Short Communication

Impact of Cadmium and Zinc on the Growth of White Clover (*Trifolium repens* L.) Shoots and Roots

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Abstract

The aim of our study was to evaluate the impact of different concentrations of cadmium (Cd) and zinc (Zn) on the growth of white clover (*Trifolium repens* L.) shoots and roots in the first stages of its life. An inhibitory effect of all investigated concentrations of Cd on the growth of shoots and roots was ascertained. A significant ($p < 0.05$) inhibitory effect on the growth of shoots and roots was determined at 0.1, 0.3 and 0.5 mM concentrations of Cd. Low (0.025 mM and 0.05 mM) concentrations of the Zn cause growth promotion of shoots and roots. Higher (0.1, 0.3, 0.5 and 1.2 mM) concentrations of Zn led to a significant ($p<0.05$) inhibitory effect on the growth of shoots and roots. Low concentrations (0.025 and 0.05 mM) of Zn enhanced root growth by 1.3% and 6.2%, while higher (0.1, 0.3, 0.5, and 1.2 mM) concentrations of this metal led to root growth inhibition by 27.7%, 60.0%, 77.6%, and 86.3%, respectively. The results of linear regression analysis confirm the inhibitory impact of different concentrations of Cd and Zn on white clover growth, and the inhibition increases with higher concentration.

Keywords: *Trifolium repens*, cadmium, zinc, impact, growth of roots, growth of shoots

Introduction

One of the most dangerous features of heavy metals is their accumulation in living organisms. Even if their concentration in the soil is relatively low, over time it can reach harmful levels in plant tissues. Many heavy metals are trace elements essential for plant life processes; however, a large amount of them can be toxic to plants [1-8].

Cadmium (Cd) is one of the most important heavy metals of concern for food-chain contamination. It is a non-essential heavy metal entering the environment from agricultural, mining and industrial activities, and is also released with the exhaust gases of automobiles. This heavy metal can be accumulated by plants to levels that are toxic to humans and animals [3, 5, 8-10]. Zinc (Zn) is a trace element essential for plant growth, referred to as microelement. Its absence causes growth problems for plants. However, if the concentration of Zn is higher than 100 ppm, it becomes toxic to plants and inhibits their growth [5, 6, 11]. At higher concentrations essential and non-essential for plant growth, heavy metals become highly phytotoxic [1, 5, 6].

Plants have developed complex mechanisms to control the access of heavy metals to their tissue and its accumulation. Thus, the impact of heavy metals on plants should be widely studied [2, 6], because knowledge on plant-metal

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interactions is important to ensure human and environmental safety [3, 12, 13].

Plants are among the most sensitive indicators of environmental pollution by heavy metals [14]. A lot of studies have been carried out and research papers examining the impact of heavy metals on different plant species have been published [3, 5, 7, 9, 11, 15]. There are some papers on the effect of heavy metals and the environmental stress caused by this effect on *Trifolium repens* growth and development [16-19]. However, the impact of different concentrations of cadmium and zinc on the growth of white clover (*Trifolium repens* L.) in the beginning stages of their development has not been studied. Thus, the goal of the present study was to determine the effect of cadmium and zinc on white clover growth at early stages of development.

Experimental Procedures

The experiments were carried out in the laboratory of Biotesting and Environmental Bioindication at the Faculty of Natural Sciences, Vytautas Magnus University, under controlled conditions in 2011-12. The seeds of white clover (*Trifolium repens* L.) were selected for the study of plant sensitivity to the impact of heavy metals cadmium (Cd) and zinc (Zn). This plant species was chosen owing to good germination of seeds and the rather fast growth of seedlings, as well as because the sensitivity of white clover to environmental stress caused by the impact of Cd and Zn remains insufficiently investigated.

The impact of Cd on the growth of *Trifolium repens* has been studied at 0.025, 0.05, 0.1, 0.3, and 0.5 mM concentrations of cadmium hydrosulphate ($CdSO_4 \cdot 8/3H_2O$). The impact of Zn has been studied at 0.025, 0.05, 0.1, 0.3, 0.5, and 1.2 mM concentrations of zinc hydrosulphate ($ZnSO_4 \cdot 7H_2O$).

The seeds were germinated at different concentrations of Cd and Zn in Petri dishes. The control plants were grown in distilled water. Cotton wool was placed in Petri dishes, and filter paper was laid over the wool layer. To each dish 70-80 ml of the prepared solution of heavy metal salts was added. Then 30 healthy-looking seeds of *Trifolium repens* of similar size were evenly spread on each Petri dish.

When the seeds were sown, Petri dishes were covered and placed to germinate for 24 hours in a thermostat with a constant temperature of 23±2ºC. During 24 hours of germination Petri dishes were kept covered in total darkness. After germination of the seeds the dishes were uncovered. After a 96-hour cultivation period, the length of shoots and roots was measured. The experiments were repeated 6 times; the total number of measurements amounted to 5,460.

Statistical analysis and presentation of the obtained data on the growth of shoots and roots was performed and compared with the growth in the control using STATISTICA 6.0 processing software. The dependence of the average length of shoots and roots on the concentrations of investigated heavy metals was assessed using linear multiple regression analysis, expressed by the following equation:

$$y = a + b \cdot x \quad (1)$$

...where: y – dependent variable, x – independent variable, a and b – coefficients.

The statistical significance of the weighted averages of the obtained growth parameters was determined using a t-test [20]. Data on the means of investigating parameters with confidence limits (±SE) are presented in the figures.

Results and Discussion

It was found that the average length of *Trifolium repens* shoots growing in different concentrations of cadmium varies from 2.4±0.6 to 26.5±2.5 mm. The average length of the control shoots is 27.5±3.5 mm. The growth inhibition of white clover shoots was determined even at the lowest (0.025 and 0.05 mM) Cd concentrations (Fig. 1A).

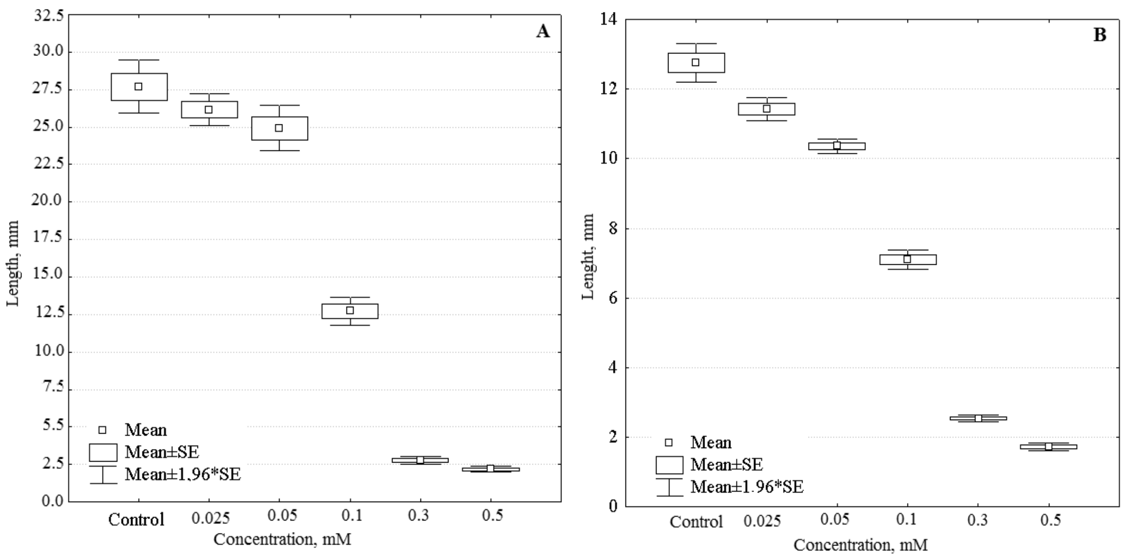

Fig. 1. Mean length of *Trifolium repens* L. shoots (A) and roots (B) grown at different Cd concentrations.

As compared to the control, the growth inhibition of shoots by 2.3% in the case of 0.025 mM concentration of Cd and by 12% at 0.05 mM concentration was determined. However, these effects are not statistically significant. At higher (0.1 and 0.3 mM) concentrations of Cd, inhibition effect was stronger – in both cases a decrease of the growth of shoots was ascertained. When the concentration of Cd was 0.1 mM, the average length of shoots (13.2±1.9 mm) was 2 times, while at 0.3 mM concentration the average length of shoots (2.7±0.9 mm) was even 10 times shorter as compared to the control. A statistically significant ($p < 0.05$) inhibitory effect on the growth of shoots was recorded at 0.1, 0.3, and 0.5 mM concentrations of cadmium; the inhibition effect comprises 54.0%, 91.9%, and 95.8%, respectively, as compared to the control.

Similar results were obtained by Anuradha and Rao [15], who stated that the toxicity of Cd inhibits seed germination by 57% in radish (*Raphanus sativus* L.). Several studies point out that Cd at concentrations from 0.3 mM to 1 mM can cause growth reduction due to alterations in the photosynthetic apparatus of plants [5, 21-23].

As in the case with the growth of *Trifolium repens* shoots, a decrease in the growth of roots at all concentrations of cadmium was recorded (Fig. 1B). The inhibitory effect of Cd on the growth of roots was stronger than on the growth of shoots. Average lengths of roots grown in different concentrations of Cd varied from 1.6±0.2 to 11.5±0.8 mm. The average length of the control roots comprises 12.3±1.3 mm. When compared to the control, an inhibitory effect of 12.5% on the growth of roots even at the lowest (0.025 mM) concentration of Cd was recorded.

In the case of 0.05 mM concentrations of Cd hydrosulphate, an inhibitory effect on the root growth reached 15.8% as compared to the control. In cases of higher concentrations of Cd salts (0.1, 0.3, and 0.5 mM), the inhibitory effect was strong and statistically significant ($p < 0.05$) – decreases in root length by 45.4%, 82.2%, and 85.7%, respectively, were detected in comparison to the control. This result indicates that with increasing Cd concentration, growth of the roots of *Trifolium repens* slows down and their length decreases. This result corroborates the findings of other researchers [24], showing that heavy metals cause the inhibition of root growth. Inhibition of root growth and the reduction of photosynthetic pigment production were also determined by many authors [5, 14, 25, 26], and were often used as parameters for the risk assessment of heavy metals. According to literature [27], even very low Cd concentrations are toxic and reduce plant growth, such as root and shoot length, as well as fresh and dry weight of roots and shoots. The results of many studies have shown that plant growth inhibition and damage to photosynthetic apparatus are common features caused by toxic heavy metal exposure [1, 3, 6, 28].

The investigation of the Zn impact on *Trifolium repens* has indicated that the mean lengths of shoot ranged from 8.4±1.2 to 32.5±3.1 mm. Average lengths of the control shoots comprised 31.6±2.5 mm. The inhibitory effect of Zn on the growth of white clover shoots was considerably lower in comparison with the effect of cadmium (Fig. 1A). Inhibitory effects on shoot growth by 3.7% were determined at 0.1 mM concentration, by 22.3% at 0.3 mM concentration, and by 49.4% at 0.5 mM concentration of zinc (Fig. 2A).

The greatest (by 73.5%) inhibitory effect on the growth of white clover shoots was determined at the concentration of 1.2 mM Zn. In all replications of the experiment the inhibitory effect of Zn on the growth of shoots was statistically significant ($p < 0.05$). Many authors point out that even trace elements essential for plant growth become toxic at higher concentrations [3, 6, 9, 14].

In contrast to the inhibitory effect of low concentration of Cd on the growth of shoots, only a growth promotion was determined at 0.025 and 0.05 mM concentrations of Zn. This may be explained by the fact that Zn is essential for plant growth [4]; therefore, low concentrations of Zn can stimulate growth. Moreover, a similar promotional effect of Zn on lettuce (*Lactuca sativa* L.) and spinach (*Spinacia oleracea* L.) was detected [28].

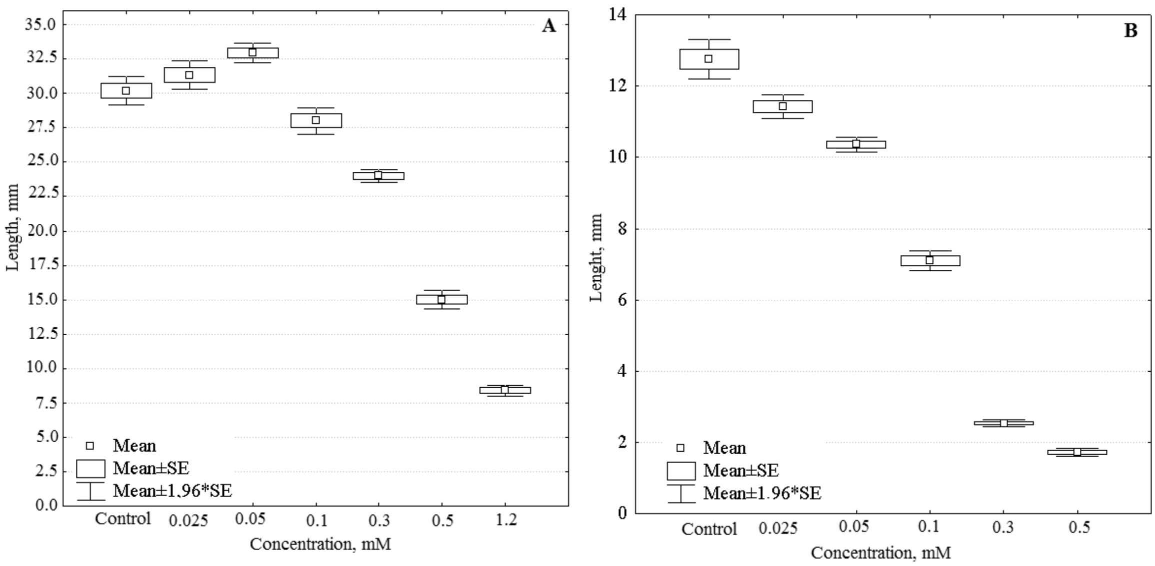

Fig. 2. Mean length of *Trifolium repens* L. shoots (A) and roots (B) grown at different Zn concentrations.

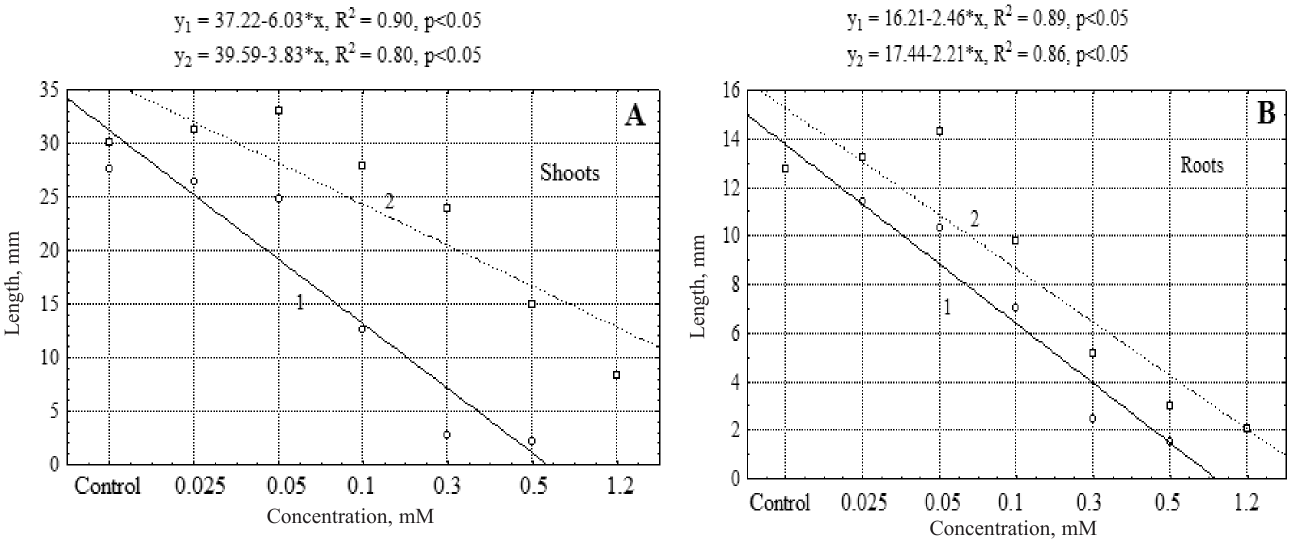

Fig. 3. Dependence of mean length of shoots (A) and roots (B) on different concentrations of Cd (1) and Zn (2); each mean value was estimated from 180 measurements.

Mean lengths of *Trifolium repens* roots after 96 h cultivation at different concentrations of Zn (Fig. 2B) ranged from 1.9±0.3 to 14.2±0.5 mm. Mean lengths of the control roots comprised 13.2±0.2 mm. The effect of Zn on white clover root growth was not as toxic as the effect of Cd (Fig. 1B). The impact of Zn on *Trifolium repens* growth can be even stimulatory. A statistically significant ($p < 0.05$) promotion of root growth by 6.2% at 0.05 mM concentration of Zn salt was determined. This stimulatory effect on root growth could be explained by minimally required plant nutrients, which were obtained from zinc [27].

Higher concentrations of Zn (surplus doses of this element) inhibited the metabolism of plants and caused significant negative effects of Zn on the growth of roots. Many vital functions of plants are disrupted, exceeding allowable limits of trace elements. The growth of white clover roots in the lowest Zn concentrations of 0.025 mM was stimulated by only 1.3%, while the growth promotion effect of white clover root by 6.2% at 0.05 mM concentration of Zn was statistically significant ($p<0.05$), as compared to the control. However, higher (0.1, 0.3, 0.5, and 1.2 mM) concentrations of Zn showed statistically significant inhibitory effects by 27.7%, 60.2%, 77.6%, and 86.3%, respectively. As mentioned by different investigators [1, 28], growth inhibition of plants is caused by the impact of heavy metals, which as micro-elements are required only in traces, but in high concentrations inhibit metabolism and reduce growth of shoots and roots in length. The investigation of Zn's effect on *Mentha spicata* L. revealed that root length of the plant, as compared to the control, decreased by almost half in growth medium with 6 µM zinc [7].

Results of linear regression analysis expressed by multiple regression models (Fig. 3) confirm that in most cases of our experiments the impact of different concentrations of Cd and Zn was inhibitory on white clover growth, and the inhibition effect increases with the increase of concentration. The significant relationships were revealed between average lengths of shoots (Fig. 3A) and different concentrations of Cd and Zn ($R^2 = 0.90$ and $R^2 = 0.80$, respectively; $p<0.05$), as well as between average length of roots (Fig. 3B) and different concentrations of investigated metals ($R^2 = 0.89$ and $R^2 = 0.86$, respectively; $p<0.05$).

Conclusions

An inhibitory effect of all assessed concentrations of Cd on the growth of white clover (*Trifolium repens* L.) shoots and roots was observed. A statistically significant ($p < 0.05$) inhibitory effect on the growth of shoots at 0.1, 0.3 and 0.5 mM concentrations of Cd was determined; the inhibition effect comprises 54.0%, 91.9%, and 95.8%, respectively, as compared to the control.

A promotion effect on the growth ($p < 0.05$) of roots (6.2%) and shoots (4.3%) at 0.05 mM concentration of Zn hydrosulphate was recorded.

In all repetitions of the experiment the inhibitory effects of Zn on the growth of *Trifolium repens* shoots at 0.1, 0.3, 0.5 and 1.2 mM concentrations were statistically significant ($p < 0.05$). The greatest (up to 73.5%) inhibitory effect on the growth of white clover shoots was determined at 1.2 mM Zn.

Results of experiments revealed that white clover (*Trifolium repens* L.) is less sensitive to the environmental stress caused by the impact of heavy metal zinc than by the impact of cadmium.

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