Metabolic responses of *Carlina acaulis* L. to chronic and acute cadmium stress: insights into chelation mechanisms, non-enzymatic antioxidants, and specialized metabolism

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Abstract

Background

Chronic and acute stress can lead to completely different metabolic responses of plants exposed to the same abiotic factor. The effect of long-term chronic cadmium stress (ChS, 0.1 μM Cd, 85 days) or short-term acute cadmium stress (AS, 10 μM Cd, 4 days) on the physiology of *Carlina acaulis* L. (Asteraceae) and selected secondary metabolites was compared to identify specific physiological and biochemical reactions.

Results

The bioconcentration of Cd in all analyzed organs was higher under AS in comparison with ChS (130 vs. 16 $\mu\text{g g}^{-1}$ DW, 7.9 vs. 3.2 $\mu\text{g g}^{-1}$ DW, and 11.5 vs. 2.4 $\mu\text{g g}^{-1}$ DW in roots, leaves, and trichomes, respectively). The high concentration of Cd in the trichomes in the AS treatment might be an anatomical adaptation mechanism. ChS evoked an increase in the root biomass, whereas its impact on shoot biomass was not significant in any treatment. The amounts of ascorbic acid and the sum of phytochelatins were higher in the shoots, whilst organic acids (malic and citric) reached higher levels in the roots of plants from the ChS treatment. Glutathione depletion occurred in the shoots, but there was no change in its root level in both treatments. The amount of chlorogenic acid, but not that of ursolic and oleanolic acids, was increased under ChS. On the other hand, AS exposure elevated the level of ursolic and oleanolic acids, but not chlorogenic acid in the shoots.

Conclusions

These data indicate that ChS and AS induce different physiological and biochemical defense mechanisms. Both chelation and enhancement of the antioxidative machinery contribute to protection of *C. acaulis* exposed to long-term (chronic) Cd exposure and alleviate Cd toxicity effectively. However, triterpene acids were elevated only under AS treatment, which may suggest an instantaneous action of these metabolites under short-term acute Cd stress.

Background

Morphological and metabolic responses of plants to Cd exposure have been widely studied over the last decades [1–3]. Thousands of experiments with various species proposed many hypotheses regarding the Cd tolerance mechanisms and Cd toxicity in plants. The main complications in the unification of plant tolerance mechanisms to Cd are the various times of exposure and applied concentrations. Sanità Di Toppi and Gabbrielli [1] pointed out that the major problem of the Cd-plant interaction is the use of a high Cd concentration for a short time, while a common concentration in standard soil is typically less than 1 μM . This implies that most scientific publications studied acute stress (high concentrations with short-time exposure), which reflects environmental conditions inadequately. However, several studies dealing

with the chronic effect of Cd (long-time, low-concentration) in terrestrial [3, 4] or aquatic plants [6, 7], including algae [8], have been published.

Cadmium ions are readily absorbed by plants; however, its physiological function is not yet known. Members of the Asteraceae family (such as chamomile and dandelion) readily accumulate considerable amounts of Cd in the shoots but also exhibit various metabolic responses triggered to counteract toxicity caused by this metal [9, 10]. Among them, changes in non-enzymatic antioxidants and chelators such as L-ascorbic acid, thiols, low molecular organic acids (LMOAs), and phenols are generally the most common in plants.

Plants belonging to the *Carlina* genus (Asteraceae family) produce various biologically active compounds [11, 12]. Hence, they have been widely used in folk medicine [13]. Moreover, some species of *Carlina*, including *C. acaulis*, are facultative metallophytes, i.e. species that tolerate soils with high content of heavy metals. Two species, *Carlina acaulis* L. and *Carlina vulgaris* L., are part of the flora of calamine areas located in the metalliferous sites of Bolesław, in Southern Poland [14, 15].

The aim of this study was to investigate similarities and differences in the responses of *C. acaulis* exposed to low Cd concentrations over a long time (chronic stress, ChS) or to high Cd concentrations over a short time (acute stress, AS). The main objectives of the study included: (i) comparison of *C. acaulis* response to ChS and AS, (ii) assessment of the Cd translocation and accumulation under different Cd doses, (iii) quantification of non-enzymatic antioxidants and chelators under ChS and AS, and (iv) evaluation of accumulation of phenolic compounds and specific triterpenes in *C. acaulis* exposed to ChS or AS.

Methods

Plant material, growth conditions, and experimental design

Achenes of *Carlina acaulis* L. (Asteraceae, voucher specimen no. 2005A) were obtained from the Botanical Garden of Maria Curie-Skłodowska University (Lublin, Poland). They were germinated on the surface of garden soil and 10-day old seedlings were transferred into polyethylene pots (0.5 L) filled with garden soil substrate. The chemical composition of the garden soil was as follows: 20.98 N-NH₄, 137.43 N-NO₃, 137.68 P-PO₄, 252.35 K, 2518 Ca, 217.00 Mg, 545.25 S, and 135.00 Cl (mg dm⁻³ of soil) with pH 6.45 and electrical conductivity of 2.27 mS cm⁻¹. After 28 days of pot cultivation, the plants were carefully washed with distilled water and transferred into pots (one plant per pot) filled with 0.5 L of half strength Hoagland's no. 2 nutrient solution. After 5 days of acclimation, the plants were divided into three groups (15 plants per treatment): i) control plants (continual hydroponic cultivation without addition of Cd), ii) chronic stress/ChS (85 days of hydroponic cultivation in the presence of 0.1 µM Cd) and ii) acute stress/AS (4 days of exposure to 10 µM Cd in hydroponics): the cultivation in hydroponic conditions lasted 90 days for all treatments (see time axes in Suppl. Fig. S1). The culture solutions were continuously aerated, evapotranspiration loss was replenished daily with distilled water, and the medium

was renewed every 14 days to prevent nutrient deficiency. Cadmium was added in the form of $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (Sigma-Aldrich, St. Louis, MO, USA). Cultivation was carried out in a growth chamber at 18/25 °C (night/day) under light-emitting diodes at photosynthetic photon flux density of $90 \mu\text{mol m}^{-2} \text{s}^{-1}$ and relative humidity of 60-65%. All treated plants were harvested after 90 days of cultivation in hydroponics. The plants were separated into shoots and roots and the fresh weight (FW) was determined. Five plants were powdered in liquid nitrogen and stored at -80 °C for determination of LMWOAs, total phytochelatins (PCs), reduced glutathione (GSH), and L-ascorbic acid (AsA). The remaining plant biomass was dried at room temperature for determination of selected secondary metabolites or oven dried at 70 °C to constant weight for quantification of Cd and other mineral nutrients. Two parallel repetitions of the experiment were performed to verify the responses of the main parameters, including: growth variations, Cd accumulation, and thiol content.

Determination of Cd and essential nutrients

Dry leaf material was divided into trichomes and parenchyma by multiple-centrifugation and mechanical shaking. The roots, trichomes, and leaves without trichomes were digested in 5 mL of a mixture of $\text{HNO}_3\text{:H}_2\text{O}$ (2:8 v/v) in a microwave digestion apparatus (TOPwave, Analytick Jena AG, Jena, Germany). The resulting clear solutions were transferred into volumetric flasks and filled up to 25 mL with deionized water. The amounts of all investigated elements were measured using ICP-OES PlasmaQuant PQ 9000 Elite (Analytik Jena AG, Jena, Germany). Effective plasma power was 1300 W and the plasma, auxiliary, and nebulizer argon flow rate were 12.0, 0.5, and 0.6 L/min, respectively. Attenuated axial direction of measurement for Ca, K, Mg and axial direction for Cd, Zn, Mn, Fe, Mo, Cu were applied. Three replicates of each sample were measured for a correct statistical analysis.

Measurement of LMWOAs, AsA, and thiols

The LMWOAs, AsA, and thiols were analyzed in the plant FW using Agilent 7100 Capillary Electrophoresis (Agilent Technologies, Santa Clara, CA, USA): the LMWOAs (malic and citric acids) according to the method proposed by Dresler et al. [16], total AsA content following Dresler and Maksymiec [17], and thiols (PCs and GSH) after monobromobimane derivatization [18].

HPLC of triterpene and phenolic acids

An aliquot (0.5 g) of dried plant material was extracted three times with 100% methanol (3×1.5 mL) in an ultrasonic bath for 30 min. The extracts were combined, filtered through 0.22 µm filters, and filled up to 5 mL in a volumetric flask. High performance liquid chromatography (HPLC) analyses were performed on a VWR Hitachi Chromaster 600 chromatograph with a PDA detector and EZChrom Elite software (Merck, Darmstadt, Germany). The RP18e LiChrosper 100 column (Merck, Darmstadt, Germany) (25 cm × 4.9 mm i.d., 5 µm particle size) was used to separate triterpenic acids – oleanolic and ursolic acid. Other technical details are the same as reported previously [12]. Chlorogenic and 3,5-dicaffeoylquinic acids were analyzed using C18 reversed-phase column Kinetex (Phenomenex, Torrance, CA, USA) (10 cm × 4.0 mm

i.d., 2,6 µm particle size) as in previous work [19]. The identification of the compounds analyzed was confirmed by comparison of the retention time and spectral similarity with standards.

Quantification of total phenolic content and antioxidant capacity

Analyses were performed in the same methanolic extract used for determination of triterpene and phenolic acids. The total (soluble) phenolics content (TPC), expressed as mg of gallic acid equivalents (GAE) per gram of air dry weight of plants was measured using the Folin-Ciocalteu reagent [10]. The antioxidant capacity, expressed as mg of trolox equivalents per gram of air dry weight, was measured using free radical 2-azino-bis-3ethyl-benzthiazoline-6 sulfonic acid (ABTS) [20].

Statistical analysis

Samples from five individual plants were assessed for each treatment, parameter, and organ (n = 5). One-way analysis of variance (ANOVA) followed by a Tukey's post-hoc test was used to evaluate the significance of differences (p<0.05) between treatments. Principal component analyses (PCA) were performed separately for shoots and roots based on all studied parameters. All statistical analyses were carried out using Statistic ver. 13.3 software (TIBCO Software Inc. 2017).

Results

Impact of chronic/acute Cd stress on the growth

The short-term 10 µM Cd stress resulted in visible necrotic symptoms on old leaves (Fig. 1). However, no decrease in the FW of plants exposed to AS (10 µM Cd) was observed in any organ (Fig. 2). On the contrary, the plants cultured at ChS (0.1 µM Cd) showed significantly higher root FW (by ca. 80%) in comparison to the control plants. Similarly, the shoots of the ChS plants had also 25% higher biomass (Fig. 2).

Accumulation of Cd and selected essential nutrients

The concentration of Cd in plant organs was significantly affected by its medium concentration and exposure time (Fig. 3). The AS treatment resulted in almost 8-fold higher Cd accumulation in the roots, compared to the ChS. Accumulation of Cd in the aboveground organs (leaves) was also ca. 2-fold higher in favor of AS, with even higher differences in the trichomes (Fig. 3). Owing to the greater increase in the root Cd content under AS, the translocation factor (TF) for the leaf/root was over three-fold lower and the trichome/leaf TF value increased about twice at AS in comparison to ChS (Suppl. Table S1).

Our results showed that the exposure to both Cd treatments resulted in a decrease in the concentrations of Ca and Mg in the roots and K and Cu in the leaves. In turn, foliar and root Mn concentrations increased considerably under ChS, whereas AS induced an increase in the Mn level only in the roots. The bioconcentrations of other elements unchanged. Moreover, Cd stress did not affect the content of the analyzed elements in trichomes. The exception was Zn, whose content decreased under ChS (Tab. 1).

Changes in antioxidants and chelators differ under acute and chronic Cd stress

The AS and ChS exposure elevated the AsA amount but reduced the GSH content in the shoots. At the same time, the root contents of these antioxidants remained unaffected (Fig. 4a, b). A repeatedly elevated AsA level was determined in the shoots of plants exposed to Cd stress, especially to ChS (Fig. 4a). Similarly to the AsA and/or GSH accumulation, the content of the sum of PCs in the shoots differed between the treatments. Our results showed that the Cd stress (both treatments) significantly elevated the sum of PCs, but the ChS-exposed plants had an over two-fold higher concentration of PCs than those grown under AS (Fig. 4c). In turn, the accumulation of citric and malic acids significantly increased in both ChS and AS shoots, and in the roots under only the ChS treatment (Fig. 5a, b).

Changes in selected secondary metabolites under various Cd exposure types

Two triterpene acids were detected in the shoots only and their accumulation was significantly enhanced under the AS treatment (Fig. 6). As for the detected phenolic acids, ChS considerably stimulated mainly the accumulation of chlorogenic acid in both organs, while the AS treatment induced an increase in the content of this acid, which however was not statistically significant (Fig. 7a). The accumulation of 3,5-dicaffeoylquinic acid was significantly elevated in the shoots only under exposure to both AS and ChS (Fig. 7b). This increase in both phenolic acids under the ChS treatment was related to higher TPC and antioxidant capacity of the roots, compared to the control, and significant elevation of TPC in the shoots compared to AS (Suppl. Fig. S2).

Principle component analysis

The PCA of the obtained variables, especially from the shoots (Fig. 8a), clearly separated the individuals into three groups according to the experimental treatments. The first PC explained 37 and 35% of total variability for the shoots and roots, respectively, while the second PC explained 17% for the shoots and 15% for the roots (Fig. 8a, b). This means that both PCs explained approx. 54% and slightly more than 50% of the total variance for the shoots and roots, respectively. In the case of the shoots, the first PC facilitated separation of both Cd-stressed groups of plants from the control, and PC1 was positively correlated with GSH, K, and Cu and negatively correlated with both LMWOAs, 3,5-dicaffeoylquinic acid, AsA, PCs, Cd, and triterpenes. On the other hand, the shoot biomass was strongly correlated with PC2. PC2 was also partially determined by the Cd, ursolic, and oleanolic acid variables, whose high contents were noted in the shoots of the AS plants (Fig. 8a). In the roots, PC1 distinguished the control and ChS plants (Fig. 8b). The first component was positively determined by the content of phenolic compounds, LMWOAs, and biomass and negatively correlated with Zn, Ca, and Mg concentrations. In turn, PC2 separates the AS roots – individuals with high Cd, Mo, Fe, Cu and partially AsA and PC content.

Discussion

Cadmium ions can induce several visual changes in plants, including reduction of growth, alteration of morphology, chlorosis/necrosis, etc. [1, 21]. Larsson et al. [22] noted that even 0.5 μM Cd can decrease

leaf area while concentrations above 2.0 μM Cd can significantly reduce chlorophyll content. In our experiments, the short-term Cd stress (AS) resulted in visible necrotic symptoms on the leaves of *C. acaulis* (Fig. 1), without an impact on plant biomass (Fig. 2). On the other hand, when plants were exposed to ChS, a growth-promoting effect of Cd ions was found, especially in the roots (Fig. 2). A similar phenomenon was observed after 14 days of exposure of *C. acaulis* to Ag ions used at a concentration of 1 μM [23]. The stimulatory effect of the sub-inhibitory concentration of non-essential toxic metals can be related to the so-called hormetic effect [24]. As indicated by Calabrese [25], hormesis is an adaptive compensatory process in response to stress and initial disruption in homeostasis. One of the mechanisms responsible for stimulation of growth evoked by the low Cd concentrations could be an increase in cell proliferation by functional substitution of Zn (by Cd), which is a cofactor of enzymes playing a major role in replication and translation [26]. Other proposed mechanisms are related to the increase in root thickness due to exodermis and endodermis modifications and to the peroxidase-mediated higher lignin synthesis [27, 28]. Recently, a presently controversial postulate has been proposed that plants have developed mechanisms that employ Cd as a beneficial element due to the positive effects of low Cd doses on plant growth and some physiological indicators [29].

Cadmium is a highly mobile element, and its progressive accumulation in relation to longer exposure time has been observed in various species [4, 10]. Our results indicate that AS caused higher accumulation of Cd in the *C. acaulis* organs probably due to damage to some components of the defense mechanisms, while plants exposed to ChS had enough time to adapt to the presence of Cd ions in the nutrient solution by modulation of the level of protective metabolites (mentioned below). The adaptation may also include an increased "barrier" effect due to root modifications (Fig. 2). As the trichome/leaf TF value for Cd under ChS was about two-fold lower than at AS (Suppl. Table S1), we assume that under a high Cd concentration (AS in our work) Cd ions can be partly detoxified by storage in the leaf trichomes. This mechanism of Cd detoxification has been proposed for various species [30-32]. It is also not excluded that a preferential location and active transport of Cd to trichomes could be only an effect of passive/diffusive transportation and higher Cd accumulation under AS (Fig. 3).

Several mechanisms of Cd effect on the uptake of essential nutrients have been postulated: competition for Ca transporters, inhibition of Fe loading to the xylem, or indirect influence on nutrient movement [33]. Our results regarding the changes in the accumulation of the selected nutrients (Tab. 1) are in agreement with previous studies which showed that two *Atriplex* species exposed to Cd contained significantly lower levels of K and Ca [34]. Interestingly, we observed that the leaves of *C. acaulis* exposed to ChS accumulated larger amounts of Ca than the control or AS-treated plants (Tab. 1). We have recently observed a similar increase in the shoot Ca level in this species under the growth-promoting concentration of Ag(II) (1 μM) [23]. Given the known role of Ca in the detoxification of Cd, the elevated amount of Ca in the leaves under low Cd stress may be an indication that some protective mechanism is activated by the plants after Cd exposure. The role of Ca in attenuation of Cd toxicity is known [34, 35] and several hypotheses have been put forward, including improved lipid peroxidation protection, competition of Ca^{2+} for the same channel transporters with Cd^{2+} , improved antioxidant enzyme activities

[36], or even excretion of Ca-Cd crystals through trichomes [30]. Reduced Mn translocation was also noted under the influence of Cd ions [37]. On the other hand, there were no significant changes in the Fe bioconcentration (Tab. 1), although the Cd-induced inhibition of root Fe(III) reductase can lead to a decrease in the Fe uptake and deficiency of the element [37]. This may indicate that the types of Cd stress used in our experiments did not cause abnormalities in the Fe balance in *C. acaulis*.

It is known that AsA and GSH are essential components of the ascorbate-glutathione pathways of ROS scavenging. Under both AS and ChS, the foliar AsA concentration increased, while the GSH level was reduced (Fig. 4a, b), indicating possible reciprocal changes between these compounds. Simultaneously, their contents in roots were not affected by Cd stress and indicated more pronounced changes in the photosynthetic tissues (Fig. 4a, b). Moreover, the highest accumulation of AsA was observed in the shoots under ChS (Fig. 4a). It has been shown that AsA protects plant cells against Cd-induced oxidative damage [38] and the age of plants has a significant impact on AsA accumulation under Cd stress [17]. Such a ChS-induced increase in the AsA level may indicate an important protective role of this antioxidant during chronic Cd exposure. At low and continuous Cd concentrations, plants have time to "acclimatize" to the stress. The lower GSH concentration in the shoots also confirms its role as a precursor for biosynthesis of PCs [18, 39], while a more intensive increase in root PCs without any impact on the root GSH content (Fig. 4b, c) may indicate enhanced biosynthesis of GSH as a protective mechanism against high accumulation of Cd in the roots (compared to shoots). PCs are considered as one of the major intercellular chelating ligands for Cd ions and their content is usually related to the metal concentration [40]. In their review papers, Sanità Di Toppi and Gabbriellini [1] and Ahmad et al. [41] pointed out that accumulation of PCs is the main mechanism allowing plant cells to cope with Cd stress and the synergistic role of PCs with antioxidants is underlined. However, they also indicated that most these investigations were focused on acute Cd stress. In our study, it was found that, even at the lower Cd accumulation in the ChS shoots, the content of PCs was over twice higher than under AS, which involved higher doses of Cd (cf. Figs 3 and 4c), suggesting that the exposure time, in addition to the applied metal dose, also plays a very important role in accumulation of PCs. Sun et al. [40] suggested that an increasing Cd concentration in the nutrient medium elevates content of PCs, but a Cd concentration above a critical value reduces their level due to severe metal toxicity. On the other hand, there is ample evidence that even 20 nM Cd can induce biosynthesis of PCs in *Ceratophyllum demersum* [7].

LMWOAs are efficient compounds in detoxification of heavy metals. They are involved in several mechanisms including: (i) reduction of metal availability by chelation with exudates; (ii) intracellular metal chelation; (iii) long-distance translocation of metals to compartments with low biological activity such as trichomes and the cell wall [42–44]. It has been found that LMWOAs are produced in response to Cd ions by various species of vascular plants [16, 44] or algae [38]. In our experiments, the concentrations of LMWOAs (citric and malic acids) considerably increased in the shoots under ChS and AS, but only under ChS in the roots (Fig. 5a, b). It seems that elevated accumulation of organic acids may be a mechanism of tolerance of chronic Cd stress, perhaps through exudation, since the ChS treatment resulted in a lower concentration of Cd in the tissues than in the AS treatment (cf. Figs 5 and 3). This phenomenon has been described as a tolerance mechanism in various species [45].

The concentration of triterpene acids (ursolic and oleanolic), which were detected in the shoots of *C. acaulis*, increased significantly but only under the AS exposure (Fig. 6). This trend is somehow different from the other secondary metabolites studied (at least for the shoots). This would suggest an instantaneous action of these compounds. The level of these acids in response to heavy metals has only rarely been discussed in the literature, mainly in relation to their antioxidant properties, which may also play a role under metal stress [46]. It has been shown that heavy metal stress increased ursolic acid in *Prunella vulgaris* [47] and Cd provoked higher accumulation of oleanolic acid in cell cultures of *Achyranthes bidentate* [48] or triterpenoid saponins in *Bacopa monnieri* [49]. However, a negative effect of Cd or Cu stress on the content of trisaccharide triterpene has also been observed in plant cultures of *Centella asiatica* [50]. In turn, Wang et al. [48] suggested that induction of oleanolic acid accumulation is related to the Cd-exposure time and probably to gene expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase in this pathway.

In our study, only the ChS exposure stimulated the accumulation of chlorogenic acid in both organs (roots and shoots) (Fig. 7a). In turn, the accumulation of 3,5-dicaffeoylquinic acid increased significantly in the shoots under both treatments (Fig. 7b). The elevated concentration of both phenolic acids at ChS was associated with higher root TPC and its antioxidant capacity (Suppl. Fig. S2) as well as stimulation of root growth (Fig. 2). Similarly, as shown by [Sofo et al. \[51\]](#), the remodeling of the root architecture and the production of some secondary metabolites may be two responses of plants exposed to metal stress. In an earlier work, Kováčik and Klejdus [9] observed significant elevation of chlorogenic acid in the related species chamomile (Asteraceae family) after prolonged exposure even to a low Cd concentration (3 μ M), indicating that chlorogenic acid has probably more general antioxidative action. On the other hand, the negative effect of multi-heavy metal stress on chlorogenic acid accumulation has also been observed in *Carlina vulgaris* plants collected from metalliferous areas [15]. The authors found that plants inhabiting heavy metal polluted areas accumulated less soluble phenolics and flavonoids and exhibited lower antioxidant capacity than plants from non-polluted regions.

Conclusions

The present study demonstrated some different physiological responses of *Carlina acaulis* to chronic (long time/low concentration) and acute (short time/high concentration) Cd stress. Although some mineral nutrients were negatively affected by Cd ions, chronic stress had less negative effects and even stimulated root growth, probably due to lower endogenous accumulation of Cd. At the same time, ascorbic acid and phytochelatin were more elevated in the shoots but the content of organic (malic and citric) acids was increased in the roots of plants from the chronic treatment. In combination with the strongly elevated chlorogenic acid level in this treatment, both chelation and enhancement of accumulation of non-enzymatic antioxidants are expected to contribute to protection in plants exposed to the long-term (chronic) Cd treatment. On the contrary, the role of triterpene acids in chronic or acute stress tolerance mechanisms was not immediately apparent. However, we suggest that under AS treatment, the instantaneous action of these metabolites can be an important physiological reaction.

Abbreviations

ABTS: 2-azino-bis-3ethyl-benzthiazoline-6 sulfonic acid; ANOVA: Analysis of variance; AS: Acute stress; AsA: L-ascorbic acid; ChS: Chronic stress; DW: dry weight; FW: Fresh weight; GAE: Gallic acid equivalents; GSH: Reduced glutathione; LMWOAs: Low molecular weight organic acids; PCA: Principal component analyses; PCs: Phytochelatins; TF: Translocation factor; TPC: Total phenolics content;

Declarations

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Authors' contributions

S.D., M.S., and J.K. conceived the experiments and the experimental design; S.D. did project administration; S.D. and M.S. cultivated the plants; S.D. performed LMWOA, GSH, AsA, and PC analysis; M.S. and M.W. analyzed the secondary metabolites; I.S. and J.S. analyzed the elements; S.D. conducted statistical analysis and data visualization; S.D. prepared the original manuscript draft; B.H.-N., M.W., I.S., and J.K., and were involved in the experimental ideas and critically revised the manuscript. All authors have read and approved the manuscript.

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Table

Table 1. Accumulation of selected mineral nutrients in the leaves (without trichomes), roots, and trichomes of *C. acaulis* exposed to long-term chronic cadmium stress (ChS, 0.1 μM Cd, 85 days) or short-term acute cadmium stress (AS, 10 μM Cd, 4 days). Data are means $\pm$ SE (n = 5); values followed by the same letter are not significantly different between treatments ($p < 0.05$, Tukey's test).

Ca mg g ⁻¹ DW				Mg mg g ⁻¹ DW			
	leaf	root	trichomes	leaf	root	trichomes	
Control	13.1 ±0.3 b	5.81 ±0.37 a	11.7 ±0.9 a	5.53 ±0.15 a	4.31 ±0.92 a	2.78 ±0.15 a	
ChS	14.6 ±0.3 a	4.16 ±0.16 b	13.6 ±0.5 a	5.58 ±0.35 a	2.44 ±0.21 b	3.07 ±0.23 a	
AS	13.7 ±0.4 b	4.12 ±0.48 b	13.7 ±0.7 a	4.96 ±0.22 a	2.41 ±0.11 b	3.04 ±0.09 a	
K mg g ⁻¹ DW				Fe µg g ⁻¹ DW			
	leaf	root	trichomes	leaf	root	trichomes	
Control	85.0±3.0 a	46.5 ±3.2 a	28.6 ±2.0 a	79.9 ±3.3 a	3153 ±682 a	129.8 ±6.3 a	
ChS	74.8 ±4.4 b	42.4 ±1.8 a	29.7 ±3.7 a	72.8 ±6.7 a	3535 ±517 a	116.8±17.6 a	
AS	70.9 ±1.9 b	45.6 ±3.9 a	28.2 ±1.2 a	76.8 ±3.4 a	3463 ±325 a	128.5±6.4 a	
Cu µg g ⁻¹ DW				Mn µg g ⁻¹ DW			
	leaf	root	trichomes	leaf	root	trichomes	
Control	6.13 ±0.34 a	12.1 ±1.3 a	6.71 ±0.85 a	7.02 ±0.72 b	5.80 ±0.86 b	7.06 ±1.40 a	
ChS	4.82 ±0.37 b	10.6 ±1.9 a	4.91 ±0.47 a	9.92 ±1.15 a	10.10 ±0.92 a	7.65 ±1.42 a	
AS	4.56 ±0.53 b	11.8 ±1.3 a	5.35 ±0.49 a	7.35 ±1.03 b	9.13 ±1.32 a	6.96 ±0.68 a	
Zn µg g ⁻¹ DW				Mo µg g ⁻¹ DW			
	leaf	root	trichomes	leaf	root	trichomes	
Control	22.4 ±2.2 a	64.0 ±2.9 a	25.2 ±2.8 a	4.45 ±0.91 a	11.51 ±4.00 a	2.60 ±0.48 a	
ChS	20.1 ±0.8 a	28.1 ±1.7 b	17.6 ±1.9 b	3.09 ±0.52 a	8.79 ±4.03 a	2.58 ±0.39 a	
AS	21.5 ±1.8 a	53.0 ±6.6 a	22.0 ±3.1 ab	2.94 ±0.38 a	11.26 ±1.79 a	2.39 ±0.21 a	

Figures

Control

10 cm

Chronic Cd stress

Acute Cd stress

Figure 1

Phenotype of *Carlina acaulis* at the end of the treatments with various Cd concentrations and exposure time.

Figure 2

Effect of long-term chronic cadmium stress (ChS, 0.1 μ M Cd, 85 days) or short-term acute cadmium stress (AS, 10 μ M Cd, 4 days) on the fresh weight of *C. acaulis* organs. Data are means $\pm$ SE (n = 8-10); values followed by the same letter are not significantly different ($p < 0.05$, Tukey's test).

Figure 3

Effect of long-term chronic cadmium stress (ChS, 0.1 μM Cd, 85 days) or short-term acute cadmium stress (AS, 10 μM Cd, 4 days) on the Cd accumulation in the roots, leaves (*leaves without trichomes), and trichomes of *C. acaulis*. Data are means $\pm$ SE (n = 5); values followed by the same letter are not significantly different (p < 0.05, Tukey's test).

Figure 4

Effect of long-term chronic cadmium stress (ChS, 0.1 μ M Cd, 85 days) or short-term acute cadmium stress (AS, 10 μ M Cd, 4 days) on: (a) AsA; (b) GSH; and (c) sum of PC content in the shoots and roots of *C. acaulis*. Data are means $\pm$ SE (n = 5); values followed by the same letter are not significantly different (p < 0.05, Tukey's test).

Figure 5

Effect of long-term chronic cadmium stress (ChS, 0.1 μM Cd, 85 days) or short-term acute cadmium stress (AS, 10 μM Cd, 4 days) on: (a) malic acid; and (b) citric acid content in the shoots and roots of *C. acaulis*. Data are means $\pm$ SE ($n = 5$); values followed by the same letter are not significantly different ($p < 0.05$, Tukey's test).

Figure 6

Effect of long-term chronic cadmium stress (ChS, 0.1 μ M Cd, 85 days) or short-term acute cadmium stress (AS, 10 μ M Cd, 4 days) on ursolic and oleanolic acids content in the shoots of *C. acaulis*. Data are means $\pm$ SE (n = 5); values followed by the same letter are not significantly different (p < 0.05, Tukey's test).

Figure 7

Effect of long-term chronic cadmium stress (ChS, 0.1 μM Cd, 85 days) or short-term acute cadmium stress (AS, 10 μM Cd, 4 days) on: (a) chlorogenic acid and (b) 3,5-dicaffeoylquinic acid in the shoots and roots of *C. acaulis*. Data are means $\pm$ SE ($n = 5$); values followed by the same letter are not significantly different ($p < 0.05$, Tukey's test).

Figure 8

Scaled scatter plot of principal component analysis of selected secondary metabolites (ChA – chlorogenic acid; CQA - 3,5-dicaffeoylquinic acid; UA – ursolic acid; OA – oleanolic acid; TPC – total phenolic content), thiol-peptides (GSH – glutathione; PCs – phytochelatin), organic acids (MA – malate acid; CA – citrate acid), antioxidant capacity (ABTS), biomass, macro- and microelements in the shoots

(a) and roots (b). The length of lines shows a correlation between original data and principal component axes.

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