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EFFECT OF CADMIUM AND ZINC ON METABOLISM OF SPINACH

WPŁYW KADMU I CYNKU NA METABOLIZM SZPINAKU

Abstract: Changes in metabolism of spinach (*Spinacia oleracea* L., 'Matador' cv.) plants exposed to stress caused by two heavy metals: cadmium and zinc were studied. The distribution of these elements in biomass (in root, leaf blade, petiole), chlorophyll content and activity of glutamate 5-kinase [E.C.2.7.2.11] the enzyme catalyzing the first step of proline biosynthesis were investigated in pot experiments. Results of the experiment revealed the toxic effects of cadmium and zinc at both tested levels (2.5, 25 mg Cd and 50, 500 mg Zn · kg^{-1}) for spinach. Under these conditions, decrease of glutamate kinase activity in spinach plants grown on contaminated treatments compared with untreated control was found. Allosteric regulation of glutamate kinase activity by free proline creates a possibility for an increase in glutamic acid content due to the synthesis of glutathione and subsequently phytochelatines in plant cells. For this reason the rates of Cd and Zn applied into soil decreased the glutamate kinase activity. The higher doses of both metals negatively affected yield of spinach dry biomass, but only Cd dose (25 mg Cd · kg^{-1} soil) was associated with the significant inhibition of aboveground biomass compared with control treatment (by 14 %). The highest contents of both elements were analyzed in blades of spinach leaves. Chlorophyll content was decreased only by higher cadmium dose.

Keywords: chlorophyll, glutamate kinase, toxic elements, stress, spinach

Cadmium is a widespread heavy metal released into the environment by energy production, metal-working industries, traffic, and phosphate fertilizers. Zinc toxicity is a problem in areas of natural Zn deposits, spoil heaps from mining and around zinc

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smelters. The increase of Zn and Cd accumulation in plant biomass can also be due to the application of sewage sludge to the soil [1, 2].

Heavy metals such as cadmium and zinc were found to induce oxidative stress in plants. Because of their atomic properties, heavy metals can induce uncontrolled redox reactions that will lead to the oxidation of membranes and proteins and the disruption of cellular homeostasis [3]. Plants can accumulate heavy metals in their tissues due to their great ability to adapt to variable properties of the environment. Cadmium and zinc can easily interact with iron, one of the most important elements for plant growth and metabolism. According to Siedlecka and Krupa [4] strong Fe-dependency to Cd mobility within the plant and adaptation of photosynthetic dark phase to Cd stress were found. Zinc is known to replace Fe from chelate complexes forming corresponding heavy metal chelates [5]. Cadmium inhibited the oxidative mitochondrial phosphorylation, reduced activity of plasma membrane ATPase and strongly affected the activity of several enzymes, such as glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, Rubisco and carbonic anhydrase. Cd ions can inhibit the activity of several antioxidative enzymes [6].

Metabolisms of glutathione, organic acids, peroxidases, stress proteins, proline and related amino acids, as well as mechanisms of compartmentalization, lignification, and root development are affected by the presence of the toxic elements and many other stress agents. Accumulation of free proline in plants has been often reported as a response to a wide range of environmental stresses including presence of heavy metals [7]. The glutamate kinase enzyme catalyzed the first step of proline biosynthesis, ie the conversion of glutamic acid to γ-glutamyl phosphate. Determination of glutamate kinase activity seems to be a suitable tool for monitoring the stress-induced changes of plant metabolism during the whole plant growth period [8].

This study was focused on the investigation of changes in the plant metabolism under a chronic stress caused by contaminants. The glutamate kinase activity [E.C.2.7.2.11] was investigated as a plant response to stress caused by Cd and Zn application into soil.

Material and methods

Adaptation of spinach (*Spinacia oleracea* L., 'Matador' cv.) plants to excessive cadmium and zinc levels in soil was investigated in pot experiment repeated for three years. For experiment, spinach seeds (20 seeds obtained from SEMO Ltd. Smržice, Czech Republic) were sown into plastic pots containing soil mixture as specified below. The plants (10 plants per pot) were cultivated from April to June under natural light and temperature conditions at the experimental hall of the Czech University of Life Sciences in Prague, Czech Republic. The water regime was controlled and the soil moisture was kept at 60 % MWHC.

For culivation of spinach plants, 5 kg of chernozem soil (pH_{KCl} = 7.2, C_{ox} = 1.83 %, CEC = 258 mval · kg^{-1}) was thoroughly mixed with 0.5 g N, 0.16 g P, and 0.4 g K applied in the form of ammonium nitrate and potassium hydrogen phosphate for control treatment and with the same amount of nutrients plus toxic element (Cd in $CdCl_2$ or Zn in $Zn(CH_3COO)_2 \cdot 2H_2O$) for treated variants. Two rates of Cd (Cd1 = 2.5, Cd2 = 25

mg · kg^{-1}) and Zn (Zn1 = 50, Zn2 = 500 mg · kg^{-1}) were applied. Each treatment was performed in five replications. Spinach plants were planted up to the stage of full leaves development.

The dried aboveground spinach biomass was used for determination of total Cd and Zn contents. The plant material was decomposed by a dry ashing procedure. The ash was dissolved in 1.5 % HNO_3 [9].

Varian SpectrAA-400 (Australia) atomic absorption spectrometer with a GTA-96 graphite tube atomizer was applied for Cd determinations. A pyrolytically-coated tube with a L'vov platform was used for all Cd measurements. Flame atomization (air-acetylene flame) was applied (Varian SpectrAA-300 atomic absorption spectrometer, Australia) for Zn determinations. The quality of the plant and soil analyses was verified using the RM 12-02-03 Lucerne.

Glutamate kinase was isolated from acetone-dried spinach fresh aboveground biomass and was extracted from 1 g of acetone powder by 10 cm^3 phosphate buffer (50 mM, pH 7.6) for 30 min and centrifuged (2 ^{o}C, 22000 g) for additional 20 min. Solid ammonium sulphate was added (2.12 g to every 10 cm^3 filtered supernatant) and the mixture was centrifuged (2 ^{o}C, 22000 g) for 30 min. The pellets were suspended in 50 mM phosphate buffer (pH 7.6). After incubation the activity of the enzyme was determined spectrophotometrically by modification of the hydroxamate method [10].

Chlorophyll was extracted from spinach fresh aboveground biomass by acetone. Contents of chlorophylls *a* and *b* were determined spectrophotometrically at wavelength λ = 644 and 663 nm.

Results and discussion

Plant responses to the excessive cadmium and zinc contents in soil were assessed on the basis of decreased spinach dry matter after application of higher Cd and Zn rates (Fig. 1) and increased concentrations of these elements in the aboveground biomass

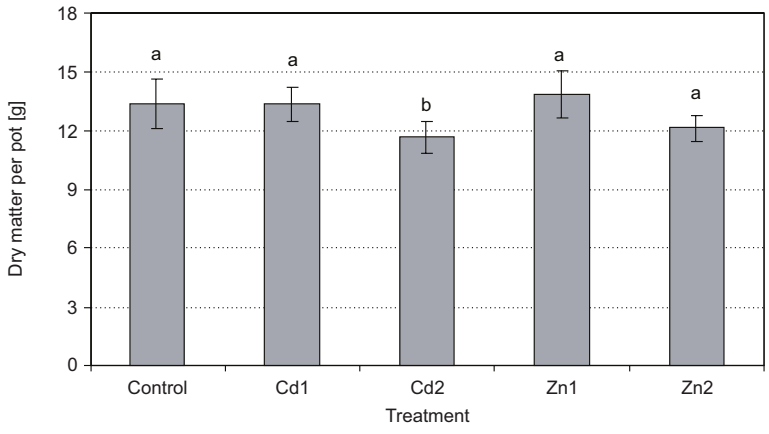

Fig. 1. Yield of spinach dry matter [g per pot]; upper letters refer to the effects of Cd and Zn addition – the same letters mean not significant difference at the level $P < 0.05$

(Table 1). Both lower Cd and Zn rates did not affected yield of spinach dry matter. The higher Cd dose (25 mg Cd · kg^{-1} soil) was associated with the significant inhibition of aboveground biomass. The biomass yields of Cd2 treatment was reduced to ca 14 % in contrast to control treatment. The yield inhibition by Zn2 level was much less pronounced (9 %).

Table 1

Cd and Zn contents in spinach aboveground biomass [mg · kg^{-1}]

Treatment	Cd content	Zn content
	[mg · kg^{-1}]	
Control	0.44 ± 0.01	53.9 ± 3.4
Cd1	1.99 ± 0.12	
Cd2	7.30 ± 0.21	
Zn1		95.0 ± 5.8
Zn2		158.5 ± 7.2

Application of both rates of cadmium and zinc led to the increase of both elements contents in plant biomass in the pot experiment in contrast to control treatment (Table 1). Compared with the untreated control, the cadmium content in leaves was enhanced up to 4.5–fold (Cd1) and 16.6-fold (Cd2), while the zinc content in aboveground biomass was increased 1.8-fold (Zn1) and 2.9-fold (Zn2). Our data correspond with those by Tlustoš et al [11] who reported that excessive amounts of toxic elements in contaminated soil inhibited plant growth and development due to their phytotoxicity. Kabata-Pendias and Pendias [12] reviewed plant response to increased levels of Cd in soil and showed a great difference in the ability of various plant species to absorb this metal. The highest Cd concentration was determined in leafy and root vegetables.

The results of distribution of cadmium and zinc in spinach biomass showed the concentrations of both elements as following leaf blade > root > petiole (Table 2). Tlustoš et al [13] have presented similar results for spinach. According their results 63.8 % of Cd content was found in spinach aboveground biomass and 36.2 % in roots.

Table 2

The distribution of cadmium and zinc in spinach biomass

Treatment	Cd	Zn
	[mg · kg^{-1}]	
Root		
Control	0.27 ± 0.01	34.9 ± 2.6
Cd1	1.41 ± 0.06	
Cd2	5.47 ± 0.05	
Zn1		65.7 ± 3.7
Zn2		131.5 ± 3.5

Table 2 contd.

Treatment	Cd	Zn
	[mg · kg^{-1}]	
Leaf blade		
Control	0.75 ± 0.01	90.7 ± 1.3
Cd1	2.90 ± 0.02	
Cd2	7.59 ± 0.07	
Zn1		121.3 ± 3.7
Zn2		276.7 ± 3.1
Petiole		
Control	0.43 ± 0.01	30.9 ± 5.4
Cd1	1.50 ± 0.02	
Cd2	4.82 ± 0.08	
Zn1		63.7 ± 4.0
Zn2		95.3 ± 6.7

Determination of chlorophyll content confirmed its decrease only on Cd2 and Zn2 treatments (Table 3). Our previous results (Cd rate was 90 mg · kg^{-1}) showed damage caused by cadmium led to the bleaching of chlorophylls [8]. In accordance with these data, cadmium and zinc treatments considerably inhibited growth and progressively reduced chlorophyll contents of duckweed plants [14]. Moreover, the cadmium-treated plants were found to be completely photobleached in those experiments.

Table 3

Chlorophyll contents in spinach leaves [mg · g^{-1}]

Treatment	Chlorophyll *a*/Chlorophyll *b*	Chlorophyll *a*	Chlorophyll *b*
		[mg · g^{-1}]	
Control	2.759	0.080 ± 0.004	0.029 ± 0.003
Cd1	2.793	0.081 ± 0.007	0.029 ± 0.005
Cd2	3.182	0.070 ± 0.010	0.022 ± 0.006
Zn1	2.964	0.083 ± 0.006	0.028 ± 0.005
Zn2	3.076	0.080 ± 0.009	0.026 ± 0.002

Plant stress evoked by cadmium and zinc treatments was associated with a reduced activity of glutamate kinase (Fig. 2), the enzyme catalyzing the first step of proline biosynthesis from L-glutamate. Activity of this enzyme is regulated in plants via a feedback mechanism by content of proline [15]. Results of the glutamate kinase activity showed a strong effect of both elements. Effect of zinc as an essential element in the glutamate kinase activity was significantly lower compared with Cd toxic element. Allosteric regulation of glutamate kinase activity by free proline enables to increase the

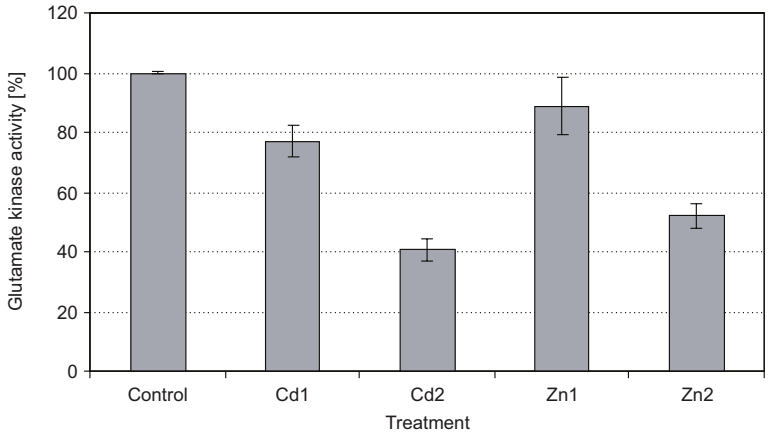

Fig. 2. Changes of glutamate kinase activities of spinach; activity of glutamate kinase of untreated control = = 100 %

content of glutamate that is required for the formation of a peptide bond between the g-carboxyl group of glutamate and the α-amino group of cysteine and used in synthesis of phytochelatins via glutathione in plant cells [8, 16].

Conclusions

1. Application of cadmium and zinc led to the increase of both elements contents in plant biomass in the pot experiment in contrast to control treatment.

2. The higher doses of both metals negatively affected yield of spinach dry biomass, but only Cd dose (25 mg Cd · kg^{-1} soil) was associated with the significant inhibition of above-ground biomass compared with control treatment (by 14 %). The yield inhibition by Zn2 level was much less pronounced (9 %).

3. The results of distribution of cadmium and zinc in spinach biomass showed the concentrations of both elements as following leaf blade > root > petiole.

4. Determination of chlorophyll content confirmed its decrease only on Cd2 and Zn2 treatments.

5. Allosteric regulation of glutamate kinase activity by free proline creates a possibility for an increase in glutamic acid content due to the synthesis of glutathione subsequently phytochelatines in plant cells. For this reason the rates of Cd and Zn applied into soil decreased the glutamate kinase activity.

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WPŁYW KADMU I CYNKU NA METABOLIZM SZPINAKU

Abstrakt: Badano zmiany metabolizmu roślin szpinaku (*Spinacia oleracea* L., odmiany 'Matador') eksponowanych na stres powodowany przez dwa metale ciężkie: kadm i cynk. W doświadczeniu wazonowym badano rozmieszczenie tych pierwiastków w biomasie (w korzeniach, blaszkach i ogonkach liściowych), zawartość chlorofilu oraz aktywność kinazy glutaminianowej [E.C.2.7.2.11], enzymu katalizującego pierwszy stopień biosyntezy proliny. Wyniki doświadczenia wykazały toksyczny wpływ kadmu i cynku na szpinak w obydwu zastosowanych dawkach (2,5 i 25 mg Cd oraz 50 i 500 mg Zn · kg^{-1}). W tych warunkach stwierdzono spadek aktywności kinazy glutaminianowej w roślinach szpinaku rosnących w obiektach zanieczyszczonych metalami w porównaniu z obiektem kontrolnych, bez ich dodatku. Regulacja allosteryczna aktywności kinazy glutaminianowej przez wolną prolinę indukuje możliwość zwiększenia zawartości kwasu glutamiowego w wyniku syntezy glutationu, a następnie fitochelatyn w komórkach roślin. Z tego względu dawka Cd i Zn zastosowana do gleby zmniejszyła aktywność kinazy glutaminianowej. Większa dawka obydwu metali ujemnie oddziaływały na plon suchej masy szpinaku, ale tylko dawka 25 mg Cd · kg^{-1} gleby wywołała znaczne zahamowanie (o 14 %) wzrostu nadziemnej biomasy w porównaniu z obiektem kontrolnym. Największe zawartości obydwu metali stwierdzono w blaszkach liściowych szpinaku. Zawartość chlorofilu zmniejszyła się tylko po zastosowaniu większej dawki kadmu.

Słowa kluczowe: chlorofil, kinaza glutaminianowa, pierwiastki toksyczne, stres, szpinak