v). CEC was calculated according to the method of Metson (1956), using a 1 mol dm^{-3} ammonium acetate solution at pH 7. The soil texture was obtained from the dry weight of standard fractions (coarse sand, 0.2–2 mm; fine sand, 0.2 mm–50 μm; coarse silt, 20–50 μm; fine silt, 2–20 μm; and clay, <2 μm) defined in the French norm NF X31-107 (AFNOR, 2003), and by plotting the results on the USDA textural classification triangle (USDA, 2015).

2.3. Sampling campaigns and meteorological data collection

One sampling campaign was carried out every three weeks (26th June, 15th July, 6th August, 26th August, 16th September and 21th October), covering the whole production cycle of the plants. At each harvest, four plants (randomly selected in advance) were fully harvested in each plot. Each plant was in-situ separated in