Polish Journal of Environmental Studies Vol. 6, No. 6 (1997), 47 - 50

Biochemical Indicators of Environmental Contamination with Heavy Metals

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Received 28 May, 1997
Accepted 8 August, 1997

Abstract

Changes in activity of one of the most important enzymes of nitrogen compound metabolism – glutamate dehydrogenase (GDH EC1.4.1.2-4) under the conditions of variable concentrations of Cd^{2+}, Zn^{2+}, Pb^{2+} were investigated. Dry matter increase and glutathione (GSH) levels varying under these conditions were also analyzed. The obtained results show a close relationship between the concentration of the investigated metal ions, especially Cd^{2+}, and the metabolism of primary amino acids. The increase of the heavy metal concentration resulted in a many-fold GDH activity increase, significant decrease of the seedling mass, and lowering of the GSH level. The increase of GDH activity may attest to the important role of that enzyme in catabolic processes intensified under these conditions, or to the increasing importance of the reductive amination of 2-oxoglutarate in the states of physiological stress caused by the presence of heavy metals. The observed relationship concerns the plant's photosynthetizing organs rather than its root system.

Keywords: heavy metals, glutamate dehydrogenase, glutathione.

Introduction

Heavy metal, present in the environment in excess create stressful conditions for plants. Particularly harmful are those which inhibit biochemical processes, in synergistic or antagonistic interactions. Most often the toxic action of heavy metals reveals itself in the inhibition of plant growth [1, 2, 3]. This results from their effect on the key metabolic processes including photosynthesis, respiration, transpiration, NO_3^- uptake and reduction, as well as NH_4^+ assimilation [4-8]. Many authors report the effect of heavy metals on enzyme activities [5, 8-11]. Under *in vitro* conditions, most susceptible to the action of heavy metal ions are the enzymes with cysteine and histidine residues as the contact amino acids of the active centre [11-14]. This group of enzymes includes, among others, nitrate and nitrite reductases, glutamine synthetase and glutamate dehydrogenase [5, 11-15].

However, changes in enzyme activities observed at the *in vitro* conditions resulting from the presence of heavy metals are not always similar to changes observed in *in vivo* conditions. Plant cells subjected to the action of heavy metals created some stress avoidance and tolerance mechanisms. The mechanisms involve, among others, protecting protoplasts against metals by making their penetration more difficult, removing metals from the sites of metabolic activity, accelerating the synthesis of proteins less susceptible to certain metals, and changing directions of metabolic pathways [16]. The uptaken metal ions may be immobilized in the cell wall, glyoxysomes or vacuoles [16]. The heavy metals which occur in the cells show a tendency to form strong covalent bonds with hydrosulphide compounds [17-18]. Some metals occuring in the cells are nearly totally complexed by phytochelatines forming metal-phytochelatine bonds [17-20]. Glutathione participate in the formation of these complexes and some heavy metals play the part of the activators of phytochelatine synthetase [18-19].

The present work aims at demonstrating that GDH, as one of the key enzymes of NH_4^+ assimilation, may play the part of a biochemical index of environmental contamination by heavy metals.

Material and Methods

Material

The investigation was carried out on the roots and shoots of 3 week old seedlings of *Triticale var. Malno*, cultivated in the hydroponic system with root aeration and with constant concentration of macro and microelements [21].

Samples of roots and shoots grown on the medium without the additional heavy metal ions were described as control. In the remaining combinations three different concentrations of three heavy metal ions (Cd^{2+}, Pb^{2+}, Zn^{2+}) as nitrate were added. The concentrations are presented in Table 1.

The following conditions were maintained during plant growth: pH 6.0, day temperature 22-24°C, night temperature 18-20°C, photoperiod of 14h, light intesity 30 W · m^{-2}, 40 seedlings for 5l medium, exchange of medium every 5 days.

Analytical Methods

Extraction and quantitative determinations of the glutamate dehydrogenase in the dialysed preparations were performed using the Barash method [22], based on measurement of absorbance decrease at 340 nm.

Protein was determined according to Lowry et al. [23] with the modification for plant proteins [21]. Glutathione was extracted with 5% trichloracatic acid and estimated as described by Smith et al. [24]. NH_4^+ content was determined according to Hoffman's method [25].

Dry matter was determined by drying the samples for 2h at 70°C and then for 3h at 105°C.

Extraction and determinations of heavy metals were carried out according to Gworek [26]. Plant material was washed, dried at 60°C, milled and then ashed in a muffle furnace at 480°C. The ash was dissolved in concentrated HCl and silica precipitated by adding boiling water. After concentrating to an organic phase with methylobutylic ketone, metal ions were determined by atomic absorption spectrophotometry.

Results and Discussion

The use of various concentrations of heavy metals in the media significantly affected their concentrations in the seedling shoots and roots (Table 2). The obtained results indicate the existence of large differences in the ability of *Triticale* seedlings to uptake and accumulate particular cations. Out of three analyzed cations cadmium was most easily incorporated. A similar phenomenon was observed

Table 1. Concentration of heavy metal ions in medium.

Metal ions	mg · l^{-1}		
	1	2	3
Cd^{2+}	0.02	0.25	5.00
Pb^{2+}	8.00	100.00	200.00
Zn^{2+}	4.00	16.00	100.00

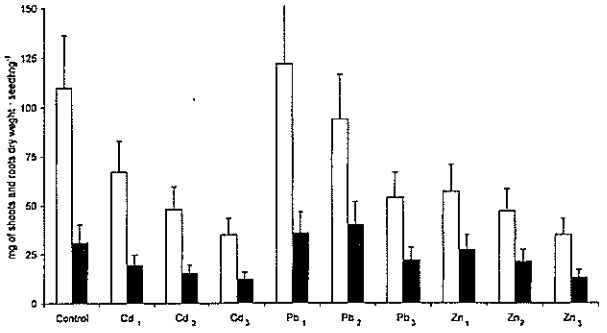


Fig. 1. Effect of increasing concentration of Cd^{2+}, Pb^{2+} and Zn^{2+} on dry matter yield of *Triticale* sedlings shoots (□) and roots (■). Vertical bars represent ± SE from 4 independent experiments.

in many species of higher plants [27-28]. Most of the bound cations accumulated in the roots and only to a small extent were they transfered to the seedling shoots. Pb^{2+} uptake was the lowest while Cd^{2+} was the highest. Large differences between the roots and green parts of seedlings in heavy metals accumulation may indicate the existence of mechanisms limiting the transportation of them from the roots to photosynthesizing parts, as demonstrated for other plant species [14, 27-28].

The effect of heavy metals on seedling growth was also demonstrated: the yield of shoot and root dry matter depended on the type and concentration of these ions (Fig. 1). The feeding of the seedlings with Cd^{2+} or Zn^{2+} even at low concentrations caused an inhibition of plant growth as compared to the control samples. The increase of the ion concentrations in the nutrient and thus inside the plants intensified that process. The comparison of leaf and root growth inhibition caused by the investigated ions shows that despite their significant accumulation in roots and shoots, the former did not react with as high a reduction of dry matter yield as the shoots. The ratio of green part dry matter to that of roots independently on the cation used decreased with its increasing concentration. The stimulating effect of low concentrations of Pb^{2+} on the dry matter yield of both shoots and roots seems to be an interesting phenomenon. Similar phenomenon has been described by other authors [8].

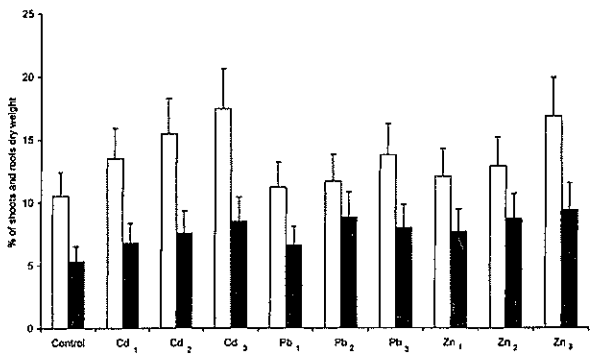


Fig. 2. Effect of increasing concentration of Cd^{2+}, Pb^{2+} and Zn^{2+} on per cent content of dry weight of *Triticale* sedlings shoots (□) and roots (■).Vertical bars represent ± SE from 4 independent experiments.

Table 2. Contents of Cd^{2+}, Pb^{2+} and Zn^{2+} in shoots and roots of *Triticale* seedlings after 21 day growth on particular media. (See Table 1). Data represent the mean (± SE) from at least three experiments.

Metal ions in the medium	mg · g^{-1} dry weight	
	shoots	roots
Cd^{2+}_0 (Control)	< 0.001	< 0.001
Cd^{2+}_1	0.104 ± 0.011	1.054 ± 0.085
Cd^{2+}_2	0.191 ± 0.021	2.493 ± 0.201
Cd^{2+}_3	0.299 ± 0.028	4.486 ± 0.262
Pb^{2+}_0 (Control)	< 0.001	< 0.001
Pb^{2+}_1	0.023 ± 0.002	21.968 ± 1.705
Pb^{2+}_2	0.119 ± 0.008	52.140 ± 2.810
Pb^{2+}_3	0.205 ± 0.017	93.865 ± 9.519
Zn^{2+}_0 (Control)	0.047 ± 0.005	0.031 ± 0.002
Zn^{2+}_1	1.057 ± 0.009	18.875 ± 1.175
Zn^{2+}_2	1.547 ± 0.011	36.032 ± 2.137
Zn^{2+}_3	4.501 ± 0.320	47.036 ± 2.684

Comparing metal concentrations in the nutrient causing nearly 50% growth inhibition of the shoots, it could be stated that Cd^{2+} is 800 times more toxic than Pb^{2+}, but 16 times more so than Zn^{2+}. Similar experiments performed on wheat revealed that Cd^{2+} present in the soil caused a significant decrease of accumulation of macro and microelements, including Ca^{2+}, K^+, Na^+ and Fe^{2+} while Pb^{2+} stimulated the uptake of Ca^{2+} into the plants [3, 28-29]. It is worth noting that the increase of the investigated heavy metal concentrations resulted in the increase of the per cent contents of dry matter of both parts of the seedlings (Fig. 2). This seems to be connected to the disturbance of the water balance in plants caused by the heavy metal ions [8, 30].

The increase of heavy metal concentrations in plants significantly affected the activity of glutamate dehydrogenase (Figs. 3 and 4). The activity of that enzyme expressed to both: g of fresh weight and mg of protein increased with the growth of the heavy metal contents. Relative increase of enzyme activity was higher in the plant shoots than in roots (and particularly high in the presence of Cd^{2+}). Such a high increase of the GDH activity with the simultaneous high growth inhibition of the green part dry matter could indicate that the activity level of that enzyme is an indicator of the intensifying catabolic processes. However, recent data indicate that GDH can play a significant role in the process of NH_4^+ assimilation at the conditions of physiological stress [15].

The determination of NH_4^+ contents in plants served for the interpretation of the GDH activity changes. As this enzyme presents the inductive properties its activity which was demonstrated depends on the intracellular concentration of NH_4^+ as one of the substrates in many plant species (31-32). Comparison of the NH_4^+ levels in the shoots and roots of plants fed with Cd^{2+}, Pb^{2+}, and Zn^{2+} demonstrates that changes in the NH_4^+ contents in the former were relatively low, while a considerable decrease of the NH_4^+ levels were observed in the roots (Table 3) as compared to the control plants, amounting in the case of Cd^{2+} to about 70%. Therefore, the small changes in heavy metal levels and the simultaneous significant increases of GDH activities may suggest the lack of a simple dependence between enzyme activity and the concentration of this substrate. On the other hand, lower NH_4^+ levels observed may result from the inhibition of the nitrate reduction process catalyzed by the nitrate and nitrite reductases as it is known that the heavy metal ions inhibit a number of key metabolic processes, including, among others, the nitrate reductase [4-8].

One of the defensive mechanisms of plants against the toxic properties of heavy metals is the synthesis of metalopeptides of a low oxidation-reduction potential, which might contain the cysteine-like compounds [17-20]. Thus, glutathione should play an important role in the process of the detoxication of heavy metals. The level of this compound in the *Triticale* seedlings depended on the type and concentration of the heavy metal ions (Table 3). Out of the analyzed heavy metals, Cd^{2+} caused the deepest drop of the glutathione level, which was similar in both shoots and roots. This increased as the Cd^{2+} concentration in the plant was higher. It may be connected with the activation by Cd^{2+} of phytochelatine synthetase. That enzyme uses GSH as a substrate in the formation of phytochelatines [18]. It is relatively difficult to explain the observed increase of the GSH level in the shoots of seedlings fed with Pb^{2+}. That phenomenon accompanies the above described growth stimulation of the seedling green organs in the presence of low concentrations of that metal.

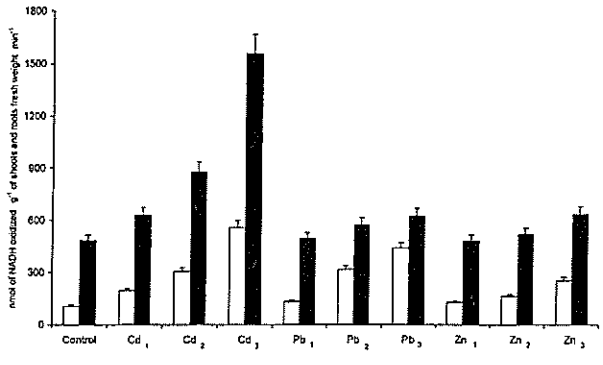


Fig. 3. Changes in the GDH – NADH total activity in shoots (□) and roots (■) of *Triticale* seedlings growing in medium containing three concentrations of Cd^{2+}, Pb^{2+} and Zn^{2+} and without heavy metal ions. Vertical bars represent ± SE from at least 3 independent experiments.

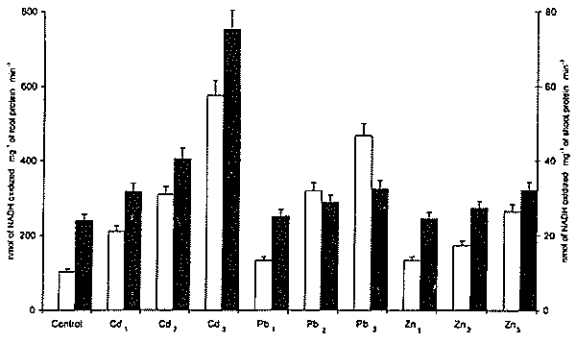


Fig. 4. Changes in the GDH – NADH specific activity in shoots (□) and roots (■) of *Triticale* seedlings growing in medium containing three concentrations of Cd^{2+}, Pb^{2+} and Zn^{2+} and without heavy metal ions. Vertical bars represent ± SE from at least 3 independent experiments.

Table 3. Effect of Cd^{2+}, Pb^{2+} and Zn^{2+} concentration on NH_4^+ and glutathione levels in *Triticale* seedling shoots and roots. (See Table 1). Data represent the mean (± SE) from at least three experiments.

Metal ions in the medium	µg · g^{-1} dry weight			
	NH_4^+		glutathione	
	shoots	roots	shoots	roots
Control (without metal ions)	442 ± 27	1182 ± 46	380 ± 51	237 ± 45
Cd^{2+}_1	605 ± 24	932 ± 33	290 ± 81	97 ± 16
Cd^{2+}_2	401 ± 31	860 ± 43	149 ± 45	89 ± 30
Cd^{2+}_3	325 ± 30	333 ± 18	77 ± 6	83 ± 28
Pb^{2+}_1	401 ± 22	731 ± 40	472 ± 120	251 ± 95
Pb^{2+}_2	440 ± 30	700 ± 36	583 ± 25	126 ± 38
Pb^{2+}_3	492 ± 22	687 ± 35	455 ± 32	116 ± 36
Zn^{2+}_1	432 ± 19	1084 ± 38	306 ± 50	263 ± 80
Zn^{2+}_2	423 ± 23	704 ± 38	309 ± 40	269 ± 67
Zn^{2+}_3	442 ± 30	605 ± 27	304 ± 26	268 ± 58

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