South African Journal of Botany 94 (2014) 204–209

Contents lists available at ScienceDirect

South African Journal of Botany

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

Ammonium ions affect metal toxicity in chamomile plants

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ARTICLE INFO

Article history:
Received 24 April 2014
Accepted 30 June 2014
Available online 23 July 2014

Edited by RA Street

Keywords:
Heavy metals
Mineral nutrition
Oxidative stress
Phenolics

ABSTRACT

The impact of ammonium presence ($+NH_4^+$) or absence ($-NH_4^+$) on cadmium (Cd) and nickel (Ni) toxicity in chamomile (*Matricaria chamomilla*) was studied. Elimination of ammonium elevated tissue water content, amount of soluble proteins and Fv/Fm ratio though responses differed between Cd and Ni. Antioxidative enzyme activities (ascorbate- and guaiacol-peroxidase) were also more enhanced in Cd – NH_4^+ than in Ni – NH_4^+ treatments (in comparison with respective $+NH_4^+$ counterparts). Thiols rather decreased in shoots and increased in roots in the absence of ammonium. At the level of coumarin-related metabolites, NH_4^+ absence typically elevated amount of herniarin glycoside precursor while "stress" coumarin derivative umbelliferone decreased, indicating an additional negative impact of ammonium presence. Subsequently, total soluble phenols and PAL activity were not enhanced by ammonium absence. Mineral nutrients were not considerably affected with the exception of Fe and K that increased preferentially in the roots in the absence of ammonium. These data clearly show that NH_4^+ presence is stressful alone or in the presence of metals and that ammonium is not a suitable alternative source of nitrogen.

1. Introduction

Ammonium (NH_4^+), in addition to nitrate, is the main form of inorganic nitrogen (N) absorbed by plants (Hachiya and Noguchi, 2011). It is directly incorporated into the organic form of amino acids by enzymatic reactions (Gouia et al., 2003). On the other hand, high doses of ammonium are toxic to many plants species-specifically (Domínguez-Valdivia et al., 2008) though recent improvements may allow its use as fertilizer (Xie et al., 2013).

Owing to N importance in plant metabolism, their limited availability results in reduced growth and lower yield of plants (Kováčik et al., 2007; Giorgi et al., 2009). At the same time, the shift from N-based to C-based compounds (mainly phenolics) is usually observed (Kováčik et al., 2011; Rubio-Wilhelmi et al., 2012a; Rubio-Wilhelmi et al., 2012b). This effect of N deficiency on phenolic content is typical because depletion of other macronutrients such as potassium does not elevate phenols (Nguyen et al., 2010). It should be noted that these responses were observed in plants cultured in nitrate solutions while the impact of NH_4^+ nutrition has only partially been studied (Domínguez-Valdivia et al., 2008; Kováčik and Klejdus, 2014).

Increasing industrial production elevates the release of heavy metals into the environment, thus increasing risk for human health. Among others, nickel (Ni) and cadmium (Cd) are potentially dangerous because of their accumulation in the food chain. Ni is an essential 'ultramicronutrient' while Cd has no known physiological function. Both these metals are divalent and are unable to catalyze the generation of reactive oxygen species via Fenton–Haber–Weiss reactions (Gajewska and Skłodowska, 2010; Kováčik et al., 2012).

Environmental stress may elevate oxidative damage in plants including excess of metals (Gajewska and Skłodowska, 2010) and ammonium (Domínguez-Valdivia et al., 2008). It is also known that metals stimulate NH_4^+ formation (Chien and Kao, 2000) thus elevating risk of damage. Therefore antioxidative enzymes (such as peroxidases) and antioxidants (such as phenols or thiols) are important components that protect cells under stressful conditions (Kováčik et al., 2014).

Chamomile is a widely-used medicinal plant and also a "nitrogen-loving" plant (Schilcher et al., 2005). Previous study also showed that NH_4^+ is formed in nitrate-deficient plants (Kováčik and Klejdus, 2014). Though ammonium is formed under excess of various metals (Chien and Kao, 2000; Gajewska and Skłodowska, 2009; Gajewska et al., 2009), its impact on metal toxicity was only partially studied (Nasraoui et al., 2012). It can be assumed that this direct source of nitrogen for organic compounds such as amino acids could improve plant metabolism. Furthermore, absence of ammonium in relation to metal toxicity was not yet investigated. We therefore studied impact of NH_4^+ presence or absence on Cd and Ni excess in chamomile and various physiological and metabolic processes were monitored.

2. Material and methods

2.1. Plant culture, experimental design and statistics

Chamomile seedlings were pre-cultured in sand and further cultured in nitrate-containing Hoagland solution over 3 weeks followed by 2 weeks of adaptation to ammonium nitrogen prior to the start of

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http://dx.doi.org/10.1016/j.sajb.2014.06.016
0254-6299/

the treatment as described in detail previously (Kováčik and Klejdus, 2014). Because ammonium is toxic to many plants, they were not cultured in NH_4^+ alone from the beginning of cultivation (Domínguez-Valdivia et al., 2008). After this adaptation, plants were further cultured in ammonium-containing medium (+NH_4) or in ammonium-deficient medium (−NH_4) with the addition of cadmium ($CdCl_2 \cdot 2½H_2O$) or nickel ($NiCl_2 \cdot 6H_2O$) in a concentration of 60 μM. This concentration of metals has no impact on plant growth under the given cultivation conditions (Kováčik et al., 2011) and the N-deficient medium contained an equimolar amount of salt but without N (Kováčik and Klejdus, 2014). The whole experiment therefore involved 6 treatments designed as C + NH_4/C − NH_4 (control), Cd + NH_4/Cd − NH_4 and Ni + NH_4/Ni − NH_4 and plants were analyzed after 7 days of exposure.

For fresh mass-requiring parameters, individual shoots (whole basal leaf rosettes) or roots were powdered using liquid N_2 and fresh material was extracted as described below. Dry samples (dried at 75 °C to constant weight) were analyzed for total phenols, reducing sugars and minerals. Processing of all samples involved homogenization with inert sand using mortar and pestle to achieve complete tissue disruption. Data were evaluated using ANOVA followed by a Tukey's test (MINITAB Release 11, Minitab Inc., State College, Pennsylvania) at $P < 0.05$. The number of replications (n) in tables/figures denotes individual plants measured for each parameter. Two boxes (each containing 25 plants) were used for each treatment, thus the whole experiment included 12 boxes. Two independent repetitions of the whole experiment were performed in order to check reproducibility.

2.2. Assay of physiological parameters

Fresh and dry masses were measured in order to determine the plant water content [100 − (dry mass × 100 / fresh mass)] allowing recalculation of parameters measured in fresh samples.

The potential quantum yield of photosystem II (PSII) was measured using a Plant Stress Meter (PSM Mark II, Biomonitor, SCI AB, Umeå, Sweden) with a sensor diameter of 5 mm, and results were expressed as Fv/Fm (Kováčik et al., 2012).

Reducing sugars were extracted from dried material and determined colorimetrically using reaction with arsenomolybdate according to Somogyi–Nelson's method; quantification was based on a standard curve prepared with different concentrations of glucose (Kováčik et al., 2011).

2.3. Enzymatic activities

Fresh tissue was homogenized with small amount of inert sand using cold mortar and pestle in 50 mM potassium phosphate buffer (pH 7.0, 1 g FW/5 ml). After centrifugation, supernatants were used to measure enzyme activities of ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) as the oxidation of ascorbate and guaiacol at 290 and 470 nm, respectively (Kováčik et al., 2011; Kováčik et al., 2009).

Activity of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) was determined as the production of *trans*-cinnamic acid from phenylalanine using a slightly modified HPLC method with homogenates prepared with sodium borate buffer (pH 8.7) and UV detection performed at 275 nm (Kováčik et al., 2007; Ferrarese et al., 2000).

2.4. Assay of metabolites

Soluble proteins were quantified according to the Bradford method (Bradford, 1976) in homogenates prepared with 50 mM potassium phosphate buffer mentioned above and bovine serum albumin as standard (595 nm).

Total soluble phenols (extracted with 80% methanol from dry material) were quantified using Folin–Ciocalteu method with gallic acid as standard at 750 nm (Kováčik et al., 2011). Coumarin-related metabolites [(*Z*)- and (*E*)-2-ß-D-glucopyranosyloxy-4-methoxycinnamic acids (GMCAs), herniarin and umbelliferone] were estimated by gradient HPLC and quantified as described previously (Repčák et al., 2009).

Total thiols (amount of non-protein –SH group) were quantified using Ellman's reagent in homogenates prepared with 50 mM potassium phosphate buffer and cysteine for calibration at 412 nm (Kováčik et al., in press).

2.5. Quantification of ammonium and minerals

Ammonium concentrations were determined in samples extracted with deionized water (0.2 g FW/2 ml) using Nessler's reagent (detection at 425 nm) with NH_4Cl for calibration (Rubio-Wilhelmi et al., 2012b).

Mineral nutrients were quantified by AAS after mineralization of dry material with HNO_3 and H_2O_2 mixture as reported earlier (Kováčik et al., 2012).

3. Results and discussion

3.1. Absence of NH_4^+ improved physiological parameters

Excess of ammonium is usually toxic for plant growth though various species are variably sensitive: for example, spinach was highly sensitive while pea showed higher tolerance (Domínguez-Valdivia et al., 2008). In the present study, growth was not altered by co-application of NH_4^+ with metals owing to shorter exposure time (data not shown) while removal of NH_4^+ ameliorated some visual symptoms such as brown color of roots: in comparison with nitrate-fed plants, chamomile is clearly an NH_4^+-sensitive species (Kováčik and Klejdus, 2014). Decrease of NH_4^+ content was observed after its elimination

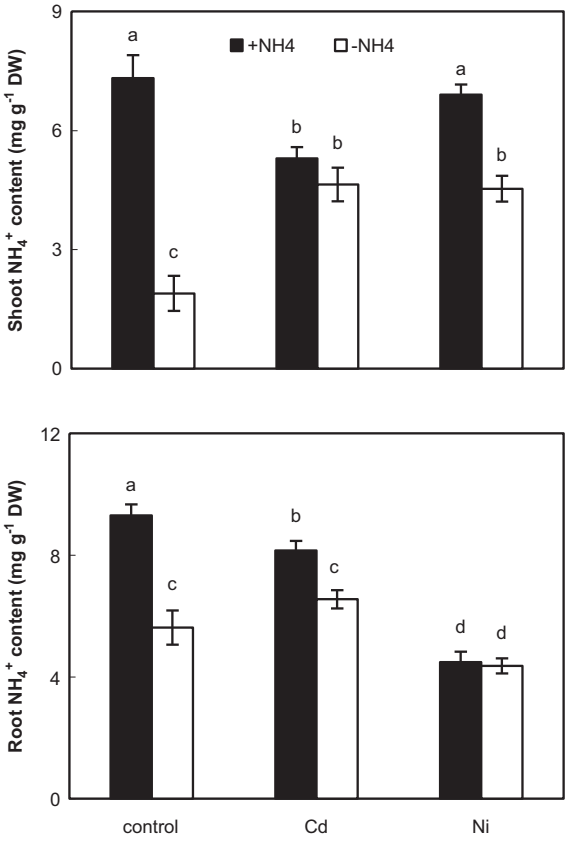

Fig. 1. Amount of ammonium ions in *Matricaria chamomilla* plants cultured in cadmium (Cd) or nickel (Ni) enriched solution (60 μM) in the presence (+NH_4) or absence (−NH_4) of ammonium nitrogen over 7 days. Data are means ± SDs shown as bars ($n = 4$). Values within each graph followed by the same letter(s) are not significantly different according to Tukey's test ($P < 0.05$).

Table 1
Selected physiological parameters of *Matricaria chamomilla* plants cultured in cadmium (Cd) or nickel (Ni) enriched solution (60 μM) in the presence (+NH_4) or absence (−NH_4) of ammonium nitrogen over 7 days. Data are means ± SDs ($n = 20$ for tissue water content and $n = 3$ for all other parameters). C − control. Values within rows followed by the same letter(s) are not significantly different according to Tukey's test ($P < 0.05$).

	C + NH_4	C − NH_4	Cd + NH_4	Cd − NH_4	Ni + NH_4	Ni − NH_4
Shoot						
Tissue water content (%)	83.38 ± 0.54 d	85.84 ± 0.41 b	83.81 ± 0.88 cd	87.48 ± 0.34 a	84.14 ± 0.40 cd	84.66 ± 0.52 c
Soluble proteins (mg g^{-1} DW)	68.5 ± 3.38 c	82.6 ± 4.51 b	72.8 ± 6.29 c	96.4 ± 3.97 a	76.1 ± 3.92 bc	82.5 ± 3.17 b
Reducing sugars (mg g^{-1} DW)	31.6 ± 1.29 c	32.1 ± 0.94 c	33.9 ± 2.21 bc	36.9 ± 1.45 b	43.8 ± 1.22 a	33.7 ± 1.91 bc
APX activity[a]	83.3 ± 2.23 c	81.6 ± 5.49 c	86.1 ± 3.96 c	161.5 ± 20.3 a	106.7 ± 10.6 bc	119.4 ± 14.2 b
GPX activity[b]	0.63 ± 0.054 b	0.34 ± 0.038 c	0.33 ± 0.031 c	0.62 ± 0.064 b	0.79 ± 0.077 b	1.35 ± 0.131 a
Fv/Fm	0.64 ± 0.028 b	0.78 ± 0.016 a	0.58 ± 0.035 b	0.76 ± 0.024 a	0.60 ± 0.034 b	0.78 ± 0.029 a
Root						
Tissue water content (%)	92.35 ± 0.26 b	92.69 ± 0.31 ab	93.33 ± 0.52 ab	93.61 ± 0.16 a	90.52 ± 0.84 c	92.50 ± 0.28 b
Soluble proteins (mg g^{-1} DW)	46.1 ± 2.50 bc	34.9 ± 3.37 d	40.8 ± 2.67 cd	58.2 ± 5.03 a	49.7 ± 3.43 ab	46.4 ± 3.92 bc
Reducing sugars (mg g^{-1} DW)	41.2 ± 1.73 ab	42.0 ± 2.64 a	36.7 ± 2.06 b	40.8 ± 1.54 ab	42.3 ± 0.95 a	44.6 ± 1.52 a
APX activity[a]	339.5 ± 21.8 b	234.6 ± 22.7 c	344.9 ± 13.2 b	346.7 ± 24.1 b	518.5 ± 18.2 a	325.2 ± 19.9 b
GPX activity[b]	5.05 ± 0.22 bc	3.12 ± 0.21 e	4.27 ± 0.28 d	8.93 ± 0.26 a	4.49 ± 0.34 cd	5.21 ± 0.24 b

[a] nmol min^{-1} mg^{-1} protein.
[b] μmol min^{-1} mg^{-1} protein.

from the culture medium as naturally expected (Fig. 1) while metals modified this change. Preferentially in shoots, both Cd and Ni elevated NH_4^+ amount that is in accordance with reports from various species where metals stimulated NH_4^+ accumulation (Gajewska and Skłodowska, 2009; Gajewska et al., 2009; Gouia et al., 2000). In the roots, changes of NH_4^+ content were less expressive and rather a decrease was observed, indicating differences between above- and below-ground organs (Fig. 1).

Measurement of maximal efficiency of PSII photochemistry (Fv/Fm ratio, Table 1) is a sensitive parameter allowing detection of potential toxicity of applied stressors. In chamomile, even low shoot-accumulated metals such as Cu depleted this parameter while Cd had a lower impact (Kováčik et al., 2008). In this view, NH_4^+ excess is as toxic as excess of metals because all NH_4^+-containing variants have lower Fv/Fm value than respective −NH_4^+ counterparts (Table 1). Reducing sugars, mainly glucose in the assay we used, are basal substrates for respiration. Among +NH_4^+ treatments, Ni evoked elevation while a previous study with nitrate nutrition showed that Ni depleted them (Kováčik et al., 2011). In contrast to the mentioned study, elimination of N (ammonium) from the culture medium did not affect significantly this parameter and only Ni treatments differed (Table 1). This is another indication that Ni and Cd effects are different and further analyses of individual sugars are needed to highlight the impact of N nutrition on primary metabolites. However, ammonium nutrition followed by deficiency had a lower impact on C-based metabolites than nitrate deficiency (Kováčik et al., 2011) as discussed below in terms of phenolic metabolites.

Antioxidative enzymes represent essential protection against oxidative stress stimulated also by the excess of metals (Gajewska and Skłodowska, 2010). This was observed in chamomile exposed to Cd

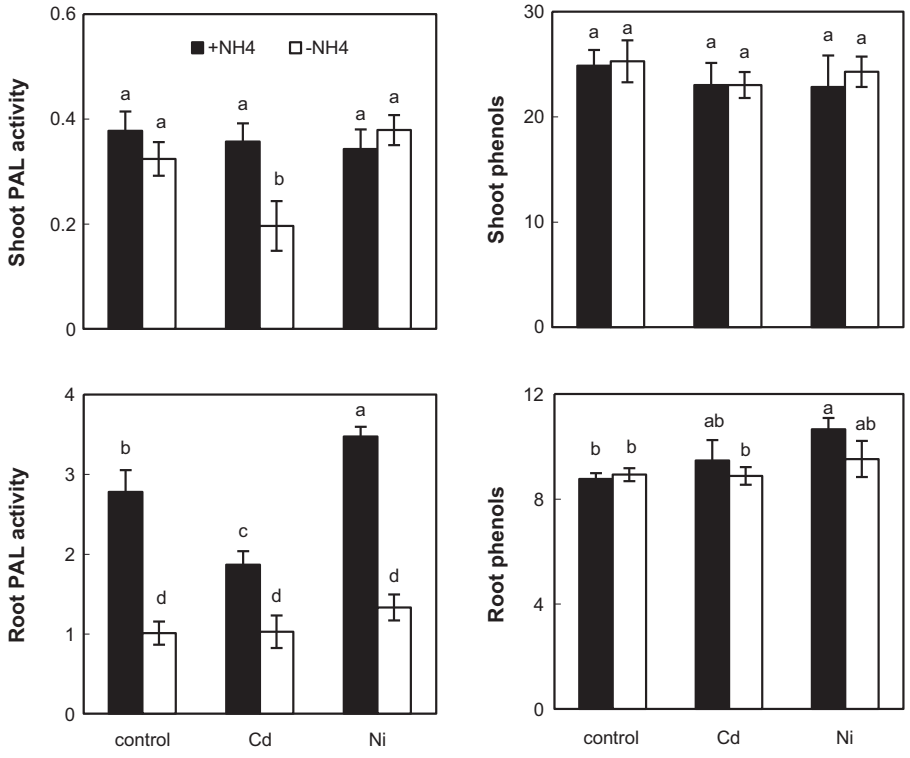

Fig. 2. Activity of phenylalanine ammonia-lyase (nmol min^{-1} mg^{-1} protein) and accumulation of total soluble phenols (mg g^{-1} DW) in *Matricaria chamomilla* plants cultured in cadmium (Cd) or nickel (Ni) enriched solution (60 μM) in the presence (+NH_4) or absence (−NH_4) of ammonium nitrogen over 7 days ($n = 4$). Other details are as in Fig. 1.

and Ni and elimination of nitrate almost universally elevated GPX activity (Kováčik et al., 2011). Present study showed that GPX and APX activities were rather depleted if C + NH_4^+ and C − NH_4^+ treatments are compared (Table 1), suggesting that the absence of stress factor (NH_4^+ ions) does not require higher antioxidative protection. In accordance, APX activity was elevated in ammonium-sensitive spinach while the role of GPX was not apparent (Domínguez-Valdivia et al., 2008). On the other hand, absence of ammonium in combination with metals evoked increase in GPX activity mainly (in comparison with metal + NH_4^+) that is an indication of involvement in response to metal excess being similar to mentioned study with nitrate deficiency (Kováčik et al., 2011). Despite this similarity, basic physiological parameters such as tissue water content typically increased after elimination of NH_4^+ (Table 1). It means that ammonium excess rather than metal presence had a negative impact on plant vitality.

3.2. Phenolic metabolism was not stimulated by ammonium absence

The PAL enzyme is a pivotal step in the biosynthesis of phenols that is regulated by nitrate deficiency (Kováčik et al., 2007). Surprisingly, the present data revealed an increase of neither PAL activity nor the sum of soluble phenols in ammonium-deficient solutions (Fig. 2). This could be evoked, the most probably, by the fact that PAL enzyme releases not only cinnamic acid but also ammonium that is subsequently recycled by GS/GOGAT enzymatic system (Kováčik et al., 2007 and the references therein). Because NH_4^+ content was still high enough or even elevated in metallic treatments without NH_4^+ ions in the medium, this could be a reason for the rather depleted PAL activity and negligible responses of total phenols (Fig. 2). Notwithstanding this, the sum of soluble phenols was still higher in NH_4^+-cultured than in nitrate-cultured chamomile (Kováčik and Klejdus, 2014), indicating direct stimulatory effect owing to toxicity of ammonium ions.

Among coumarin-related metabolites, responses of herniarin and its glycoside precursors (GMCAs) to metals and NH_4^+/deficiency were similar to nitrate-fed and nitrate-starved plants (Kováčik et al., 2011). We note that these metabolites are not included in "total soluble phenols" (owing to the absence of free hydroxyl group on aromatic ring). On the other hand umbelliferone is typical "stress" metabolite of chamomile (Repčák et al., 2009) and its higher content in all + NH_4^+ treatments confirm toxicity of ammonium excess (Table 2).

Overall, changes of phenolic metabolites in response to NH_4^+ and its deficiency indicate either sufficient pool of NH_4^+ (thus lowering need for the production of NH_4^+ by PAL enzyme) or their consumption by enzymes such as GPX mentioned above that increased preferentially under metal excess with the absence of NH_4^+. Just soluble GPX serves as a scavenger of H_2O_2 in plants (Kováčik et al., 2011 and the references therein). Despite these multiple relations, ammonium presence had a rather negative impact on phenolic metabolism that could be an additional reason for NH_4^+ toxicity to chamomile.

3.3. Proteins and thiols responded differentially to NH_4^+ removal

Nitrogenous metabolites such as proteins are depleted or tended to be depleted in N-deficient plants, depending mainly on the duration of deficient conditions (Giorgi et al., 2009; Kováčik et al., 2011; Rubio-Wilhelmi et al., 2012a; Rubio-Wilhelmi et al., 2012b). This fact

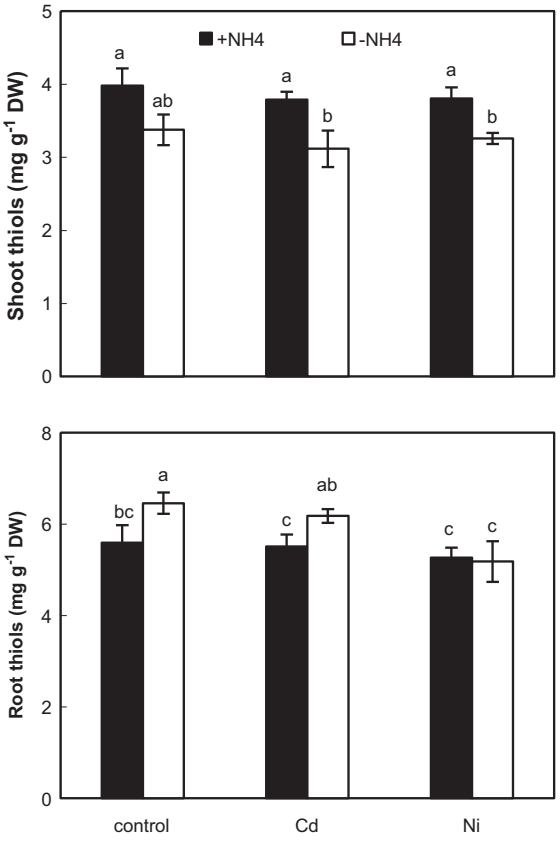

Fig. 3. Amount of non-protein thiols in *Matricaria chamomilla* plants cultured in cadmium (Cd) or nickel (Ni) enriched solution (60 μM) in the presence (+NH_4) or absence (−NH_4) of ammonium nitrogen over 7 days. Data are means ± SDs shown as bars ($n = 4$). Other details are as in Fig. 1.

was also observed in chamomile though absolute quantity of proteins differed if nitrogen was applied as nitrate or ammonium (Kováčik and Klejdus, 2014). Soluble proteins were altered more expressively in comparison with nitrate-fed plants (Kováčik et al., 2011) and even an increase (Cd) or no alteration (Ni) was observed in metallic treatments without NH_4^+ presence (Table 1). This could indicate restoration of proteosynthetic activity including antioxidative enzymes mentioned above. Further proteomic analyses are needed to highlight these changes. In agreement, Cd in combination with lower NH_4^+ concentrations elevated soluble proteins more than a higher NH_4^+ dose in *Arabidopsis* (Nasraoui et al., 2012) and soluble proteins were lower in spinach plants cultured with 3 mM NH_4^+ than in respective nitrate-fed plants (Domínguez-Valdivia et al., 2008). Besides, Cd-induced NH_4^+ accumulation was related to a decrease of some N-metabolizing enzymes such as glutamine synthetase leading to lower protein content in detached leaves (Chien and Kao, 2000).

Thiols are efficient antioxidants and metal-chelating compounds formed under an excess of various metals (Kováčik et al., 2014). Their sum was depleted in all treatments in the absence of ammonium while roots revealed stimulation (Fig. 3). However, it is visible that

Table 2
Accumulation of coumarin-related metabolites (μg g^{-1} DW for umbelliferone and mg g^{-1} DW for GMCAs and herniarin) in *Matricaria chamomilla* shoots cultured in cadmium (Cd) or nickel (Ni) enriched solution (60 μM) in the presence (+NH_4) or absence (−NH_4) of ammonium nitrogen over 7 days. Data are means ± SDs ($n = 3$). C − control, GMCAs − *Z*- and *E*-2-*β*-D-glucopyranosyloxy-4-methoxycinnamic acids. Other details are as in Table 1.

	C + NH_4	C − NH_4	Cd + NH_4	Cd − NH_4	Ni + NH_4	Ni − NH_4
GMCAs	8.67 ± 0.35 c	18.1 ± 1.41 a	8.63 ± 0.26 c	11.5 ± 0.60 b	8.30 ± 0.42 c	15.3 ± 1.32 a
Herniarin	1.20 ± 0.25 a	1.29 ± 0.22 a	0.92 ± 0.10 ab	0.83 ± 0.05 b	0.73 ± 0.05 b	0.86 ± 0.06 ab
Umbelliferone	70.5 ± 6.52 a	35.4 ± 5.14 b	41.6 ± 4.27 b	19.6 ± 2.01 c	68.1 ± 3.47 a	34.8 ± 4.03 b

Table 3
Accumulation of selected mineral nutrients in *Matricaria chamomilla* plants cultured in cadmium (Cd) or nickel (Ni) enriched solution (60 μM) in the presence (+NH_4) or absence (−NH_4) of ammonium nitrogen over 7 days. Data are means ± SDs ($n = 3$). C — control. Other details are as in Table 1.

	C + NH_4	C − NH_4	Cd + NH_4	Cd − NH_4	Ni + NH_4	Ni − NH_4
Shoot						
K (mg g^{-1} DW)	79.5 ± 3.17 b	75.1 ± 1.44 b	97.3 ± 2.28 a	80.8 ± 1.77 b	79.0 ± 4.42 b	77.2 ± 6.11 b
Na (mg g^{-1} DW)	5.54 ± 0.07 a	5.36 ± 0.21 a	5.29 ± 0.33 a	5.23 ± 0.16 a	5.41 ± 0.16 a	5.10 ± 0.12 a
Ca (mg g^{-1} DW)	7.54 ± 0.41 b	9.38 ± 0.13 a	6.16 ± 0.49 c	6.68 ± 0.32 bc	6.08 ± 0.53 c	6.95 ± 0.75 bc
Mg (mg g^{-1} DW)	4.95 ± 0.14 a	5.43 ± 0.20 a	5.04 ± 0.31 a	4.51 ± 0.25 a	4.97 ± 0.41 a	4.80 ± 0.30 a
Fe (mg g^{-1} DW)	0.17 ± 0.014 a	0.16 ± 0.015 a	0.14 ± 0.015 a	0.15 ± 0.009 a	0.14 ± 0.012 a	0.16 ± 0.022 a
Zn (μg g^{-1} DW)	49.2 ± 3.55 a	36.2 ± 1.51 b	41.2 ± 2.07 ab	44.3 ± 2.31 ab	40.6 ± 4.86 ab	44.5 ± 5.14 ab
Cu (μg g^{-1} DW)	13.8 ± 0.41 a	12.7 ± 2.77 ab	11.4 ± 0.80 ab	9.08 ± 1.16 b	10.2 ± 1.54 ab	9.43 ± 1.61 b
Root						
K (mg g^{-1} DW)	52.4 ± 3.72 c	65.2 ± 3.03 b	62.3 ± 4.54 b	75.5 ± 3.37 a	34.8 ± 2.50 d	57.1 ± 2.32 bc
Na (mg g^{-1} DW)	8.01 ± 0.70 a	6.42 ± 0.23 b	8.44 ± 0.49 a	6.21 ± 0.50 b	6.40 ± 0.34 b	6.23 ± 0.33 b
Ca (mg g^{-1} DW)	5.25 ± 0.16 b	5.43 ± 0.15 ab	5.97 ± 0.29 a	5.40 ± 0.25 ab	4.01 ± 0.28 c	5.17 ± 0.18 b
Mg (mg g^{-1} DW)	1.64 ± 0.10 a	1.72 ± 0.11 a	1.61 ± 0.09 a	1.62 ± 0.07 a	1.25 ± 0.07 b	1.58 ± 0.14 a
Fe (mg g^{-1} DW)	2.59 ± 0.17 e	4.76 ± 0.25 d	5.53 ± 0.26 c	7.78 ± 0.27 a	2.76 ± 0.18 e	6.54 ± 0.26 b
Zn (μg g^{-1} DW)	96.5 ± 6.95 b	134.0 ± 18.7 a	70.8 ± 4.16 c	67.7 ± 4.14 c	56.8 ± 7.08 c	58.9 ± 4.51 c
Cu (μg g^{-1} DW)	21.2 ± 2.01 ab	19.5 ± 2.30 b	23.7 ± 2.47 ab	23.8 ± 3.37 ab	18.5 ± 1.34 b	27.4 ± 3.36 a

above- and below-ground organs respond differentially to ammonium presence but impact of metals was not evident. Assay of individual compounds could elucidate this observation.

3.4. Mineral nutrients were more affected in roots

Previous study showed a slight increase in Cd accumulation but Ni uptake was not altered in ammonium-cultured plants while nitrate-fed ones revealed depletion (Kováčik et al., 2011). We therefore investigated other mineral nutrients. Shoots in Cd + NH_4^+ treatment contained more K^+ than control (C + NH_4^+) while Cd − NH_4^+ depleted this value (Table 3). This could be related to the above-mentioned alteration of Cd content and it was recently reported that increasing NH_4^+ dose elevated Cd uptake in *Arabidopsis* (Nasraoui et al., 2012). Responses of shoot and root tissues were rather different, for example in terms of K, Fe and Zn accumulation. Changes of Fe amounts were the most expressive in the roots owing to its increase after elimination of ammonium in all treatments (Table 3) which is clearly an ammonium-induced response because an earlier study showed depletion of Fe in nitrate-deficient plants (Kováčik et al., 2011). This could be, at least partially, a reason for enhanced GPX activity owing to Fe presence in its structure. Overall, changes of mineral nutrients involved response to ammonium rather than to metal presence and majority of minerals were present in quantities similar to those observed in nitrate-fed plants (Kováčik et al., 2011).

4. Conclusions

Physiological and metabolic responses of plants cultured in ammonium-nitrogen solutions with the addition of heavy metals revealed that NH_4^+ rather than metal excess was crucial negative factor. Elimination of ammonium improved plant vitality (increase in tissue water content, Fv/Fm ratio, amount soluble proteins) and suppressed typical stress responses (depletion of coumarin derivative umbelliferone). Additionally, enzymatic activities differed in relation to applied metal (Cd and Ni) while phenols or thiols were not extensively different. In this view, application of ammonium is not a suitable tool for eventual amelioration of metal toxicity in plants with sub-optimal nitrogen nutrition.

Disclosure statement

The authors declare that there are no conflicts of interest.

Role of the funding source

Sponsors had no involvement in the present study.

Acknowledgment

The work was supported by OP Education for Competitiveness (European Social Fund and the State Budget of the Czech Republic) CZ.1.07/2.3.00/30.0017 Postdocs in Biological Sciences at MENDELU.

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