The effects of nickel exposure on physiological functioning of mustard greens and mustard spinach

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Abstract

Nickel (Ni) is a plant nutrient, which plays a role in small amounts in, for example, urease. The impact of elevated Ni^{2+} concentrations (1-10 µM) in the root environment on physiological functioning of mustard greens (*Brassica juncea)* and mustard spinach (*Brassica rapa*) was studied. The enhanced levels of Ni^{2+} became swiftly phytotoxic (≥ 5 µM) and resulted in decreased biomass production, an increased pigment concentration, a higher fluorescence level and increased leaf chlorosis. The effect on mustard greens and mustard spinach was similar with only small differences in the size of the effect. The nitrate and sulfate concentrations in both shoot and root for *B. rapa* and *B. juncea* were decreased for increasing Ni^{2+} concentrations. In comparison with previous research on copper and zinc, the most similar effects on nitrate and sulfate concentrations can be found in nickel and zinc, especially in the root. The water-soluble non-protein thiol content of the root and shoot were increased in both species, with the exception of the water-soluble non-protein thiol content in the root of *B. juncea* at 10 µM Ni^{2+}.

Key words: abiotic stress / *Brassica juncea* / *Brassica rapa* / metal tolerance / nickel / toxic metals

1 Introduction

Nickel (Ni) is considered as an essential mineral for some plants or even an essential mineral for all plants (Seregin and Kozhevnikova 2006; Chen et al. 2009; Iori et al. 2013). However, the amount of Ni required for normal growth of (some) plants is very low. Nickel has different functionalities as raw material in the metallurgical and electroplating industries and as part of electrical batteries. The concentration of Ni in soils may rise as the consequence of anthropogenic activities. Examples of Ni enhancing anthropogenic activities are mining, smelting activities, industrial waste, fertilizer application and vehicles emissions. Through these anthropogenic activities more and more nickel could end up in the soils and in (ground)water. In the food chain excess concentrations of nickel could harm living organisms. Severe damage caused by nickel to fish, mammals and plants has been reported. The content of nickel in the food chain raises questions about the nickel concentrations that may turn toxic to living organisms, especially plants (Chen et al. 2009; Draszawka-Bołzan 2013; Iori et al. 2013).

Nickel is a constituent in several enzymes, for example in the active center of urease, an enzyme with the function to convert urea as nitrogen source (Draszawka-Bołzan 2013). A deficiency of nickel can result in visible symptoms of stress and disruption of metabolism of ureides, organic acids and amino acids at the leaf level. On the other hand, extremely high soil Ni concentrations can cause farmland to become unsuitable for growing crops, vegetables and fruits (Chen et al. 2009).

In the last few years the toxic effects of copper and zinc on growth and metabolism in Brassicaceae species have been studied (Shahbaz et al. 2010; Shahbaz et al. 2014; Stuiver et al. 2014). However, the (toxic) levels and effects of nickel on Brassicaceae species remained so far underexposed. In previous research copper (Cu) proved to be toxic in the root environment at ≥ 2 µM for Chinese cabbage (*Brassica pekinesis*). For zinc (Zn), the seedlings of Chinese cabbage were negatively affected in growth and metabolism to elevated Zn^{2+} concentrations in the root environment at ≥ 2 µM (Shahbaz et al. 2014; Stuiver et al. 2014).

Two different Brassicaceae species were used for this study, mustard greens (*Brassica juncea*) and mustard spinach (*Brassica rapa*). Both species are, as well as other Brassicaceae species, fast growing vegetable crops with a preference for nitrate as nitrogen source and a high sulfur requirement for growth (Aghajanzadeh et al. 2014; Stuiver et al. 2014). The two Brassicaceae species are described by a low (*Brassica rapa*) and high (*Brassica juncea*) glucosinolate content. The aim of the study was to gain insight in the effects of elevated Ni^{2+} concentrations (1 to 10 µM) in the nutrient solution on physiological functioning, including growth, distribution of sulfur and nitrogen and chlorophyll content. These concentrations (1 to 10 µM Ni^{2+}) were used as starting point from the previous studies on zinc and copper.

2 Material and methods

2.1 Plant material and growth conditions

Two different Brassica species, mustard greens (*Brassica juncea*) and mustard spinach (*Brassica rapa*), were germinated in vermiculite for 10 days in a climate-controlled room. Ten-day-old seedlings were transferred to an aerated 25% Hoagland nutrient solution (pH 5.9-6.0), consisting of 1.25 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.25 mM KH_2PO_4, 0.5 mM $MgSO_4$, 11.6 µM H_3BO_3, 2.4 µM $MnCl_2$, 0.24 µM $ZnSO_4$, 0.08 µM $CuSO_4$, 0.13 µM Na_2MoO_4, and 22.5 µM Fe^{3+}-EDTA, containing supplemental concentrations of 0, 1, 2, 5 and 10 µM $NiSO_4$ in 30 L containers in a climate-controlled room for 10 days. Day and night temperatures were 22 and 18˚C (± 1 ˚C), relative humidity was 60-70% and a photoperiod of 14h. Each container included 20 sets of plants per container, ten plants per species, three plants per set.

2.2 Plant harvest and growth analysis

All plants were harvested on day 10 of exposure, with exception of the plants for the analysis of water-soluble non-protein thiol concentrations. The shoots and roots were separated and weighed. For the analysis of pigments and anions, plant material was frozen in liquid N_2 directly after harvest and stored at -80 ˚C. For the analysis of water-soluble non-protein thiol concentrations, freshly harvested plant material of both species, harvested on day 12, was used. For the determination of the dry matter for both species, plant tissue was dried at 80 ˚C for 72h. Fresh shoot- and root-biomass production was calculated by subtracting pre-exposure weight from that after Ni^{2+} exposure. Shoot/root biomass ratio was calculated from the fresh shoot and root weights after exposure. Growth rate of the whole plant (g g^{-1} day^{-1}) was calculated on a fresh-weight basis.

2.3 Analysis pigment, fluorescence, water-soluble non-protein thiols, sulfate and nitrogen concentrations

Pigments were extracted from whole shoots and the chlorophyll a, b and carotenoids concentrations were measured as described by Lichtenthaler (1987). Fluorescence was measured with a PAM fluorescence meter. Sulfate and nitrate were extracted from frozen plant material and determined refractrometrically after HPLC separation (Shahbaz et al. 2010). Water-soluble non-protein thiols were extracted from fresh shoots and roots and the total water-soluble non-protein thiols concentrations were determined according to De Kok et al. (1988).

2.4 Statistical analysis

Statistical analysis was performed with an Student's t-test. Different letters indicate significant differences at $P < 5\%$ between different treatments.

3 Results

3.1 Plant growth, pigments and fluorescence

Exposure of mustard greens and mustard spinach seedlings for 10 days to elevated Ni^{2+} concentrations in the nutrient solution resulted in a significant reduction in growth for both species at ≥ 5 µM Ni^{2+} (Table 1). The shoot biomass production was for both species negatively affected. The shoot biomass production of *B. rapa* was slightly more affected than *B. juncea*, however both species have a significant reduction at ≥ 5 µM Ni^{2+}. The root biomass production was also for both species negatively affected. The root biomass production of *B. rapa* was as well slightly more affected than *B. juncea*, however both species have a significant reduction at ≥ 5 µM Ni^{2+}. The shoot/root ratio was decreased for both species (Table 1). The dry matter content of the shoot was increased for both species at ≥ 10 µM Ni^{2+}. The dry matter content of the root was differently affected for both species. The dry matter content of the root for *B. rapa* was significantly affected at ≥ 5 µM Ni^{2+}, however the dry matter content of root for *B. juncea* was only barely affected (Table 1).

Exposure of plants to 5 µM Ni^{2+} and 10 µM Ni^{2+} resulted in leaf chlorosis for both *B. rapa* and *B. juncea*. The leaf chlorosis for 5 µM Ni^{2+} was more visible for *B. rapa* in comparison with *B. juncea*. The chlorophyll concentration for both species was lightly decreased for 2 µM Ni^{2+}, but increased for 5 µM Ni^{2+} and 10 µM Ni^{2+} (Table 2). The chlorophyll a : b ratio was not strongly affected for *B. rapa* and *B. juncea*. The chlorophyll : carotenoid ratio was decreased for *B. rapa*, however for *B. juncea* the chlorophyll : carotenoid ratio was almost unaffected (Table 2).

The level of fluorescence was affected at ≥ 2 µM Ni^{2+} for *B. rapa* and *B. juncea*. The level of fluorescence for *B. rapa* was stronger affected by the elevated Ni^{2+} concentrations compared to *B. juncea*. The fluorescence level of *B. rapa* was especially for 5 µM Ni^{2+} strong affected. It was not possible to measure the fluorescence level for both species for 10 µM Ni^{2+}, due to the very small surface area of the leaves (Table 2).

Table 1: Impact of Ni^{2+} exposure on biomass production of mustard greens and mustard spinach. Ten-day-old seedlings of both species were grown on 25% Hoagland solution containing supplemental concentrations of 0, 1, 2, 5 and 10 µM $NiSO_4$ for ten days. The initial shoot and root fresh weights were 0.027 ± 0.007 and 0.012 ± 0.004 g for *B. juncea* and 0.032 ± 0.007 and 0.011 ± 0.004 g for *B. rapa* respectively. Data on biomass production, growth rate, and shoot : root ratio (on fresh weigt basis) represent the mean of three independent experiments with 10 measurements with three shoots and roots each (± SD). Data on dry matter content represent the mean of three independent experiments with three measurements with 3 shoots and roots each (± SD). Data on pigment concentrations represent the mean of the two measurements with three shoots each (± SD). Different letters indicate significant differences between treatments (P < 5%, student's t-test).

	Ni^{2+} concentration / µM				
	0	1	2	5	10
Brassica juncea					
Shoot					
Biomass production (g fresh weight)	1.86 ± 0.87a	2.05 ± 1.10a	1.99 ± 1.08a	1.36 ± 0.57b	0.34 ± 0.08c
Dry matter content (% of fresh weight)	9.54 ± 0.82ab	8.95 ± 0.76a	9.54 ± 0.85ab	9.65 ± 0.82b	13.66 ± 0.02c
Root					
Biomass production (g fresh weight)	0.40 ± 0.21ab	0.45 ± 0.26a	0.43 ± 0.21a	0.31 ± 0.11b	0.08 ± 0.02c
Dry matter content (% of fresh weight)	7.10 ± 0.54a	6.85 ± 1.05ab	6.47 ± 0.83b	7.04 ± 0.73ab	6.55 ± 1.40ab
Plant					
Growth rate ($g\ g^{-1}\ day^{-1}$)	0.40 ± 0.03a	0.40 ± 0.04a	0.40 ± 0.02a	0.37 ± 0.04b	0.23 ± 0.06c
Shoot : root ratio	4.8 ± 0.4a	4.6 ± 0.4ab	4.4 ± 0.7bc	4.5 ± 0.8ab	4.1 ± 0.6c
Brassica rapa					
Shoot					
Biomass production (g fresh weight)	3.92 ± 2.30a	4.26 ± 3.47a	3.35 ± 2.03a	0.93 ± 0.32b	0.33 ± 0.11c
Dry matter content (% of fresh weight)	8.54 ± 0.64a	8.09 ± 0.37a	8.87 ± 0.60b	10.42 ± 0.67c	14.41 ± 2.58d
Root					
Biomass production (g fresh weight)	0.88 ± 0.66a	0.99 ± 0.87a	0.79 ± 0.39a	0.29 ± 0.12b	0.09 ± 0.06c
Dry matter content (% of fresh weight)	5.95 ± 1.20ab	5.84 ± 0.43a	5.82 ± 0.52a	6.46 ± 0.77b	8.32 ± 2.81c
Plant					
Growth rate ($g\ g^{-1}\ day^{-1}$)	0.45 ± 0.02a	0.45 ± 0.03a	0.44 ± 0.02a	0.33 ± 0.03b	0.23 ± 0.04c
Shoot : root ratio	4.9 ± 1.1a	4.3 ± 0.6b	4.0 ± 0.4c	3.4 ± 0.6d	4.2 ± 1.3bc

Table 2: Impact of Ni^{2+} exposure on pigment concentrations and fluorescence of mustard greens and mustard spinach. Ten-day-old seedlings of both species were grown on 25% Hoagland solution containing supplemental concentrations of 0, 1, 2, 5 and 10 µM $NiSO_4$ for ten days. Data on pigment concentrations represent the mean of two measurements with three shoots each (± SD). Data on fluorescence represent the mean of two measurements with ten shoots each (± SD). It was not possible to measure the fluorescence level for both species for 10 µM $Ni^{2+,}$ due to the small surface area of the leaves. Different letters indicate significant differences between treatments (P < 5%, student's t-test).

	Ni^{2+} concentration / µM				
	0	1	2	5	10
Brassica juncea *Shoot*					
Chl a+b (mg / g^{-1} fresh weight)	0.52 ± 0.10a	0.59 ± 0.27abc	0.42 ± 0.04b	0.55 ± 0.15ac	0.68 ± 0.16c
Chl a/b	2.63 ± 0.14ab	2.44 ± 0.36a	2.64 ± 0.39ab	2.77 ± 0.18b	2.39 ± 0.82ab
Chl a+b / Car	5.11 ± 0.48ab	5.51 ± 0.87a	5.21 ± 0.95ab	4.69 ± 0.23b	5.78 ± 1.90ab
Fluorescence	0.85 ± 0.02a	0.85 ± 0.01a	0.83 ± 0.02b	0.83 ± 0.02b	-
Brassica rapa *Shoot*					
Chl a+b (mg / g^{-1} fresh weight)	0.30 ± 0.12ab	0.30 ± 0.13ab	0.28 ± 0.07a	0.40 ± 0.07b	0.52 ± 0.08c
Chl a/b	2.95 ± 0.31ab	2.99 ± 0.22a	2.66 ± 0.26b	3.52 ± 0.97a	3.04 ± 1.35ab
Chl a+b / Car	4.72 ± 0.43a	4.73 ± 0.43a	5.02 ± 0.36a	3.99 ± 0.60b	4.10 ± 0.56b
Fluorescence	0.84 ± 0.01a	0.84 ± 0.03a	0.82 ± 0.02b	0.77 ± 0.04c	-

3.2 Sulfate and nitrate concentrations

The sulfate and nitrate concentrations of seedlings of both mustard greens and mustard spinach decreased almost consistent when exposed to elevated Ni^{2+} concentrations for 10 days in the nutrient solution. The roots and shoots of both species were differently affected upon Ni^{2+} exposure (Table 3). The sulfate and nitrate concentrations in the roots were more affected than the sulfate and nitrate concentrations in the shoots. Overall the nitrate concentrations for the shoot for *B. juncea* were lower than nitrate concentrations of the shoots for *B. rapa*. The sulfate concentrations for the shoot of the two different species were higher in *B. juncea*. The sulfate and nitrate concentrations for the roots were more similar between *B. juncea* and *B. rapa*, even though the roots of *B. rapa* were more affected by the elevated Ni^{2+} concentrations than the roots of *B. juncea*. The sulfate : nitrate ratio for the shoot was increased for 10 µM Ni^{2+} for both species. However, the sulfate : nitrate ratio for the root was more stable or even decreasing for *B. rapa* and *B. juncea* (Table 3).

Table 3: Impact of Zn^{2+} exposure on sulfate and nitrate concentrations of mustard greens and mustard spinach. Ten-day-old seedlings of both species were grown on 25% Hoagland solution containing supplemental concentrations of 0, 1, 2, 5 and 10 µM $NiSO_4$ for ten days. Sulfate and nitrate concentrations represent the means of two independent experiments with 28 to 30 shoots and roots each, pooled from two experiments (± SD). Different letters indicate significant differences between treatments (P < 5%, student's t-test).

	Ni^{2+} concentration / µM				
	0	1	2	5	10
Brassica juncea					
Shoot					
Sulfate (µmol g^{-1} fresh weight)	17.0 ± 2.3ac	14.6 ± 3.8abc	16.01 ± 2.9abc	12.6 ± 2.4b	13.8 ± 2.9bc
Nitrate (µmol g^{-1} fresh weight)	61.8 ± 14.3ac	55.1 ± 21.7abc	60.0 ± 17.1abc	42.1 ± 14.3b	62.5 ± 18.9c
Nitrate : sulfate ratio	3.60 ± 0.4a	3.69 ± 0.8ab	3.63 ± 0.5a	3.28 ± 0.6a	4.53 ± 0.9b
Root					
Sulfate (µmol g^{-1} fresh weight)	14.4 ± 3.2a	10.3 ± 5.6b	10.8 ± 4.8b	8.9 ± 3.2b	8.4 ± 2.7b
Nitrate (µmol g^{-1} fresh weight)	47.5 ± 9.3a	35.1 ± 18.1b	39.6 ± 14.0ac	33.5 ± 12.3c	27.4 ± 11.2c
Nitrate : sulfate ratio	3.33 ± 0.3a	3.65 ± 0.5ab	3.85 ± 0.4b	3.74 ± 0.3b	3.17 ± 0.4a
Brassica rapa					
Shoot					
Sulfate (µmol g^{-1} fresh weight)	13.6 ± 5.5a	11.4 ± 1.2a	11.5 ± 0.9a	12.1 ± 1.1a	7.5 ± 1.8b
Nitrate (µmol g^{-1} fresh weight)	73.6 ± 12.2a	70.8 ± 15.7a	66.2 ± 14.4a	64.1 ± 13.0a	58.8 ± 22.2a
Nitrate : sulfate ratio	5.84 ± 1.1a	6.21 ± 1.1a	5.81 ± 1.5a	5.36 ± 1.3ab	7.83 ± 2.7ac
Root					
Sulfate (µmol g^{-1} fresh weight)	14.7 ± 4.8a	11.6 ± 0.7ab	10.2 ± 1.6b	8.8 ± 3.5bc	7.7 ± 1.6c
Nitrate (µmol g^{-1} fresh weight)	43.1 ± 5.4a	35.2 ± 7.87b	37.3 ± 1.7b	23.9 ± 9.9c	19.1 ± 8.1c
Nitrate : sulfate ratio	3.23. ± 0.9ab	3.08 ± 0.9ab	3.75 ± 0.64a	2.75 ± 0.9b	2.49 ± 1.0b

3.3 Water- soluble non-protein thiols

The water-soluble non-protein thiol content of the shoot increased with the Ni^{2+} concentration for both species, *B. rapa* and *B. juncea* (Fig. 1). The effect of the elevated Ni^{2+} concentrations on the root was different in comparison with the effect on the shoot. The water-soluble non-protein thiol content of the root increased until the level of 5 µM Ni^{2+}, however at the level of 10 µM Ni^{2+} the water-soluble non-protein thiol content of the root decreased significantly for *B. juncea*. The water-soluble non-protein thiol content level of *B. juncea* and *B. rapa* was similar in the shoot. For the roots, the water-soluble non-protein thiol content level in *B. juncea* was higher in comparison with *B. rapa* (Fig. 1).

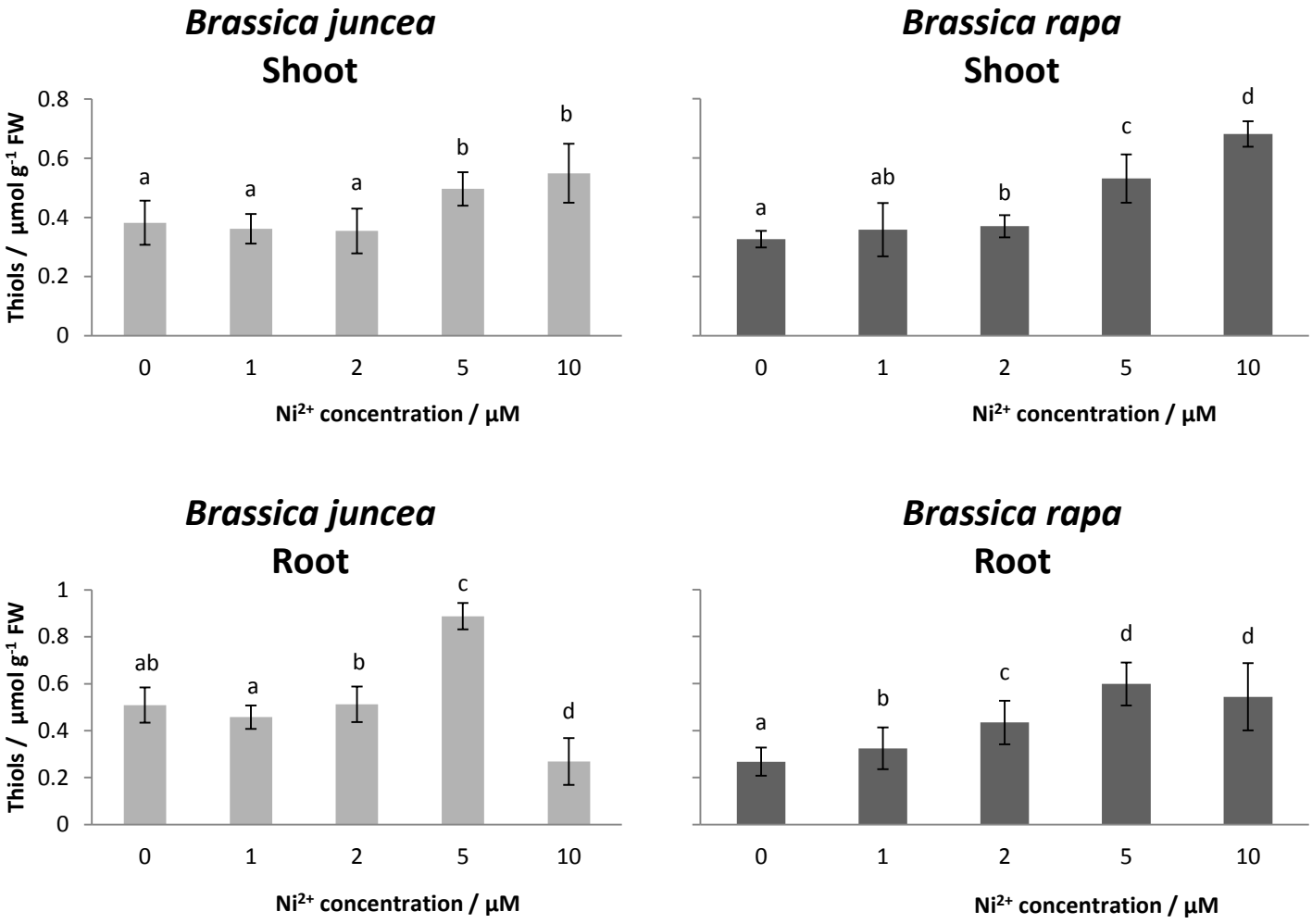

Figure 1: Impact of Ni^{2+} exposure on total water-soluble non-protein thiol concentrations of mustard greens (grey) and mustard spinach (black). Ten-day-old seedlings of both species were grown on 25% Hoagland solution containing supplemental concentrations of 0, 1, 2, 5 and 10 µM $NiSO_4$ for twelve days. Data represent the means of two measurements on three plants each (± SD). Different letters indicate significant differences between treatments (P < 5%, student's t-test).

4 Discussion

The toxic effects of heavy metals, like nickel are mainly manifested as inhibition of the growth of plants. The plant growth inhibition caused by heavy metals may be result from immediate inhibition of cell divisions and general metabolic disorder (Seregin and Kozhevnikova 2006). Seedlings of mustard greens and mustard spinach were negatively affected to elevated Ni^{2+} concentrations in the nutrient solution for growth rate. The growth rate was for both species significant affected at ≥ 5 µM Ni^{2+} (Table 1). The differences in growth and chlorosis became visible after the fifth day of exposure in the nutrient solution. Plants grown with nickel in the concentrations of 5 µM Ni^{2+} and 10 µM Ni^{2+} were much smaller in size and had smaller leaves. At first view *B. rapa* plants looked more affected than plants of *B. juncea* at 5 µM Ni^{2+} with smaller leaves and more chlorosis. The effect of the elevated Ni^{2+} on the growth of both species was quite similar to the sensitivity of Chinese cabbage for Cu^{2+} and Zn^{2+} concentrations, however the effect of Cu^{2+} and Zn^{2+} in the previous research was already significant at ≥ 2 µM Ni^{2+} (Shahbaz et al. 2014; Stuiver et al. 2014). Plant growth inhibition by copper and zinc occurs at lower metal concentrations in comparison with nickel. In the previous research on zinc, the shoot/root ratio decreased at the toxic Zn^{2+} levels (≥ 5 µM Zn^{2+}). However, the shoot/root ratio in the research on copper increased. The shoot/root ratio decreased at toxic Ni^{2+} levels, especially for *B. juncea*, comparable to the decrease of the shoot/root ratio in the research on Zn^{2+}. The decrease of the shoot/root ratio is caused by the raised decrease in shoot growth (Table 1). Copper has more effect on the shoot in contrast to zinc and nickel, which have more effect on the root. The higher decrease in shoot growth doesn't seem to match with an earlier described specific characteristic of nickel, the inhibition of root branching (Seregin and Kozhevnikova 2006; Shahbaz et al. 2014; Stuiver et al. 2014). However, by observations of the roots there seemed to be differences in the root branching for the different nickel concentrations, but these could be caused by the overall decline in biomass.

The decrease in biomass production was in the previous research in zinc and copper attended by a decrease in pigment concentration (Shahbaz et al. 2014; Stuiver et al. 2014). The elevated nickel concentrations didn't negatively affect the pigment concentration in *B. rapa* and *B. juncea* (Table 2). The pigment concentration of *B. rapa* was more positively affected than the pigment concentration of *B. juncea*, which had a more stable pigment concentration. The chlorosis was in the observations more visible for *B. rapa* than for *B. juncea*. The chlorosis seemed to be more developed in younger leaves, especially for *B. rapa*. Upon Cu^{2+}exposure, chlorosis also started more in younger leaves. However, for Zn^{2+} exposure chlorosis started in older leaves. The chlorophyll a:b ratio is hardly affected by the elevated Ni^{2+} concentrations for both *B. juncea* and *B. rapa* (Table 2). This is similar for the previous research on Zn^{2+}, but the chlorophyll a:b ratio was significant decreased for toxic copper concentrations (Shahbaz et al. 2010; Shahbaz et al. 2014; Stuiver et al. 2014). The higher pigment concentrations in combination with the observed chlorosis suggest that the chlorosis isn't the consequence of pigment degradation. This was also observed for high copper concentrations, which arose the assumption that chlorosis was caused by hindered chloroplast development (Shahbaz et al. 2010; Shahbaz et al. 2014; Stuiver et al. 2014). The level of fluorescence is significant affected at ≥ 2 µM Ni^{2+} for *B. rapa* and *B. juncea* (Table 2). This gives a sign of the increase of stress for both *B. rapa* and *B.*

juncea at Ni^{2+} concentrations of ≥ 2 µM, beside the visual signs like chlorosis and smaller leaves that occurred at ≥ 5 µM Ni^{2+}.

Both sulfate and nitrate concentrations of seedlings of mustard greens and mustard spinach decreased or remained unaffected when exposed to elevated Ni^{2+} concentrations (Table 3). In both root and shoot, the sulfate and nitrate concentrations decreased in *B. rapa* and to a lesser extent in *B. juncea*. This is in several ways different in comparison with the previous research on Chinese cabbage for Cu^{2+} and Zn^{2+} concentrations (Shahbaz et al. 2014; Stuiver et al. 2014). For sulfate, the concentrations in the shoot were for both elevated Cu^{2+} and Zn^{2+} levels enhanced. The elevated Ni^{2+} levels on the other hand decreased the sulfate level in the shoot (Table 3). The sulfate concentration in the root was unaffected in the research with Zn^{2+}. An elevated copper concentration resulted in a slight increase in the sulfate concentration in the roots. The nitrate concentrations in the shoot were differently affected for elevated Zn^{2+} and Cu^{2+} levels. In the shoot, the nitrate concentration for Zn^{2+} was decreased, but for Cu^{2+} the nitrate concentration in the shoot was increased. The effects of Cu^{2+} and Zn^{2+} on the nitrate concentration in the root are similar. In both studies the nitrate concentration in the root decreased (Shahbaz et al. 2014; Stuiver et al. 2014). Overall, the heavy metals zinc and nickel share the most similar effects on nitrate and sulfate concentrations, especially in the root. In the shoot, the only big difference between the effects of nickel and zinc is the effect on the sulfate concentration in the shoot. The effects on the sulfate concentration of copper and nickel were completely different. For nitrate, the effect in the concentration is almost the same for nickel and copper. The overall decreasing effect of nickel on both nitrate and sulfate in shoot and root, with the exception of the nitrate concentration at 10 µM Ni^{2+} in the shoot of juncea, doesn't seem to suggest a specific effect of nickel on sulfate or nitrate, but a nonspecific decreasing uptake of the roots. The different effects of heavy metals on the sulfate concentrations and the role of transporters in this process needs to be further resolved.

The water-soluble non-protein thiol content of the shoot increased with the Ni^{2+} concentration for both species, *B. rapa* and *B. juncea* (Fig. 1). In the previous studies on zinc and copper the water-soluble non-protein thiol content of the shoot was also increased for elevated Cu^{2+} concentration (at ≥ 2 µM Cu^{2+}), but only slightly enhanced for elevated Zn^{2+} concentrations (Shahbaz et al. 2010; Stuiver et al. 2014). In the root, the water-soluble non-protein thiol content was increased for elevated Ni^{2+} concentrations, however at the level of 10 µM Ni^{2+} the water-soluble non-protein thiol content of the root decreased significantly for *B. rapa*. The high water-soluble non-protein thiol content for *B. juncea* at 5 µM Ni^{2+} is similar at 10 µM Ni^{2+} (Fig. 1). For elevated Zn^{2+} concentrations, the water-soluble non-protein thiol content in the root was strongly enhanced at ≥ 1 µM Cu^{2+}. In the study on copper influence, the water-soluble non-protein thiol content in the root was also strongly increased. The effect of the different metals on the water-soluble non-protein thiol content in the root comparable, except for the lower value for *B. juncea* at 10 µM Ni^{2+}, which could be due to the very small biomass of the samples. Also other measurements indicate some anomalous values for 10 µM Ni^{2+}, probably caused by the extreme small and strong affected plants. The accumulation of water-soluble non-protein thiols hat occurs in varying degrees in the root and shoot for toxic heavy metal levels for the three studies on different heavy metals might be partially explained by the enhancement of

phytochelatin content in plants. However, a primary role of phytochelatins in the detoxification of (heavy) metals seems to be very restricted and doubtfull (Shahbaz et al. 2010; Shahbaz et al. 2014; Stuiver et al. 2014). The increase of water-soluble non-protein thiols may indicate a function of phytochelatin synthase (thiol-rich compounds synthesis with glutathione as precursor) in metal micronutrient homeostasis for non-toxic levels of (heavy)metals, including nickel, copper and zinc (Schat et al. 2002). For the detoxification of high excessively accumulated levels of metal micronutrients other mechanisms of detoxification seem to play an essential role in contrast to the accumulation of phytochelatins (Schat et al. 2002; Gasic and Coban 2007). The nature of higher levels of water-soluble non-protein thiols in combination with heavy metals like zinc, copper and nickel needs further to be evaluated.

5 Conclusions

Elevated Ni^{2+} concentrations (≥ 5 µM) in the root environment were toxic for both mustard greens and mustard spinach. The enhanced Ni contents caused a lower biomass production, an increased pigment concentration, a higher fluorescence level and increased leaf chlorosis. The nitrate and sulfate concentrations in both root and shoot for *B. rapa* and *B. juncea* were decreased for higher Ni2+ levels. The water-soluble non-protein thiol content in the shoot was increased for both species for elevated Ni concentrations. In the root the water-soluble non-protein thiol content was also increased, however at the level of 10 µM Ni^{2+} the thiol content decreased significantly for *B. juncea*.

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