EREM 76/1
Journal of Environmental Research, Engineering and Management
Vol. 76 / No.1 / 2020
pp. 58–64
DOI 10.5755/j01.erem.76.1.22072

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Received 2018/11 | Accepted after revision 2020/01

http://dx.doi.org/10.5755/j01.erem.76.1.22072

Response of Chamomile *Matricaria Recutita* to Low and Moderate Soil Cadmium Pollution

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The aim of this study was to evaluate the effects of soil cadmium (Cd) pollution to the growth and quality of medicinal herbs plant chamomile (*Matricaria recutita* L.) and their extracts. The plants were grown in soils contaminated with Cd (3, 6, 12 mgCd/kg) for four weeks. The morphological, physiological, biochemical parameters and the content of Cd in chamomile tissues and infusions were determined. The results of this study showed that soil contamination with Cd had no adverse effect to the aboveground biomass of chamomile, but slightly (up to 25 %) reduced root biomass. Cd treatment significantly reduced the number of flowers and their dry weight (1.9-2.7-fold). Cd soil pollution had no significant adverse effect on the content of photosynthetic pigments, though induced lipid peroxidation and an increase in MDA content was recorded. Cadmium concentrations in the plant tissues and extracts increased along with Cd concentration in the soil. It was found that Cd concentrations in chamomile flowers and infusions were above the maximum allowable Cd levels in medicinal plants and drinking water. This study demonstrated that the use of chamomile products may pose a risk to human health even if chamomiles are growing at environmentally relevant Cd soil concentration.

Keywords: bioaccumulation, cadmium, chamomile, growth, infusion, soil pollution.

Introduction

Chamomile (*Matricaria recutita* L.) is widely used in various medicinal products for external use and as medicinal tea or refreshment drink. Medicinal products (teas, drinks, tinctures, etc.) prepared from wild plants are generally considered to be harmless, however scientific data show that these plants may contain toxic substances (such as heavy metals, pesticides), if they are grown in a contaminated environment. Accumulated compounds in plant tissues could be extracted during the infusions preparation and could pose a human health risk. Even commercially available herbal products might contain relatively high concentrations of heavy metals (Arpadjan et al., 2008) and heavy metal poisoning from traditional herbal products was reported (Ernst, 2002).

Metal contamination in the environment can cause adverse effects in biota. Anthropogenic heavy metal emission to the atmosphere is up to several times higher than their emissions from natural sources (Shadid et al., 2017). Cd is widespread non-essential highly phytotoxic metal. Mining, industrial discharges, waste disposal in landfills, sewage sludge application, agricultural usage of pesticides and fertilizers are the main sources of Cd soil pollution. The background Cd concentrations usually are up to 0.2-0.3 mg/kg and levels in agricultural or industrial areas may be strongly elevated (Sauvé et al., 2000). Due to high Cd mobility and water solubility it easily enters the plants via roots (Gallego et al., 2012). Additional sources of cadmium for plants are atmospheric deposition, pesticides and fertilizers that can be assimilated via foliar uptake (Shadid et al., 2017). Cd disturbs enzymes activity, photosynthesis, nutrient uptake and translocation in plants, induces oxidative stress (Sandalio et al., 2001, López-Millán et al., 2009) and these adverse effects may result in plant growth or yield reduction.

Some medicinal plants were reported to accumulate heavy metals in belowground and aboveground tissues (Harris et al., 2011; Kalny et al., 2007; Zheljazkov et al., 2008) and several species were reported as hyperaccumulators (Wei et al., 2008). Heavy metal accumulation could lead to undesirable changes in medicinal plants yields, quality and contents of essential oils (Zheljazkov et al., 2006). Sometimes the content of heavy metals found in medicinal plants is close to or even above their maximum allowable level (MAC) (Caldas, Machado, 2004, Rubio et al., 2012). According to the World Health Organization (WHO) data, approximately 70 % of the world population use medicinal plants for primary health care and this raises the concern about the safety of medicinal herbs use. The WHO set the maximum level of Cd in medicinal plants of 0.3 mg/kg (WHO, 1999). Most studies analysing heavy metal (especially Cd) impact to medicinal plants imitate short-term (up to several days) plants exposure to heavy metal under hydroponic conditions and focus on plants growth parameters and metals uptake from solution. Though integrated assessment of heavy metal soil pollution impact to biochemical, physiological and morphological endpoints is lacking. In the present work, the effects of low and moderate soil cadmium pollution to the growth of chamomile (*Matricaria recutita* L.) were examined. Secondly, we measured Cd damaged to cells membranes, Cd accumulation in different chamomile organs (roots and flowers) and estimated the amount of Cd released from plants into water extracts.

Materials and Methods

Chamomile (*Matricaria recutita* L.) were grown in 10 cm diameter pots filled with peat substrate mixed with sand (9:1) in climate-controlled chambers: temperature 22±1 °C in the daytime and 16±1 ° C at night, photoperiod – 14 hours, relative humidity – 65%, light intensity – 14000 Lx. Eight weeks after

plants emergence soil was spiked with Cd solution (as $CdSO_4*5H_2O$) to obtain the final 3, 6 and 12 mg Cd/kg in soil (dry weight). The same volume of distilled water was added to the control samples. All treatments were done in triplicate. The lowest tested Cd concentrations represents the maximum allowable concentration (MAC) in the soil (HN 60:2004). The plants were exposed to Cd for 4 weeks prior the harvesting.

The morphological (shoot, root and flower dry weight, number of flowers), physiological (chlorophyll a and b concentration), biochemical (malondialdehyde (MDA) concentration) parameters and Cd concentrations in roots, flowers and infusions were evaluated.

For dry weight assessment, the plants were dried at 60°C for 48-72 h up to constant weight. The number of flowers was determined by taking 3 plants from each pot and calculating the average flowers number per one plant. Content of chlorophylls (a, b) and carotenoids was determined spectrophotometrically after extraction of leaves in 100 % acetone (von Wettstein, 1957). Concentration of MDA, the end-product of lipid peroxidation, was assessed by reaction with thiobarbituric acid (Buege and Aust, 1978).

For Cd analysis in plant material, plant samples were dried for 24-48 h at 70°C temperature and digested using Milestone Ethos One closed vessel microwave system (with HNO_3 and H_2O_2). For the quantitative determination of Cd in plant material (roots, flowers) and flowers infusion a Shimadzu AA-6800 atomic absorption spectrometer was used.

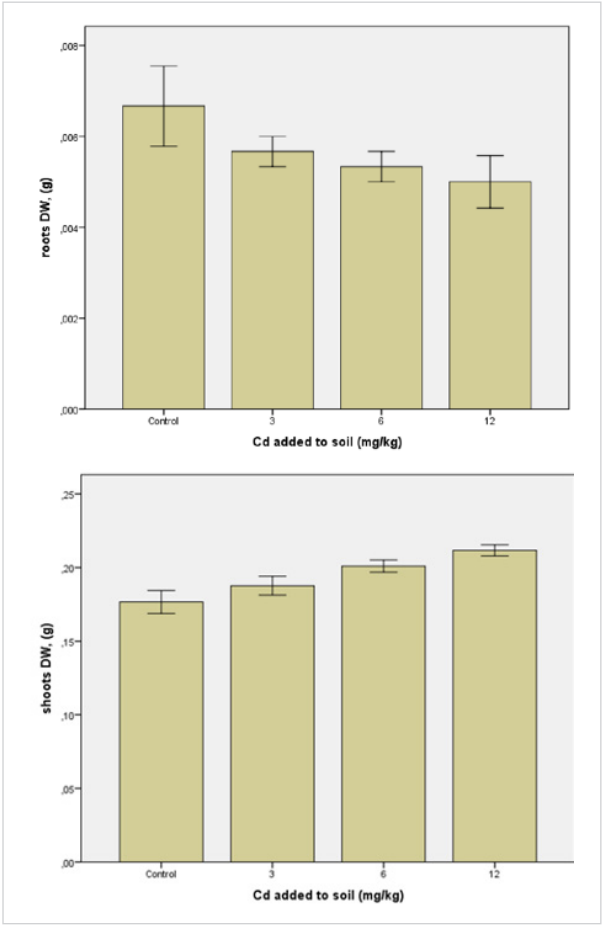
For infusions preparation, sample of 0.1 g dried chamomile flowers was infused with 100 ml of boiling distilled water, then left for an infusion for 15 minutes. After 15 minutes the extract was filtered and stored at 4°C until chemical analysis. Maximum allowable Cd quantity in drinking water of 5 µg/l (HN 24: 2003) was used for the assessment of chamomile infusions safety.

A one-way analysis of variance (ANOVA) was used to assess the metal effect on estimated endpoints. Significant differences between control and samples, treated with Cd, were determined by the Mann Whitney U-test and were considered to be significant at $p < 0.05$. Spearmen correlation was used for the analysis of relationship between Cd soil concentration and different plant parameters. The statistical analysis was carried out using Statistica and SPSS software.

Results and Discussion

The dry weight of chamomile roots grown in the soil polluted with Cd was by 15-25 % lower than that of control plants. Soil Cd pollution had no inhibitory effect on the dry weight of chamomile shoots (Fig. 1). Results indicated that low soil Cd pollution (3 mg/kg) did not affect significantly the weight increment of chamomile. However, the concentrations above the MAC started to induce a reduction in root growth, though did not affect the aboveground biomass growth. Chamomile root and shoot dry weight was reduced up to 60% after the treatment with 3-60 µM in medium growth (7 days treatment) (Pavlovič et al., 2006). A reduction in chamomile root and shoot biomass was also recorded under field conditions near ferrous metallurgical plant (Stancheva et al., 2014).

Fig. 1. *Root and shoot dry weight of chamomile M. recutita grown for 4 weeks in soil polluted with Cd*



Cd had a significant effect on the flowering of chamomile, i.e. number of flowers per plant and dry weight of flower (ANOVA, F_{number}=23.01, $F_{dry\ weight}$=11.24, p<0.05) (Fig. 2). Number of flowers in Cd polluted soil was reduced by 10-31 %, indicating that Cd has adverse effect on the reproduction of chamomile. Cd pollution resulted not only in the reduced flowering of plants, but the flowers were of lower dry weight as well. In the treatment with 3 mgCd/kg (the MAC level) the dry weight of chamomile flower was almost twofold lower than that of control (t-test, p<0.05). The flower dry weight decreased with Cd concentration in the soil (r_s=0.80, p<0.05) and in the treatment with the highest Cd concentration the dry weight was by 64 % lower compared to control level. Reduced chamomile flowering imply that the reproductive growth stage is very sensitive to heavy metal pollution and Cd may further reduce seeds number. Our results indicate that chamomiles growing in the soils with low Cd pollution (not exceeding MAC) might have lower reproduction and this may lead to reduced population. Though some studies did not reveal the negative heavy metal impact on these endpoints (Stancheva et al., 2014).

Content of photosynthetic pigments (chlorophylls a, b and carotenoids) was not adversely affected and no reduction in their concentrations was recorded (Fig. 3). Even more, a slight increase in chlorophyll a concentration was observed. Our results contradict to other studies where negative Cd impact on plant physiology was observed (Žaltauskaitė, Šliumpaitė, 2013a; 2013b; Kummerová et al., 2010), though are in line with those reporting slight increase in chlorophyll

Fig. 2. *Number of flowers and dry weight of flowers of chamomile M. recutita grown for 4 weeks in soil polluted with Cd*

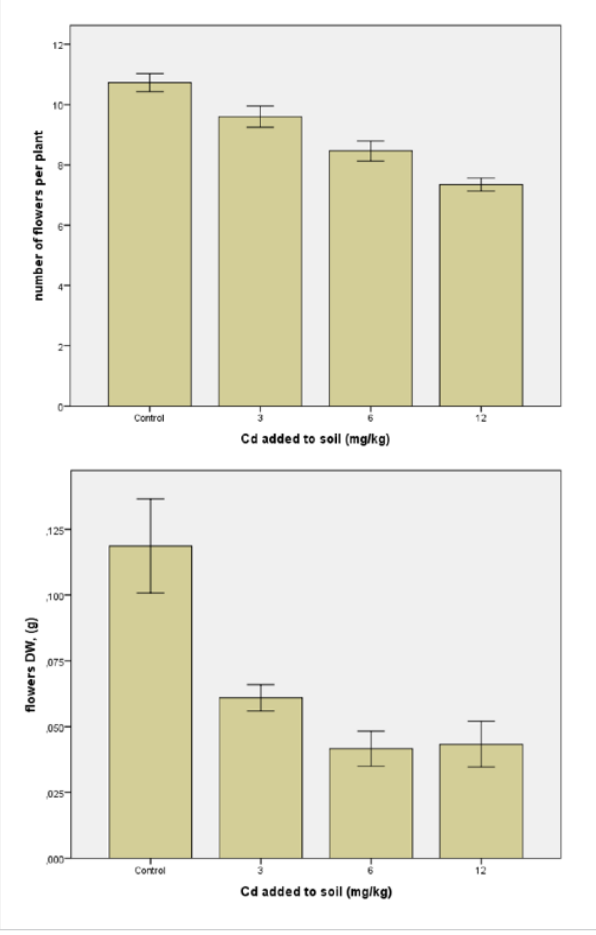
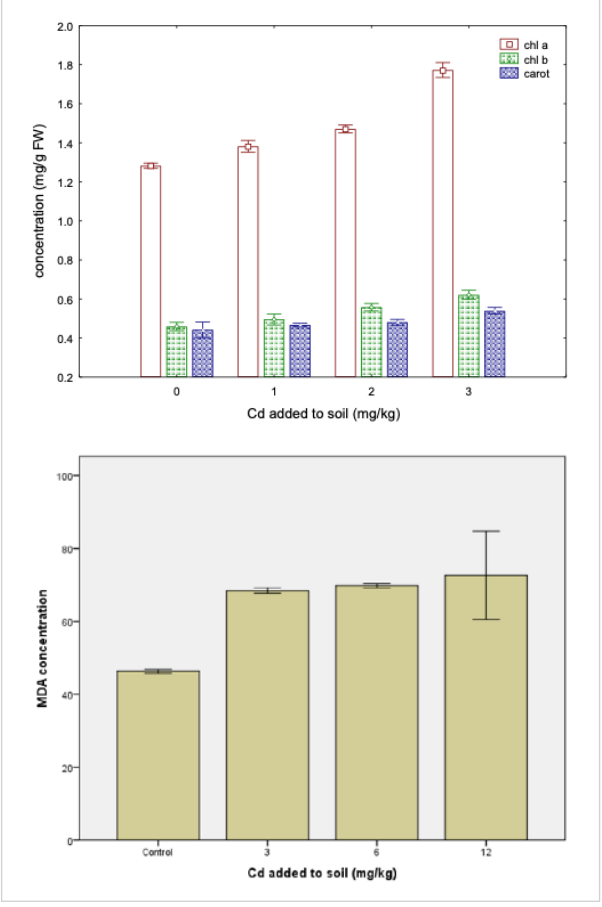


Fig. 3. *The content of chlorophyll a, b and carotenoids (left) and MDA concentration (right) in the leaves chamomile M. recutita grown for 4 weeks in soil polluted with Cd*



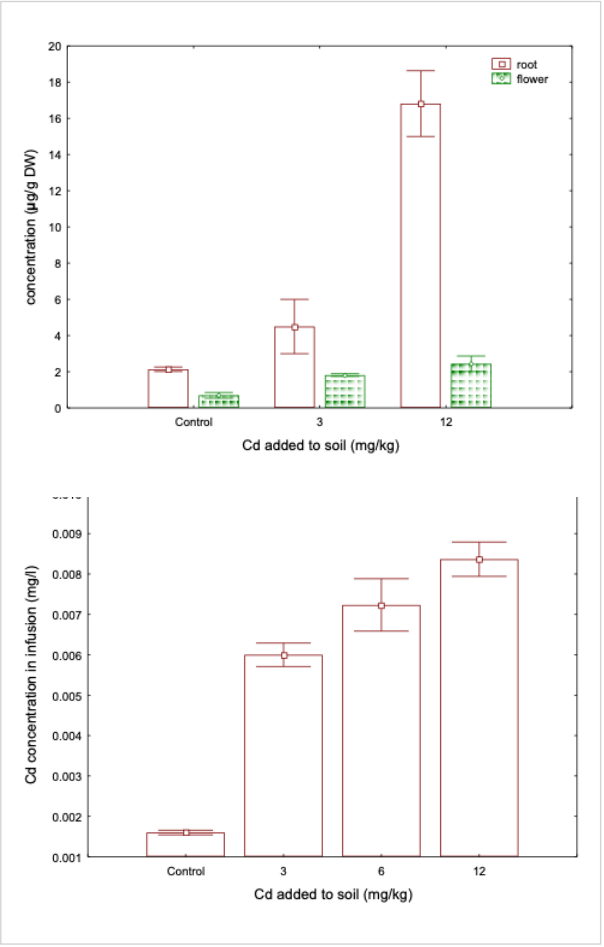
concentration in plants growing in contaminated soil (Karavaev et al., 2001). High concentrations of heavy metals weaken the activity of photosynthesis enzymes, also block electron transport in the chain, resulting in a decrease in total chlorophyll content (Thapar et al., 2008). An increase in carotenoids can be interpreted as a protective plant reaction to oxidative stress caused by heavy metals (Yu et al., 2007).

Cd induced lipid peroxidation in the tissues of chamomile (ANOVA, F=3.96, p<0.05) (Fig. 3). Concentration of MDA increased with Cd concentration in the soil (r_s=0.76, p<0.05). Cd was shown to produce reactive oxygen species (ROS) in the plants and induced oxidative stress (Sandalio et al., 2001; Dong et at., 2006; Martinez-Peñalver et al., 2012). However, no increase in MDA was detected in *M. chamomilla* after 10 10 days treatment with 120 µM Cd in hydroponics (Kováčik et al., 2006).

The amount of Cd accumulated in chamomile roots and flowers was affected by external Cd concentration in the soil (ANOVA, F_{root}=33.51, F_{flower}=10.80, p<0.05) (Fig. 4). Content of Cd both in roots and flowers increased along with Cd soil concentration (r_s=0.96, p<0.05). Concentrations of bioaccumulated Cd in roots were always significantly higher than that in flowers (p < 0.05). The translocation of Cd from roots to flowers was in the range of 10.8-63.0%. Our results are in a good agreement with other studies showing that the roots of medicinal plants are primary target of Cd accumulation and the transportation to the shoots is limited (Zheljazkov et al., 2008; Affholder et al., 2013; Žaltauskaitė et al., 2017). Chamomile was reported to accumulate Cd preferentially in roots rather in aboveground tissues (Kováčik et al., 2006, Kumerova et al., 2010, Pavlovič et al., 2006). Though, Sandalio et al. (2001) have shown that plants may accumulate Cd in roots in a non-active form reducing Cd toxicity for roots. The Cd concentrations in flowers of chamomile exceeded the limits of 0.3 mg Cd/kg recommended for medicinal plants (WHO, 1999).

Transfer of Cd during chamomile infusion process was analysed (Fig. 4) and it was observed that soil Cd concentrations highly affected Cd concentrations in infusions (ANOVA, F=50.95, p<0.01). Cd level in herbal

Fig. 4. *Cd concentration in roots and flowers (left) and infusions of chamomile M. recutita grown for 4 weeks in soil polluted with Cd*



infusion increased along with Cd soil concentration (r_s=0.90, p<0.001) and Cd concentrations in flowers (r_s=0.94, p<0.01). It was shown that Cd could be recovered to 15-21 % in the infusions of chamomile (Chizzola et al., 2008), though Arpadjan et al. (2008) reported only 9-11% recovery in infusions. The level of Cd in infusions exceeded the maximum allowable Cd level in drinking water (HN 24: 2003). Comparative study of traditional and herbal teas has shown that herbal teas had higher Cd content than traditional teas (De Oliveira et al., 2018). Our results indicate that chamomile grown in Cd polluted soils are not safe for use and it may pose the risk for humans.

Conclusions

The results of our study showed that low and moderate soil contamination with Cd had no adverse effect to the aboveground biomass of chamomile, however slightly impaired root biomass production (15-25 %). Cd pollution reduced chamomile flowering (10-31 %), flowers dry weight (up to 64%) and induced oxidative stress. Cadmium concentrations in the chamomile tissues and infusions increased along with Cd concentration in the soil. It was found that Cd concentrations in chamomile flowers and infusions were above the maximum allowable Cd levels in medicinal plants and drinking water. This study demonstrated that the use of chamomile products may pose a risk to human health and it is important to consider possible Cd intake from chamomile tea consumption.

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