DOI:10.1021/jf902645c

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Cadmium and Nickel Uptake Are Differentially Modulated by Salicylic Acid in *Matricaria chamomilla* Plants

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Chamomile (*Matricaria chamomilla*) is a widely used medicinal plant which also accumulates heavy metals in its above-ground organs. We investigated the effect of the important plant signaling molecule, salicylic acid (SA), on the accumulation of Ni or Cd, by exposing plants over 7 days to 60 μM solutions of individual heavy metals with or without 50 μM SA. Special emphasis was focused on phenolic metabolism-related parameters, not only because of their importance for growth and stress tolerance but also because phenolics are potent antioxidants in human diet. In combined treatments, SA stimulated an increase in soluble proteins of roots and reduced their water content. SA reduced total Cd in the shoot and increased Ni. Total and "intraroot" Ni decreased in Ni + SA treatment, while in the case of Cd, only "intraroot" content decreased in Cd + SA treatment, being correlated with cell wall-bound phenolic acids and lignin. SA was strongly accumulated in roots from the Ni + SA treatment, being correlated with an increase in hydrogen peroxide. In both Cd + SA and Ni + SA treatments, SA enhanced phenylalanine ammonia-lyase activity and accumulation of total soluble phenols, particularly in the roots. Here, we report for the first time that soluble phenols may be involved in Cd shoot-to-root translocation. In the case of Ni, it seems that phenols serve as a root barrier in order to prevent Ni from reaching the above-ground organs. The effects of SA on phenolic metabolism, and the signaling role of ROS in the accumulation of phenols, are discussed.

KEYWORDS: Cinnamyl alcohol dehydrogenase; heavy metals; mineral nutrients; polyphenol oxidase; shikimate dehydrogenase

INTRODUCTION

Elevated levels of heavy metals in the environment, including nickel (Ni) and cadmium (Cd), can be potentially dangerous because of their toxic effects and their accumulation in the food chain. In terms of plant metabolism, Ni is an essential "ultramicronutrient", found to be the active center of urease and the cofactor of one superoxide dismutase isoform (*1*). In contrast, Cd has no known physiological function in plants. Both these metals are divalent and are unable to catalyze the generation of reactive oxygen species (ROS) via Fenton−Haber−Weiss reactions (*2*). Notwithstanding this, they have different effects on plants since Ni shows lower toxicity in comparison with other metals (*3*).

Phenolic metabolites are widely distributed compounds involved in plant defense (*4*,*5*). They may scavenge ROS, directly or through enzymatic reactions (*6*), and may chelate metals in order to reduce the level of free metal ions (*7*,*8*). Stimulation of phenolic metabolism in response to excess Cd has also been found in chamomile (*Matricaria chamomilla* L.) (*9*, *10*) while Ni showed a less-pronounced effect (*11*) which was correlated with the activity of phenylalanine ammonia-lyase (PAL), a pivotal enzyme in the biosynthesis of phenols.

Salicylic acid (SA) is an important plant signaling molecule which can reduce symptoms of environmental stress (*12*). However, in a concentration-dependent manner, SA application can also result in oxidative damage (*13*). This indicates that SA-ameliorating effects are closely related to the applied concentration (*12*). Accordingly, in experiments with chamomile plants, 50 μM SA promoted growth (*14*) and prevented Na^+ accumulation in NaCl-exposed shoots (*15*), while 250 μM SA caused growth reduction and oxidative damage (*14*). SA can also affect the accumulation of heavy metals, as observed in pea plants; soaking of seeds in SA resulted in reduced accumulation of Cd in plants (*16*). In contrast, foliar SA application on Ni-cultured *Zea mays* has no effect on Ni content (*17*). An alleviating effect of SA through stimulation of antioxidative protection has also been described in *Oryza sativa,* but different levels of responses were

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 Published on Web 10/06/2009

observed if leaves and roots were compared (cf. refs *18* and *19*). Additionally, increase in shoot free SA has been found to be a strong predictor of Ni accumulation in hyperaccumulators and nonaccumulators in the genus *Thlaspi* (*20*).

Despite many investigations of the ameliorating effect of SA during different kinds of stress, especially at the level of antioxidative enzymes and ROS scavenging (*12*, *18*), there is no information available on the role of phenols in this process. Furthermore, explanations for the SA-evoked reduction of heavy metal content are far from being fully highlighted (*21*), providing the main stimulus for the present research. Chamomile is a suitable model plant for such studies because it accumulates considerable amounts of Cd and Ni in the above-ground organs (*10*, *22*), and at the same time, its phenolic metabolism is SA-inducible, as described previously in a time−dynamics study (*14*). Moreover, its tolerance to cultivation conditions, and increasing contamination of soils with metals, reinforces present research since even plants cultivated in noncontaminated soil may contain higher amounts of Cd than other species (*23*). We therefore tested, using chamomile cultured 6 weeks in hydroponics, the effect of 50 μM SA on the accumulation of Cd or Ni, which were applied at 60 μM. Based on the results of earlier work, these concentrations were selected to avoid damaging of the plants and consequently causing misleading results. We focused on phenolic metabolism-related parameters, not only because of their importance in growth, stress tolerance and potential heavy metal binding but also because they are potent antioxidants in human diet.

MATERIALS AND METHODS

Cultivation of Plants and Experimental Design. Twenty-one day old seedlings of *Matricaria chamomilla* L. (tetraploid 'Lutea', Asteraceae) germinated in sand (with 3−4 true leaves) were transplanted to the slightly modified Hoagland solution routinely used in our laboratory (*9*−*11*, *14*, *22*, *24*). Uniform plants were cultivated in brown plastic 5 L boxes (25 plants per box) with continual aeration of the solutions. The experiment was performed in a growth chamber under controlled conditions: 12 h day (6:00 am to 6:00 pm); photon flux density was 210 μmol m^{-2} s^{-1} PAR at leaf level supplied by cool white fluorescent tubes TLD 36W/33 (Philips, France); 25/20 °C day/night temperature; and relative humidity ~60%. In these conditions, plants form basal leaf rosettes only. Solutions were renewed weekly to prevent nutrient depletion. Plants which had been cultivated hydroponically for 6 weeks were used in the experiment and further cultured for 7 days in 60 μM Cd- or Ni-enriched solutions (added in the form of $NiCl_2 \cdot 6H_2O$ and $CdCl_2 \cdot 2^1/_2H_2O$, Lachema Brno, Czech Republic) alone or in combination with 50 μM salicylic acid (SA). Controls did not contain any additional chemicals, and pH was checked to be 6.0 ± 0.1 in all variants. Fresh and dry masses (dried at 80 °C to constant weight) were estimated in order to determine the plant water content [100 − (dry mass × 100/fresh mass)] allowing recalculation of parameters measured in fresh samples. These dried samples were further analyzed for phenolic acids and mineral nutrients including Ni and Cd. Plants for fresh mass-requiring parameters were powdered using liquid N_2 and extracted as described below. Proteins were quantified according to Bradford (*25*) using 20 μL of supernatants and bovine serum albumin as standard. For all enzymes, randomly selected control supernatants were boiled to destroy enzyme activity and to check that the observed reaction was enzymatic. Spectrophotometry was carried out with a Uvi Light XTD 2 (Secomam, ALES Cedex, France).

Quantification of Ni, Cd and Selected Mineral Nutrients. Samples for quantification of metals were prepared as described previously (*10*, *22*): dry material was kept overnight in HNO_3 and H_2O_2 mixture (10 mL + 10 mL, Suprapur, Merck) at laboratory temperature and the next day evaporated to dryness at 90 °C in a water bath (5−6 h). Dry residue was dissolved in 5% HNO_3 and diluted to a final volume of 10 mL. All measurements were carried out using an atomic absorption spectrometer AA30 (Varian Ltd., Mulgrave, Australia) and an air−acetylene flame. Samples for quantification of "intraroot" Ni and Cd were washed in 10 mM $CaCl_2$ (one root system in 300 mL) at 4 °C for 30 min in order to remove metals adsorbed to the root surface (*10*), and all other mineral nutrients were quantified in these samples. For quantification of total root Ni/Cd, samples were washed with deionized water only. Methanol-soluble and water-soluble shoot Cd and Ni contents were measured after extraction in 80% methanol and 1 mM TrisHCl buffer, respectively (*10*).

Determination of Phenolic Acids, Total Soluble Phenols, Lignin and Phenolic Metabolism-Related Enzymes. Free, glycoside-bound and cell wall-bound phenolic acids were quantified using the UPLC−MS/MS system as described previously (*26*). Briefly, glycoside-bound phenolic acids were released after acid hydrolysis of extracts with 2 M HCl and cell wall-bound phenolic acids were released after alkaline hydrolysis of methanol-insoluble root residues with 0.5 M NaOH (*14*, *27*). Total soluble phenols were extracted with 80% methanol from fresh tissue and measured using the Folin−Ciocalteu method with gallic acid as standard (*9*, *14*). Root lignin content was estimated by the thioglycolic acid reaction (*9*).

Activity of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) was determined by the production of *trans*-cinnamic acid from phenylalanine using the HPLC method with homogenates prepared using sodium borate buffer, pH 8.7 (*14*, *24*).

To determine the activities of shikimate dehydrogenase (SKDH, EC 1.1.1.25), cinnamyl alcohol dehydrogenase (CAD, EC 1.1.1.195) and polyphenol oxidase (PPO, EC 1.10.3.2) samples were homogenized in 50 mM potassium phosphate buffer (pH 7.0) containing 5 mM polyvinyl polypyrrolidone at 4 °C. Measurements and calculations were done as described earlier (*10*).

Amounts of Reactive Oxygen Species and Malondialdehyde. Hydrogen peroxide and superoxide were measured in 50 mM potassium phosphate buffer homogenates using the $TiCl_4$ and hydroxylamine methods, respectively (*24*). MDA was quantified by the thiobarbituric acid reaction (*14*).

Statistical Analyses. One-way ANOVA followed by a Tukey's test (MINITAB Release 11, Minitab Inc., State College, PA) was used to evaluate the significance of differences in the parameters ($P < 0.05$). Number of replications (n) in tables/figures denotes individual plants measured for each parameter. One box containing 25 plants was used for each variant, thus the whole experiment included 6 boxes. Two independent repetitions of the whole experiment were performed.

RESULTS

Effect of Ni, Cd and SA on Basic Growth Parameters. All the treatments have no effect on biomass accumulation after 7 days of exposure (data not shown). No visible symptoms of stress damage (i.e., chlorosis) were visible at the leaf level. In Cd and Cd + SA variants, the roots developed a brown color. Cd reduced shoot water content in both variants (Cd and Cd + SA), while in the roots, only metal + SA had a similar effect (**Table 1**). Shoot soluble proteins were not affected, and in the roots, metal + SA caused a significant increase (**Table 1**).

Distribution of Cd and Ni within Chamomile Plants. Total shoot Cd content was ca. 3 times higher in comparison with Ni (**Figure 1**). SA application reduced Cd accumulation by 41%, while in SA + Ni, an increase in Ni content by 27% was observed (**Figure 1**). Amounts of metals in methanol- and water-soluble fractions were not significantly different between SA + metal and metal alone (**Figure 1**). The Cd/Ni levels in the shoots of the control group were 0.29/3.45 μg g^{-1} DW Cd/Ni, respectively.

Total root Cd content was ca. 4 times higher than that of Ni (**Figure 1**). SA application reduced both total (−24%) and "intraroot" (−26%) content of Ni, while in the case of Cd, only "intraroot" fraction decreased in Cd + SA (−25%; **Figure 1**). Control plants contained 0.12/8.29 μg g^{-1} DW Cd/Ni in the roots.

Quantitative Changes of Salicylic Acid. In the shoots (leaf rosettes), endogenous SA content decreased in Cd + SA but increased in Ni + SA, in both the free and glycoside-bound fractions (released by acid hydrolysis) compared with the metal alone (**Table 2**). In the roots, both free and glycoside-bound

Table 1. Selected Physiological and Biochemical Parameters in *Matricaria chamomilla* Plants after 7 Days of Exposure to Different Treatments[a]

	tissue water content (%), $n = 15$	soluble proteins (mg g^{-1} DW), $n = 4$	shikimate dehydrogenase (nmol min^{-1} mg^{-1} protein, $n = 4$)	cinnamyl alcohol dehydrogenase (nmol min^{-1} mg^{-1} protein, $n = 4$)	polyphenol oxidase (UA mg^{-1} protein, $n = 4$)	lignin content (mg g^{-1} DW), $n = 4$
			Leaf Rosettes			
control	92.22 ± 0.37 a	112.5 ± 7.41 a	164.3 ± 6.13 ab	41.62 ± 4.28 a	1.43 ± 0.094 a	na[b]
SA	91.76 ± 0.40 ab	111.1 ± 13.7 a	172.0 ± 12.6 ab	38.46 ± 3.88 a	0.92 ± 0.171 b	na
Ni	92.26 ± 0.41 a	116.0 ± 26.3 a	148.2 ± 8.23 b	37.64 ± 4.56 a	1.19 ± 0.116 ab	na
Ni + SA	91.83 ± 0.38 ab	119.9 ± 8.89 a	187.2 ± 11.7 a	30.49 ± 5.92 a	1.28 ± 0.149 ab	na
Cd	91.42 ± 0.26 b	115.4 ± 10.6 a	169.4 ± 10.3 ab	38.75 ± 4.24 a	1.22 ± 0.133 ab	na
Cd + SA	91.36 ± 0.17 b	108.2 ± 8.52 a	163.9 ± 6.95 ab	33.65 ± 4.26 a	0.93 ± 0.221 b	na
			Roots			
control	95.13 ± 0.25 a	43.4 ± 4.72 cd	95.50 ± 9.80 b	88.70 ± 6.39 b	0.78 ± 0.032 b	23.17 ± 2.16 b
SA	94.96 ± 0.23 a	52.0 ± 4.41 bc	98.27 ± 10.3 b	90.76 ± 4.95 b	0.64 ± 0.085 bc	22.79 ± 1.26 b
Ni	95.02 ± 0.32 a	32.3 ± 2.72 d	95.99 ± 13.1 b	96.11 ± 13.3 b	0.56 ± 0.028 bc	22.07 ± 2.13 b
Ni + SA	94.02 ± 0.24 b	60.7 ± 7.88 b	78.27 ± 8.51 b	160.9 ± 7.42 a	0.44 ± 0.137 c	22.59 ± 2.49 b
Cd	94.82 ± 0.18 a	40.5 ± 2.05 cd	134.3 ± 9.37 a	136.3 ± 14.8 a	1.19 ± 0.199 a	28.94 ± 1.10 a
Cd + SA	94.11 ± 0.31 b	76.7 ± 5.09 a	78.76 ± 6.04 b	139.0 ± 12.9 a	0.57 ± 0.077 bc	29.06 ± 1.83 a

[a] Data are means ± SDs. Values within vertical column followed by the same letter(s) are not significantly different according to Tukey's test ($P < 0.05$); note that leaf rosettes and roots were evaluated separately. [b] Not analyzed.

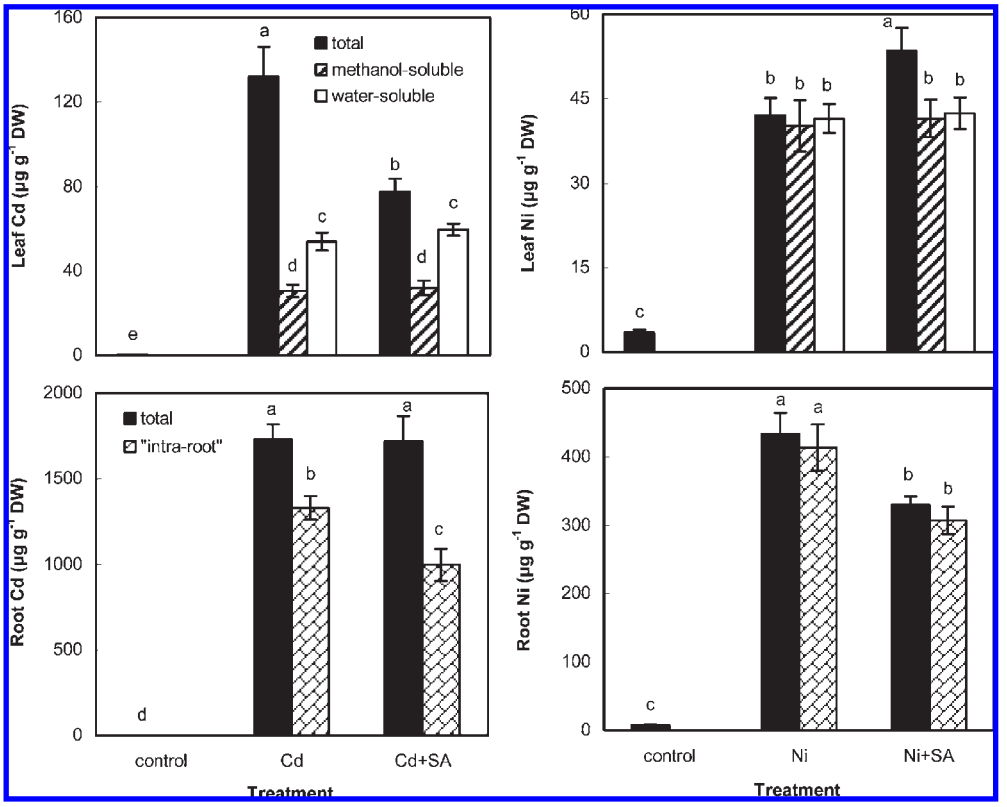

Figure 1. Accumulation of cadmium (Cd) and nickel (Ni) in the leaf rosettes and roots of *Matricaria chamomilla* plants after 7 days of exposure to different treatments. Data are means ± SDs ($n = 4$). Methanol-soluble and water-soluble Cd and Ni contents were estimated after extraction in 80% methanol and 1 mM TrisHCl buffer, respectively. "Intraroot" content was measured after washing with $CaCl_2$. Values within each graph followed by the same letter are not significantly different according to Tukey's test ($P < 0.05$).

endogenous SA was extremely elevated in the Ni + SA treatment but only slightly increased in Cd + SA, in comparison with treatments receiving the metal alone. Cell wall-bound SA (released by alkaline hydrolysis) sharply increased in Cd + SA treatment (**Table 2**).

Oxidative Status of Tissue. Neither hydrogen peroxide nor superoxide content was elevated by any treatments in the leaf rosettes (**Figure 2**). In the roots, $O_2^{\bullet -}$ accumulation was elevated in all treatments (in comparison with the control) and the highest content was recorded in the Ni + SA treatment followed by Cd + SA treatment. Accumulation of H_2O_2 followed a similar trend and was only elevated in the Ni + SA and Cd + SA treatments (**Figure 2**). Malondialdehyde accumulation was not significantly affected by any of treatments (data not shown).

Activity of PAL and Content of Total Soluble Phenols and Lignin. After 7 days of exposure, leaf PAL activity was highest in Ni + SA and Cd + SA treatments and total soluble phenols in the Cd treatment only (**Figure 3**). In the roots, PAL activity was

Table 2. Accumulation of Salicylic Acid (μg g^{-1} DW) in Different Fractions from the Leaf Rosettes and Roots of *Matricaria chamomilla* Plants after 7 Days of Exposure to Different Treatments (n = 3)[a]

	leaf rosettes	roots
	Free SA	
control	7.07 ± 0.65 b	1.13 ± 0.31 d
SA	7.23 ± 0.30 b	3.73 ± 0.45 c
Ni	4.00 ± 0.26 c	1.23 ± 0.30 d
Ni + SA	8.43 ± 0.42 b	72.4 ± 4.51 a
Cd	10.7 ± 1.45 a	3.93 ± 0.45 c
Cd + SA	6.73 ± 0.45 b	7.60 ± 0.46 b
	Acid Hydrolysis	
control	12.2 ± 1.35 b	1.17 ± 0.31 d
SA	13.6 ± 1.85 b	5.03 ± 0.42 c
Ni	6.56 ± 0.47 c	1.30 ± 0.26 d
Ni + SA	14.8 ± 1.60 b	133.8 ± 7.57 a
Cd	25.4 ± 2.11 a	13.7 ± 2.30 b
Cd + SA	12.3 ± 1.61 b	8.27 ± 0.85 b
	Alkaline Hydrolysis	
control	na[b]	1.16 ± 0.20 d
SA	na	8.40 ± 0.75 c
Ni	na	1.21 ± 0.23 d
Ni + SA	na	19.4 ± 1.76 b
Cd	na	1.09 ± 0.21 d
Cd + SA	na	263.1 ± 19.0 a

[a] Other details as in **Table 1**. [b] Not analyzed.

lower in metal + SA than in metal variant alone, being the highest in Cd treatment (**Figure 3**). On the other hand, total soluble phenol content was greater in Ni + SA and Cd + SA treatments than in metal alone, being accumulated mainly in Ni + SA (**Figure 3**).

Root lignin content was elevated only by Cd, and SA has no effect on this Cd-induced lignin deposition (**Table 1**).

Activities of Other Phenolic Metabolism-Related Enzymes. In all treatments, SKDH, CAD and PPO activities in chamomile leaf rosettes after 7 days were only slightly affected (**Table 1**). In the roots, activities of SKDH, CAD and PPO were the most enhanced in the Cd treatment and SA has no additional impact on this enhancement. In the case of Ni, only CAD activity was higher in Ni + SA treatment than in Ni treatment alone (**Table 1**).

Quantitative Changes of Phenolic Acids. Except for SA, five benzoic acid derivatives (gentisic, *p*-hydroxybenzoic, protocatechuic, vanillic and syringic acids) and five cinnamic acid derivatives (chlorogenic, caffeic, *p*-coumaric, ferulic and sinapic acids) were determined as free phenolic acids (data not shown) and glycoside-bound phenolic acids (**Table 3**). Most of the glycoside-bound phenolic acids were quantified in substantially higher levels than free phenolic acids. In the leaf rosettes, phenolic glycoside levels were mainly enhanced by Cd and to a lower extent by Cd + SA (**Table 3**). In the roots, Ni + SA showed the most pronounced effect in acid hydrolysates while Cd + SA showed the most pronounced effect in alkaline hydrolysates (**Table 3**).

Within individual compounds, gentisic acid level showed the most dramatic increase mainly in Ni + SA treatment (**Table 3**). Accumulation of chlorogenic and caffeic acids, important antioxidant compounds, was mainly affected by Cd in the leaf rosettes and by Ni + SA, particularly in the roots (**Table 3**). Protocatechuic acid, a phenol with high metal-chelating strength, was mostly unaffected by treatments, or it even slightly decreased in both leaf rosettes and roots as compared to control (**Table 3**).

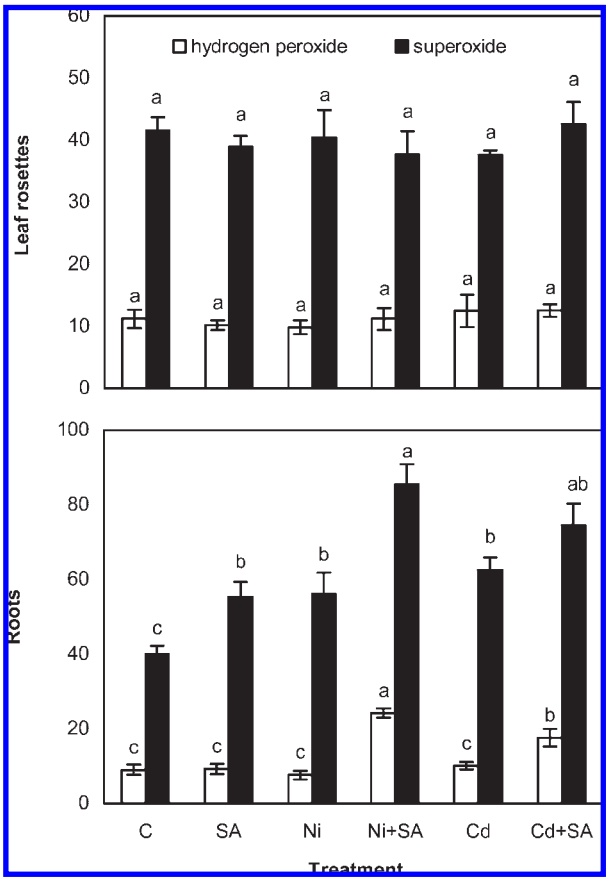

Figure 2. Accumulation of hydrogen peroxide (μmol g^{-1} DW) and superoxide radical (μg g^{-1} DW) in the leaf rosettes and roots of *Matricaria chamomilla* plants after 7 days of exposure to different treatments (n = 4). Values for either H_2O_2 or superoxide followed by the same letter(s) are not significantly different according to Tukey's test ($P < 0.05$).

Accumulation of Mineral Nutrients Related to Different Treatments. Potassium was the most affected mineral nutrient and almost the only nutrient showing a decrease in comparison with control; its decrease was more pronounced in the roots and in Ni + SA, Cd and Cd + SA treatments (**Table 4**). On the other hand, Mg was affected neither in the rosettes nor in the roots; Zn increased only in SA-treated roots (**Table 4**). Shoot Na content increased in Ni and Cd treatments but in the roots in the Cd + SA treatment only. Fe accumulation was stimulated mainly by Ni + SA in both the leaf rosettes and roots (**Table 4**). In leaves, the amount of Cu decreased slightly in the Cd treatment only, while in roots, it increased in all treatments as compared to control (**Table 4**).

DISCUSSION

Increasing industrial production and accumulation of metals in crop plants, including medicinal plants cultured for pharmaceutical purposes, is an important negative factor because of potential risk to human health (*28*, *29*). An understanding of basic metabolic processes in plants and development of potential tools for their modification are essential for production of safer foods. The use of hydroponics allows experimental comparison of the effects of treatments on roots and shoots because roots are more readily available for measurements.

In the present study, the amount of soluble proteins substantially and preferentially increased in roots treated with Cd + SA (**Table 1**). This may imply enhanced synthesis of protective

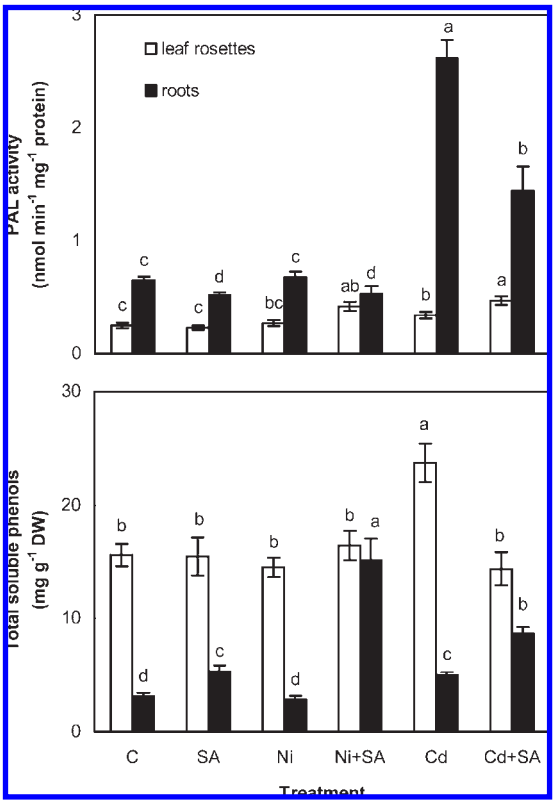

Figure 3. Phenylalanine ammonia-lyase (PAL) activity and content of total soluble phenols in the leaf rosettes and roots of *Matricaria chamomilla* plants after 7 days of exposure to different treatments ($n = 3$). Values for either leaf rosettes or roots followed by the same letter(s) are not significantly different according to Tukey's test ($P < 0.05$).

proteins such as those contributing to amelioration of oxidative damage recorded in Cd-exposed rice roots (*18*).

The decrease in total shoot Cd content and increase in total shoot Ni content were clearly correlated with changes in shoot SA (**Figure 1** and **Table 2**). This is in accordance with findings in six *Thlaspi* hyperaccumulators and nonaccumulators exposed to Ni (*20*). In the case of Cd, and consistent with our observations, pea seedlings grown from seeds presoaked with SA contained lower amounts of bound SA and shoot Cd (*16*). On the other hand, increasing exogenous Cd doses caused increase in both free and bound SA level in maize leaves (*30*), confirming both negative and positive correlation between these two parameters in different plant objects. So far, only a limited number of explanations for these observations have been proposed in terms of metal root-to-shoot translocation. For example, SA was found to induce aluminum tolerance in the roots of *Cassia tora* by modulation of citrate efflux (*21*). Decrease in total shoot Cd was correlated with depletion of total soluble phenols (if Cd and Cd + SA are compared, **Figures 1** and **3**), providing an indirect evidence that phenols may contribute to shoot-to-root retranslocation of Cd. Accordingly, large amounts of precipitated Cd in the phloem of *Arabidopsis thaliana* suggest retranslocation from the shoot (*31*). Ni in the present study evoked different responses if leaf rosettes and roots are compared, since it increased in shoots but decreased in roots (**Figure 1**). Based on enhancement of phenols in Ni + SA treatment, we assume that phenols serve as a barrier preventing shoot Ni accumulation. To support this "barrier" role of phenols, shoot Ni content increased ca. 2 times after 7 days in Ni-exposed chamomile plants cultured with an inhibitor of PAL (Kováčik and co-workers, unpublished results). In the case of copper excess, phenols have been implicated in Cu tolerance through complexation in the rhizosphere (*32*), providing evidence that phenolic compounds represent important tools for protection against heavy metal uptake. With respect to the shoot Ni increase but unchanged total soluble phenols, it may be suggested that a different mechanism was involved in the observed Ni increase. Such a mechanism may involve accumulation of some organic and amino acids, such as histidine, which are known to contribute to shoot Ni accumulation (ref *33* and the references therein). Regulation of translocation of ions to the shoots in hyperaccumulating plants probably occurs at the xylem-loading stage (ref *34* and the references therein), therefore higher exogenous metal supply might cause damage to ion-specific transporters, leading to unspecific increase in Ni in the shoot. The active role of phenols in the reduction of at least Cd uptake by chamomile shown here may also be confirmed by significantly nondifferent shoot tissue water content if Cd and Cd + SA treatments are compared (**Table 1**) since Cd root-to-shoot transport is most likely to be driven by the transpiration stream (*35*).

From the above, it can be indirectly assumed that soluble organic compounds including phenols (and regulation of their biosynthesis) serve as important regulators of heavy metal distribution. The present study emphasized this by measuring several important phenolic metabolism-related enzyme activities. Enhanced SKDH activity (a member of prechorismate pathway providing aromatic amino acids including phenylalanine) in Ni + SA variant may provide substrate for PAL as judged by a strong increase in soluble phenols (**Figure 3**); and the low PAL activity in this variant may be attributed to SA since prolonged exposure to even nontoxic SA concentrations decreases PAL activity (*14*). PAL activity has also been affected by heavy metals in a time−dynamics study, and its elevated activity after 7 days of exposure only in Cd treatments recorded in the present study fits well with our previous observations (*9*). This is another indication that Ni and Cd, although they are both divalent cations, cause different responses when translocated within the plant. This fact is also visible in terms of lignin deposition, which may restrict apoplastic passage of metal ions and radial transport in order to prevent metals from reaching the above-ground biomass (*36*). Copper, especially, is an effective inducer of lignin deposition, which correlates with low Cu content in chamomile leaf rosettes (*9*, *10*). In the present experiment, Cd has a more pronounced effect on lignin content than Ni while SA has no effect. The same was found for CAD activity (**Table 1**). It is interesting that CAD activity, which is considered as a specific marker of lignification (*37*), was also enhanced in roots exposed to Ni + SA although lignin content was not affected. This may indicate that the process of lignification was initiated already but a prolonged exposure was needed for significant lignin accumulation. At the level of specific phenolic acids, protocatechuic acid, a 3,4-dihydroxybenzoic acid with a high chelating strength (*7*), was mostly unaffected by treatments, or even slightly decreased. Instead, gentisic acid (GA), a positional isomer of protocatechuic acid, was accumulated to a high extent. It was shown that SA is converted to GA in chamomile and in tomato plants exposed to exogenous SA (*14*, *38*). An increase in GA after SA treatment was also found in the present study; however, the conversion was strongly affected by the presence of either Cd or Ni (**Table 3**). In addition, Cd treatment alone increases GA levels in roots ca. 10 times compared to control plants, while Ni treatment had no effect. Such differential regulation of GA levels suggests a specific role of GA in the response of chamomile to some heavy metals but not others. This was partially supported by experiments with infected tomato, where GA was identified as a signaling molecule which activated expression of pathogenesis-related proteins (*38*). An increase in phenolic acids bound to the cell wall, as revealed by

Table 3. Accumulation of Selected Benzoic (Gentisic−Syringic) and Cinnamic Acid (Chlorogenic−Sinapic) Derivatives (μg g^{-1} DW) in Acid Hydrolysates of 80% Methanol Extracts from the Leaf Rosettes and Roots and after Alkaline Hydrolysis of Methanol-Insoluble Root Residue in *Matricaria chamomilla* Plants after 7 Days of Exposure to Different Treatments[a]

	gentisic acid	*p*-OH-benzoic acid	protocate-chuic acid	vanillic acid	syringic acid	chlorogenic acid	caffeic acid	*p*-coumaric acid	ferulic acid	sinapic acid
Leaf Rosettes										
Acid Hydrolysis										
control	230.7 b	8.3 a	41.2 a	146.0 ab	6.2 d	12.0 bc	6.7 bc	3.7 ab	1.4 bc	6.3 ab
SA	165.2 c	4.2 c	27.1 c	173.4 a	14.1 b	6.7 d	5.6 d	4.0 a	1.6 bc	6.4 ab
Ni	82.5 d	6.4 b	37.3 ab	79.5 c	12.4 bc	8.9 cd	4.2 e	2.7 c	1.1 c	4.8 b
Ni + SA	241.6 b	7.7 ab	37.0 ab	137.5 ab	7.8 dc	12.3 bc	6.4 cd	3.3 b	1.5 bc	5.2 b
Cd	357.1 a	8.6 a	33.6 bc	116.7 bc	25.8 a	18.5 a	7.5 a	3.5 ab	2.7 a	7.1 a
Cd + SA	221.8 b	6.7 b	37.5 ab	118.2 b	27.3 a	14.1 ab	6.9 ab	3.2 b	1.9 ab	7.6 a
Roots										
Acid Hydrolysis										
control	8.6 e	5.8 e	7.1 a	15.3 a	11.7 bc	1.7 d	2.6 e	0.4 bc	3.6 d	13.4 d
SA	198.3 c	6.2 de	5.3 c	14.4 a	12.6 bc	2.9 bc	5.8 c	0.5 b	6.4 c	19.5 c
Ni	6.9 e	5.8 e	6.4 ab	15.9 a	10.3 c	2.7 c	3.9 d	0.3 c	3.7 d	20.4 bc
Ni + SA	6887.2 a	13.9 a	5.7 bc	13.3 a	26.7 a	6.6 a	19.2 a	2.1 a	18.4 a	25.3 ab
Cd	82.3 d	7.8 cd	4.9 c	16.4 a	15.9 b	3.4 b	3.5 d	0.3 c	10.2 b	19.3 c
Cd + SA	957.1 b	10.2 bc	5.8 bc	16.2 a	13.5 bc	5.0 a	8.9 b	0.6 b	13.8 b	30.2 a
Alkaline Hydrolysis										
control	nd[b]	4.5 c	4.5 c	4.7 b	3.0 c	nd	185.5 b	1.5 bc	34.2 c	2.0 b
SA	nd	8.6 b	5.7 b	9.0 b	5.4 b	nd	173.2 bc	1.4 c	60.4 b	nd
Ni	nd	5.1 c	4.6 c	6.3 b	3.5 c	nd	138.6 cd	1.4 c	36.0 c	nd
Ni + SA	5.2 b	8.3 b	3.4 d	7.6 b	4.8 b	nd	240.7 a	1.9 b	47.8 bc	4.5 a
Cd	nd	5.6 c	4.0 cd	7.1 b	nd	nd	114.5 d	1.8 b	57.2 b	nd
Cd + SA	36.5 a	41.3 a	9.2 a	15.3 a	9.7 a	nd	nd	5.2 a	140.8 a	4.9 a

[a] Data are means from three individual plants. For the lucidity of table, SDs are not shown. Other details as in **Table 1**. [b] Not detected.

Table 4. Content of Selected Mineral Nutrients in *Matricaria chamomilla* Plants after 7 Days of Exposure to Different Treatments[a]

	K (mg g^{-1} DW)	Na (mg g^{-1} DW)	Mg (mg g^{-1} DW)	Fe (mg g^{-1} DW)	Zn (μg g^{-1} DW)	Cu (μg g^{-1} DW)
Leaf Rosettes						
control	111.3 ± 7.1 a	4.36 ± 0.18 b	6.05 ± 0.57 a	0.138 ± 0.009 b	49.8 ± 6.65 a	16.9 ± 1.51 ab
SA	105.1 ± 1.7 ab	4.47 ± 0.09 b	5.92 ± 0.28 a	0.143 ± 0.006 b	50.0 ± 5.33 a	18.1 ± 0.76 a
Ni	103.3 ± 4.6 abc	5.23 ± 0.10 a	6.13 ± 0.29 a	0.149 ± 0.011 b	48.2 ± 0.92 a	16.3 ± 1.10 abc
Ni + SA	91.8 ± 4.3 cd	4.39 ± 0.07 b	6.14 ± 0.21 a	0.218 ± 0.021 a	60.2 ± 3.67 a	17.2 ± 1.21 ab
Cd	93.5 ± 2.7 bcd	5.26 ± 0.14 a	6.01 ± 0.34 a	0.133 ± 0.008 b	56.3 ± 2.91 a	13.1 ± 1.70 c
Cd + SA	90.5 ± 3.4 d	4.24 ± 0.15 b	5.73 ± 0.37 a	0.137 ± 0.014 b	58.6 ± 5.90 a	13.7 ± 0.81 bc
Roots						
control	106.0 ± 5.2 a	5.93 ± 0.54 b	4.07 ± 0.18 a	7.89 ± 0.67 b	93.6 ± 11.2 b	34.7 ± 1.72 c
SA	102.9 ± 2.4 a	5.98 ± 0.35 b	4.23 ± 0.08 a	8.18 ± 0.54 b	144.1 ± 15.7 a	63.5 ± 6.01 ab
Ni	98.5 ± 3.8 a	5.81 ± 0.64 b	4.07 ± 0.35 a	8.64 ± 1.12 b	106.2 ± 17.6 b	52.7 ± 4.46 b
Ni + SA	85.3 ± 4.6 b	6.04 ± 0.40 b	4.22 ± 0.58 a	11.7 ± 0.96 a	83.5 ± 8.9 b	73.1 ± 5.84 a
Cd	78.3 ± 2.4 b	5.79 ± 0.84 b	3.50 ± 0.31 a	8.24 ± 1.02 b	81.3 ± 3.1 b	61.3 ± 6.42 ab
Cd + SA	76.8 ± 3.6 b	7.84 ± 0.62 a	4.47 ± 0.52 a	11.6 ± 1.30 a	86.5 ± 9.5 b	58.2 ± 3.05 b

[a] Other details as in **Table 1**.

alkaline hydrolysis of methanol-insoluble root residue, was observed in Cd + SA treatment (**Table 3**). This may provide, at least partially, an explanation for total root Cd content remaining unaffected when Cd and Cd + SA are compared (**Figure 1**). The esterification of phenolic acids to the cell wall has been suggested to lead to the formation of lignin-like polymers by supplying lignin attachment sites to the matrix polysaccharides (*39*). Thus esterification and lignification may be regarded as contiguous rather than separate processes which gradually integrate (*9*), and low esterification in Ni and/or Ni + SA roots is in accordance with a decrease in both total and "intraroot" Ni since lignin content has not been affected.

In order to highlight the signal-mediated regulation of changes recorded in the present study, ROS (i.e., hydrogen peroxide and superoxide radical) were measured. Hydrogen peroxide, especially, is considered as an important signal molecule (see (*40*) for review). We previously confirmed that exogenous H_2O_2 stimulated PAL activity in chamomile roots (*24*). Besides, superoxide

was also found to be essential for PAL induction (*41*). SA application is known to stimulate an increase in H_2O_2 (mainly through depression of catalase activity) which then activates protective responses (*12*). The fact that a strong increase in root SA correlated with an increase in root H_2O_2 (and also superoxide) only in the Ni + SA treatment agrees well with this assumption and provides an explanation for the increase in root phenols through ROS-mediated PAL stimulation. The latter must occur after short-term exposure (since after 7 days when PAL was assessed in the present study, it was not enhanced). Accordingly, H_2O_2 was elevated while catalase/peroxidase activities were unchanged or depleted in (Cd + SA)-exposed rice roots after 6 days in comparison with control, while the opposite was true when Cd and Cd + SA treatments were compared (*18*).

An excess of heavy metals usually leads to the loss of important nutrients, as was also observed in chamomile plants (*10*). It was surprising to find that SA did not prevent potassium loss in the present investigation while it did in chamomile exposed to high salinity (*15*). Unchanged Mg content and a small increase in shoot Fe agree with an absence of visible chlorosis in the present study. Zn and especially Cu, being cofactors of the main superoxide dismutase isoform, were even stimulated in some treatments, suggesting that this may contribute to increase in hydrogen peroxide content just in (Ni + SA)-treated roots.

In conclusion, different relations of phenols to Cd and Ni accumulation/translocation are obvious. First, a considerable part of the shoot Cd decreased in Cd + SA treatment, and this was correlated with a decrease in shoot soluble phenols and selected phenolic acids, indicating that phenols are potential ligands for Cd in chamomile. They are probably phloem shoot-to-root retranslocated. Second, increased shoot Ni content was correlated with an increase in SA content, but total phenols and/or phenolic acids were not considerably affected. With respect to the strong enhancement of total soluble phenols and phenolic acids in the roots only in plants treated with Ni + SA, we assume that soluble phenols serve as a barrier preventing shoot Ni accumulation. Owing to this enhancement, at least a part of the Ni ions may "escape" from the roots thus contributing to shoot Ni increase. The observed decrease in root Ni supports these conclusions and indicates that above- and below-ground biomass respond differentially to Ni, which can be controlled at the xylem/phloem loading stages. Further studies focused on analyses of xylem/phloem sap and subcellular Ni and Cd localization are needed in order to identify specific phenols involved in the changes presented in this study.

ACKNOWLEDGMENT

We thank Prof. David Morris (U.K.) for constructive comments and proofreading of manuscript.

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Received July 29, 2009. Revised manuscript received September 18, 2009. Accepted September 22, 2009. This work was supported by the grant of P. J. Šafárik University rector for young scientists (to J.K., No. VVGS 4/07-08), Academy of Sciences of the Czech Republic (KAN 200380801) and Czech Ministry of Education (MSM 6198959216).