Journal of Environmental Management 180 (2016) 24–34

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Macroelemental composition of cadmium stressed lettuce plants grown under conditions of intensive sulphur nutrition

Renata Matraszek [a, *], Barbara Hawrylak-Nowak [a], Stanisław Chwil [b], Mirosława Chwil [c]

[a] Department of Plant Physiology, University of Life Sciences in Lublin, Akademicka 15, 20-950 Lublin, Poland
[b] Department of Agricultural and Environmental Chemistry, University of Life Sciences in Lublin, Akademicka 15, 20-950 Lublin, Poland
[c] Department of Botany, University of Life Sciences in Lublin, Akademicka 15, 20-950 Lublin, Poland

ARTICLE INFO

Article history:
Received 11 November 2015
Received in revised form
5 May 2016
Accepted 8 May 2016

Keywords:
Cadmium
Lactuca sativa L.
Sulphur
Macronutrients balance

ABSTRACT

Lettuce (*Lactuca sativa* L.) is moderately sensitive to cadmium (Cd) and shows high accumulation of this metal. Thus, this species is considered to be a good model for both identifying determinants controlling Cd accumulation in plant tissues and for developing breeding strategies aimed at limiting the accumulation of this metal in edible tissues. Simultaneously, lettuce is characterised by medium requirements for sulphur (S) – a macronutrient whose role is associated not only with proper growth and development, but also with stress tolerance. The common use of NPK fertilizers without sulphates (S-SO_4) together with the progressive process of reducing emissions of S compounds to the natural environment may lead to deficiency of this element in plants. The present study evaluated the changes in macronutrient content and accumulation in Cd-stressed lettuce 'Justyna' supplied with different S doses. Four concentrations of Cd (0, 0.0002, 0.02 or 0.04 mM) and three levels of S applied in the form of S-SO_4 (2, 6 or 9 mM S) were used. Cd exposure impaired the macronutrient balance and accumulation in lettuce. Intensive S nutrition to some extent alleviated Cd-induced toxicity. High S doses, especially 6 mM S, partially improved macronutrient status and restored the macronutrient balance. In Cd-stressed plants supplemented with additional S, an increase in root and shoot biomass and in the content of N, K and Mg was found, without significant changes in the Ca content. Simultaneously, the P and S contents in the biomass of both above- and underground organs remained unchanged. In the leaves, as opposite to the roots, intensive S nutrition reduced the accumulation of Cd. However, the foliar Cd concentration still exceeded the acceptable limits established for consumption. All the obtained results concerning the content of macronutrients and their ratios were referred, *inter alia*, to the standards i.e. the Diagnosis and Recommendation Integrated System (DRIS) norms.

1. Introduction

Lettuce (*Lactuca sativa* L.), family Asteraceae, subfamily Liguliflorae, native to the Mediterranean, is the most important member in the group of leafy vegetables. It is commercially produced worldwide in many countries and is also widely grown in home gardens. China (over 50% of the world production), USA (about 20%), India (5%), Spain, Iran, Italy, and Japan are among the world's largest producers (Křistková et al., 2008). Poland is ranked 38th place in global lettuce production (Rogowska et al., 2013). Lettuce is almost exclusively used in salads as a fresh vegetable, but some forms are also cooked. This vegetable is considered as one of the most healthy food. Lettuce is a good source of fibers, vitamins, especially B, A, C and K, low in calories as well as a rich source of pigments beneficial for human health (chlorophyll, carotenoids). Furthermore, it contains dietary minerals (in mg per each 100 g) such as: K – 238, Ca – 35, P – 33, Mg – 13, Fe – 1.30, Zn – 0.20, Mn – 0.18, Cu – 0.02 and 0.6 µg 100 g^{-1} of Se as well as a low amount of Na – 0.00545 g 100 g^{-1}. These minerals are essential for proper functioning of all biochemical processes of human body by performing structural and functional roles as well as serves as electrolytes (Konstantopoulou et al., 2010; Mou, 2012).

Heavy metal pollution of air, water and soils is a widespread global problem which has become a major environmental concern due to crop contamination and its hazardous effect on human

---

* Corresponding author.
E-mail addresses: renata.matraszek@up.lublin.pl (R. Matraszek), bhawrylak@yahoo.com (B. Hawrylak-Nowak), stanislaw.chwil@up.lublin.pl (S. Chwil), miroslawa.chwil@up.lublin.pl (M. Chwil).

http://dx.doi.org/10.1016/j.jenvman.2016.05.017

health. Cadmium (Cd) is one of the most toxic heavy metals. Cd contamination of large areas of land has been caused by anthropogenic activities such as mining and mineral processing of metallic ores, waste disposal, phosphate fertilizer application and wastewater irrigation (Mojiri, 2011). Over the last decades, the annual global release of this element has reached 22,000 tons (Sabeen et al., 2013). Cd is a ubiquitous non-essential metal taken up by the membrane transporters of essential elements that can interfere with the transport and physiological functions of several essential elements (Dong et al., 2006; Rivelli et al., 2014). It has been reported that Cd differently influences nutrient content and homeostasis depending on species, varieties, growth stages, and plant organ. Cd is easily extracted by plants from the environment as compared with other non-essential elements (Dede and Ozdemir, 2016). This metal is frequently accumulated by major horticultural and agriculturally important crops, and severely reduces their productivity and yield quality. Due to its high mobility, Cd very easily enters the food chain, which poses serious threat to animal and human health, mainly because of its mutagenic and carcinogenic effect as well as damage of neurological system (Godt et al., 2006).

Sulphur (S) is required at 0.1–1.0% (on a dry weight basis) for plant growth and development (Droux, 2004; Gaafar et al., 2012). The progressive process of reducing emissions of S compounds to the natural environment, the use of NPK fertilizers without S such as urea, triple superphosphate or di-ammonium phosphate instead of ammonium sulphate or ammonium superphosphate, as well as leaching sulphate ions deeper into the soil profile have limited phytoavailability of this element. S deficiency has become a recently emerging problem, which results in decreased crop yield and quality. In most plants almost all of the total S (up to 90%) is presented as cysteine (Cys) and methionine (Met). Both of these amino acids are predominantly bound to protein and are necessary for primary and secondary metabolism. Cys, as the first carbon/nitrogen-reduced S product resulting from the assimilation pathway, serves as a S donor for Met, glutathione, vitamins, cofactors, and other S compounds that play a major role in many fundamental processes, such as photosynthesis or carbon and nitrogen metabolism. Enhanced synthesis of thiol peptides, i.e. the reduced glutathione form (GSH) and phytochelatins (PCs), is considered to be an important defensive mechanism against heavy metal stress (Droux, 2004; Hawkesford and De Kok, 2007; Gaafar et al., 2012; Nazar et al., 2012). Thus, optimal S nutrition is important not only for proper plant growth and development, but is also helpful for reducing Cd translocation within the plant body as well as is required for detoxification of this heavy metal and to develop tolerance in plants against Cd toxicity (Nocito et al., 2007; Anjum et al., 2008; Gill and Tuteja, 2011).

It is reported that lettuce has a medium-low requirement for S, which is evidenced by the S/N ratio. It is assumed that S availability should be 1 unit of S to 4 units of N (Cuppett et al., 1999). Lettuce is moderately sensitive to Cd stress. This species can tolerate 3–4 $\mu g\ kg^{-1}$ Cd from soil and shows high bioaccumulation of this metal. Therefore, it is a good model both for identifying determinants controlling Cd accumulation in plant tissues and for developing breeding strategies aimed at limiting Cd accumulation in edible tissues (Tyksiński and Kardubska, 2005; Zorrig et al., 2013; Khopkar, 2015). It is well documented that Cd disturbs ion homeostasis and can induce deficiency of several macronutrients in plants (Dražić et al., 2004; López-Millán et al., 2009; Tran and Popova, 2013). So, it is possible to minimize some of the Cd-induced negative effects through the optimum use of S compounds. However, to our best knowledge, the influence of the applied exogenous S on the accumulation of macronutrients in tissues of Cd-exposed higher plants has not been studied earlier.

The aims of the present study were to determine to what extent Cd disturbs macronutrient balance in butterhead lettuce and to evaluate whether intensive S nutrition may improve the mineral status of Cd-stressed plants. Moreover, the effect of different S levels on Cd bioaccumulation was determined. We hope that these results shed light on the main strategies developed by lettuce to cope with Cd, which may be helpful in developing breeding strategies aimed at limiting accumulation of Cd in this species.

2. Material and methods

2.1. Plant material and growth conditions

The experiment was carried out in the years 2012–2014 in the Plant Physiology Department, University of Life Sciences in Lublin, Poland. The biological object of the study was butterhead lettuce (*Lactuca sativa* L. var. *capitata*) cv. Justyna. The experiment was carried out by the method of water cultures. First the seeds germinated in wet quartz sand for about three weeks. Then, the healthiest, best developed four-leaf stage seedlings were transferred 1 dm^3 glass jars (two plants per each) with Hoagland's II solution. Four Cd treatments (0, 0.0002; 0.02 or 0.04 mM, as $CdCl_2$) were applied with combinations of three S levels: 2-standard dose, 6 and 9 mM. In all experimental treatments standard S dose (2 mM S) was supplied as $MgSO_4$. In the treatments with high S doses i.e. 6 and 9 mM S the standard S dose 2 mM supplied in the form of $MgSO_4$ was additionally supplemented with appropriate amounts of S (4 and 6 mM S, respectively) in the form of Na_2SO_4.

In all experimental treatments, the level of sodium and chlorine was equalized by adding to the nutrient solution appropriate amounts of diluted NaCl and HCl; the pH of the nutritional environment was set at 5.8–6.0. The lowest Cd concentration used in the experiment (0.0002 mM) was within the values estimated for a non-polluted soil solution (from 0.00004 to 0.00032 mM Cd) (Hoseini and Zargari, 2013). The remaining two Cd concentrations (0.02 and 0.04 mM) markedly exceeded a concentration recognized as a critical Cd limit for soil solution (0.0008 mM) (De Vries et al., 2003; 2007). The S-SO_4 concentration in the standard nutrient solution, used in our experiment as control (2 mM S), is moderate. According to the data in the literature, the sulphate concentrations in natural soil solution unpolluted with heavy metals range from 0.16 to 7 mM SO_4^{2-}, in arid regions from - 3–16 mM SO_4^{2-}, whilst the SO_4^{2-} concentration of soil solution containing remnants of sulphide ore from mining operations varies from 13 up to 110 mM (Ernst et al., 2008). Plants were cultured in a controlled-climate phytotron room under the following conditions: temperature 22/18 °C (day/night), 14 h d^{-1} photoperiod, Photosynthetic Photon Flux Density (PPFD) 250–270 $\mu mol\ m^{-2}\ s^{-1}$ at the level of the tops of the plants and relative air humidity between 50 and 60%. After 14 days of growth under differentiated conditions, the plants were harvested. In order to remove the cell wall–bound Cd ions, the roots of Cd-exposed plants were desorbed in a 10 mM $CaCl_2$ solution for 10 min and then rinsed twice in distilled water, while roots from the other series – only in distilled water. The macronutrient composition of roots and leaves were determined.

2.2. Macronutrients content and bioaccumulation

The dry plant material (roots and shoots separately) was subjected to chemical analyses. The content of macronutrients was assayed with the following techniques: total nitrogen (N) with the Kjeldahl method; phosphorus (P) – with the vanadium-molybdenum method; magnesium (Mg) – colorimetrically with titanium yellow after mineralization with HNO_3 and H_2O_2 mixture; potassium (K) and calcium (Ca) – with atomic absorption