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EFFECT OF CADMIUM NITRATE ON MORPHOLOGICAL PARAMETERS OF *LUPINUS LUTEUS* L. AND *L. ANGUSTIFOLIUS* L.

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Abstract. The plants growing in the natural environment are exposed to the influence of adverse factors which can cause metabolic disturbances, inhibition of growth, damage tissues and organs. The particular threats to plants are heavy metals, especially cadmium which inhibits plant growth and development. The aim of this study was to determine the influence of cadmium nitrate at concentrations of 0.01, 0.05 and 0.1 mM on seed germination, plants length, fresh and dry weight and the water content in *Lupinus luteus* L. and *L. angustifolius* L. The cadmium which is present in soil causes inhibition of seed germination, changes in root and hypocotyl length, value of fresh to dry weight ratio and the water content in plants organs.

Key words: *Lupinus luteus, Lupinus angustifolius*, biometry, cadmium nitrate, fresh and dry weight, germination

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Introduction

Heavy metals, such as cadmium, are the most significant threat to plant organisms. Cadmium present in soil can inhibit plants metabolism (Luo *et al.* 1998; Ociepa-Kubicka & Ociepa 2012). It comes from municipal and industrial wastewater and from waste incineration. The most common forms of cadmium are salts, organic chelates and ion complexes. Cadmium is strongly sorbed by clay materials and organic substances. In this form it accumulates in the upper layers of soil, while in acid soils this element is transported into the deeper layers due to their weaker absorption. In the water, cadmium is present as ion and in the form of inorganic complexes. It gets into the water along with rainfall and through river transport, posing a risk to food chain. There are a lot of possibilities to contaminate the environment by cadmium because it accumulates easily in organism tissues. This metal is highly toxic for animals and humans so there is a need for constant monitoring of the environmental pollution, and especially of the food products (Kabata-Pendias & Pendias 1999).

The aim of this study was to determine the influence of cadmium nitrate at concentrations of 0.01, 0.05 and 0.1 mM on seed germination, changes in the organ morphology, fresh and dry weight ratio and the water content in *Lupinus luteus* L. and *L. angustifolius* L. plants.

Material and methods

In this investigation we used seeds of *Lupinus luteus* and *L. angustifolius* obtained from the National Seed Central – POLAN.

First step of the experiment was aimed at the determination of the energy and power of seed germination. For this purpose, the seeds were washed with tap water and subsequently with distilled water. The seeds were placed on Petri dishes (100 seeds in five replicates) with filter paper soaked with distilled water (control sample) and cadmium nitrate solutions at concentrations: 0.01, 0.05 and 0.1 mM. After that, the samples were incubated in an incubator at the temperature of 25°C. The seedlings were counted every 24 hours for 7 days.

In the second step, 10 most similar seedlings were chosen from each of the control samples and then planted into the pots with sand.

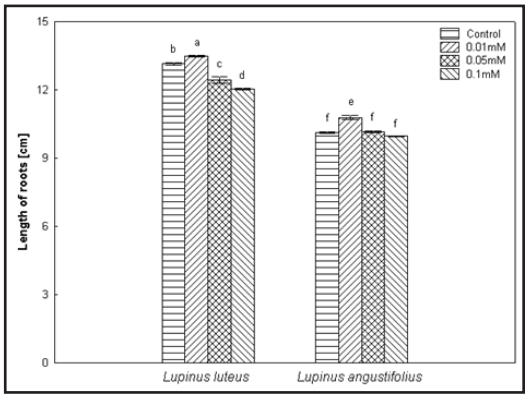

Fig. 1. Length of roots *Lupinus luteus* (**A**) and *L. angustifolius* (**B**).

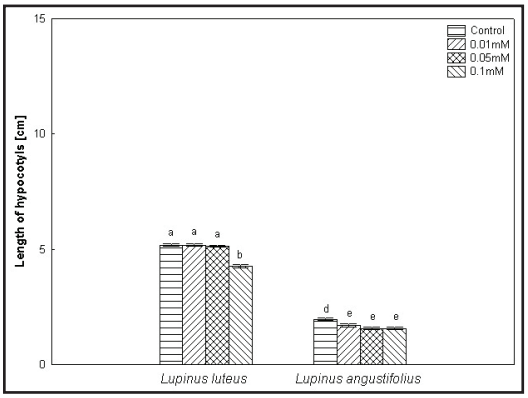

Fig. 2. Length of hypocotyls *Lupinus luteus* (**A**) and *L. angustifolius* (**B**).

The pots were placed in an incubator at the temperature of 25°C during the day and at 20°C during the night, with a light intensity of 250 μmol $m^{-2} \cdot s^{-1}$ and humidity approximately 60%. Plants were irrigated every two days with solutions of cadmium nitrate and with standard medium (Steiner) once a week.

Biometric analysis of root and hypocotyl of *L. luteus* and *L. angustifolius* was performed after 21 days. Moreover, fresh and dry weight as well as water content percentage of underground (root) and aboveground (hypocotyl, cotyledons, petioles, leaves, rest of the stalk) organs were measured.

The statistical analysis was performed using a Duncan's test. The obtained results were the average from 5 independent replicates ±SE. The differences between values within a single row were statistically significant at $p < 0.05$.

Results

The seed germination in the control sample started after 24 hours of incubation and after 6 days 100% of seeds had germinated. The presence of cadmium nitrate in the medium inhibited the germination, especially on the first day. The highest percentage of germinated seeds for *L. angustifolius* was demonstrated in cadmium nitrate solutions at a concentration of 0.1 mM comparing to the control sample. The lowest percentage of seed germination for *L. luteus* was demonstrated in the same concentration comparing to the control sample (Tab. 1). In the case of the root length of yellow and blue lupine plants, growth stimulation was observed in the plants watered with a concentration of 0.01 mM. The other concentrations inhibited root growing of *L luteus*, while in the case of *L. angustifolius* we did not observe any statistical differences (Fig. 1). The biometric measurements of *L. luteus* hypocotyls revealed the highest inhibition at the highest concentration of cadmium nitrate. The other concentrations did not affect the hypocotyl significantly comparing to the control sample. However we observed a significant inhibition of hypocotyl growth of *L. angustifolius* at all tested concentrations (Fig. 2). The fresh weight values were decreasing with increasing concentration of cadmium nitrate for *L. luteus* comparing to the control sample. In the case of *L. angustifolius* the lowest values were observed in the control sample comparing to all concentrations of cadmium nitrate. The growth of hypocotyl of *L. luteus* was inhibited, while the growth of hypocotyl of *L. angustifolius* was stimulated in comparison to the control sample. The fresh weight values of petioles in *L. luteus* were higher at concentration of 0.01 mM than at the other cadmium nitrate concentrations (0.05 and 0.1 mM). The fresh weight values of petioles in *L. angustifolius* were lower than in the control sample. The fresh weight values of leaves in *L. luteus* were significantly lower

Tab. 1. Effect of cadmium nitrate on the percent of germinated seeds of *Lupinus luteus* (**A**) and *L. angustifolius* (**B**).

Time [h]	Control		$Cd(NO_3)_2$ [mM]					
			0.01		0.05		0.1	
	A	B	A	B	A	B	A	B
24	19	12	0	0	0	0	0	0
48	45	54	52	44	50	41	62	70
72	89	88	87	77	88	93	84	92
96	95	95	92	80	88	94	88	94
120	99	99	93	81	89	95	88	94
144	100	100	94	90	90	95	89	95
168	100	100	94	90	90	95	89	95

Tab. 2. Fresh weight [mg] in plant organs of *Lupinus luteus* (**A**) and *L. angustifolius* (**B**) irrigated with cadmium nitrate solution.

Organ	Control		$Cd(NO_3)_2$ [mM]					
			0.01		0.05		0.1	
	A	B	A	B	A	B	A	B
Root	1648.91^{a} ±0.39	487.68^{h} ±2.21	1361.77^{b} ±2.30	715.21^{e} ±0.24	1003.68^{d} ±0.23	633.23^{f} ±0.20	1074.91^{c} ±0.48	559.85^{g} ±0.27
Hypocotyl	206.89^{b} ±0.24	114.23^{h} ±0.11	204.236^{c} ±0.22	141.27^{f} ±0.47	237.266^{a} ±0.42	135.75^{g} ±0.20	201.66^{d} ±0.33	145.11^{e} ±0.17
Petioles	191.66^{f} ±0.25	376.63^{a} ±0.54	194.95^{e} ±0.04	303.09^{d} ±0.57	150.34^{g} ±0.33	305.29^{c} ±0.19	151.93^{g} ±0.27	313.39^{b} ±0.19
Leaves	610.38^{d} ±0.39	630.47^{b} ±1.63	692.81^{a} ±0.31	524.62^{h} ±0.20	586.27^{e} ±1.84	527.41^{g} ±0.29	622.23^{c} ±0.42	530.06^{f} ±0.31
Rest of stalk	319.50^{b} ±0.51	187.08^{e} ±0.08	347.81^{a} ±0.28	147.39^{g} ±0.50	273.75^{d} ±0.22	151.81^{f} ±0.21	315.47^{c} ±0.29	122.83^{h} ±0.15

Tab. 3. Dry weight [mg] in plant organs of *Lupinus luteus* (**A**) and *L. angustifolius* (**B**) irrigated with cadmium nitrate solution.

Organ	Control		$Cd(NO_3)_2$ [mM]					
			0.01		0.05		0.1	
	A	B	A	B	A	B	A	B
Root	220.88^{b} ±0.16	305.06^{a} ±0.67	125.92^{d} ±2.30	200.23^{c} ±0.42	125.92^{f} ±0.32	123.69^{g} ±0.38	149.90^{e} ±0.21	70.84^{h} ±0.16
Hypocotyl	29.12^{a} ±0.13	14.62^{d} ±0.17	29.37^{a} ±0.40	10.27^{f} ±0.22	26.81^{b} ±0.11	11.88^{e} ±0.27	24.99^{c} ±0.03	11.76^{e} ±0.22
Petioles	25.99^{b} ±0.14	68.94^{a} ±0.13	25.12^{c} ±0.06	24.67^{c} ±0.21	17.80^{g} ±0.20	21.71^{e} ±0.28	19.78^{f} ±0.20	23.95^{d} ±0.05
Leaves	104.99^{b} ±0.30	161.432^{a} ±0.38	46.85^{h} ±0.24	60.02^{e} ±0.07	91.09^{d} ±0.40	51.70^{g} ±0.35	101.85^{c} ±0.09	59.70^{f} ±0.37
Rest of stalk	38.85^{b} ±0.51	26.3^{e} ±0.07	42.23^{a} ±0.32	12.06^{f} ±0.05	30.49^{d} ±0.21	11.18^{g} ±0.11	38.13^{c} ±0.09	8.48^{h} ±0.31

Tab. 4. Water content [%] in plant organs of *Lupinus luteus* (**A**) and *L. angustifolius* (**B**) irrigated with cadmium nitrate solution.

Organ	Control		$Cd(NO_3)_2$ [mM]					
			0.01		0.05		0.1	
	A	B	A	B	A	B	A	B
Root	86.60^{b} ±0.008	87.46^{a} ±0.32	86.56^{b} ±0.02	72.00^{e} ±0.06	87.45^{a} ±0.03	80.47^{d} ±0.06	86.50^{c} ±0.02	87.35^{a} ±0.03
Hypocotyl	85.92^{g} ±0.05	87.20^{f} ±0.14	85.62^{g} ±0.18	92.41^{a} ±0.15	88.70^{d} ±0.06	91.25^{c} ±0.20	87.20^{e} ±0.15	91.90^{b} ±0.16
Petioles	86.44^{f} ±0.09	74.39^{h} ±0.07	87.12^{e} ±0.03	91.86^{c} ±0.07	88.16^{d} ±0.13	92.89^{a} ±0.09	86.98^{e} ±0.14	92.36^{b} ±0.01
Leaves	82.80^{g} ±0.05	81.69^{g} ±0.04	93.24^{a} ±0.03	88.53^{d} ±0.02	84.46^{e} ±0.50	90.20^{b} ±0.07	83.63^{f} ±0.20	88.85^{c} ±0.08
Rest of stalk	87.84^{e} ±0.06	85.94^{f} ±0.04	87.86^{e} ±0.09	91.82^{c} ±0.03	88.86^{d} ±0.08	92.64^{b} ±0.08	87.91^{e} ±0.03	93.10^{a} ±0.25

at concentration of 0.05 mM whereas these values in *L. angustifolius* were lower at all tested concentrations comparing to the control sample. The remaining parts of *L. luteus* were growing better at 0.01 mM concentration of cadmium nitrate but they were growing worse at other concentrations. On the other hand we observed the inhibition of the growth of other parts of sprout of *L. angustifolius* at all tested concentrations (Tab. 2). The dry weight values of both *Lupinus* species were decreasing with increase of cadmium nitrate concentration. The *L. luteus* dry weight values were higher at a concentration of 0.01 mM than in the control sample. In general the dry weight values of *L. angustifolius* were higher than values of *L. luteus* except for hypocotyls, in which those values were lower at all tested concentrations (Tab. 3). The water content in both plants ranged from 70 to 90%. These parameters were significantly higher at all tested concentrations comparing to the control sample (Tab. 4).

Discussion

The seed germination and the root growth are the two most critical and most sensitive to pollution plant developmental stages (CHANG *et al.* 1997). The germination stage is the most intense process, when the reserve substances are easily broken down into simple compounds, which are easily absorbed (LEWICKI 2010). According to BARANOWSKA-MOREK (2003) the effective barrier for plants against the penetration of heavy metals during the germination stage is seed integument. DRAB *et al.* (2011) showed that salts with a high concentration of selected metals had the most negative impact on seed germination of *Brassica napus* L. subsp. *oleifera* (Moench) DC. and *Sinapis alba* L. In the present study, we observed an inhibition effect of cadmium compounds on number of germinated seeds (Tab. 1). Cadmium is easily absorbed by the root system and leaves proportionally to its concentration in the environment despite the fact that plants do not need cadmium to grow. The toxic effect of cadmium manifests in chlorosis of leaves, browning and reddening of veins, twisting and premature falling of leaves and the growth inhibition (KABATA-PENDIAS & PENDIAS 1999). WANG & ZHOU (2005) suggested that plants response to heavy metals is associated with their environmental requirements, defense mechanisms, concentration of the solution and the age of the plant. According to KOSYNETS *et al.* (2012) changes in root surface of *Lolium multiflorum* Lam. were associated not only with stressful factor concentration but with the exposure time ad well. Cadmium had different effect on the root and hypocotyl growth of *Lupinus luteus* and *L. angustifolius*. It inhibited the growth of root of *L. luteus* but had no effect on *L. angustifolius* (Fig. 1). In contrast, the hypocotyl growth

inhibition was observed in *L. angustifolius*, while in *L. luteus* the largest differences were observed at a concentration of 0.1 mM, compared to control plants (Fig. 2). Jasiewicz (1993) did not confirm the significant influence of low concentrations of cadmium on onion and radish (from 0.25 to 64.00 $mg \cdot kg^{-1}$ of soil), however high concentrations of this element (about 64 $mg \cdot kg^{-1}$ of soil) caused the inhibition of the growth of root and leaves in radish. Ciećko *et al.* (2000) in turn, observed decreasing in the yield of carrot grown in the soil contaminated with cadmium (25 $mg \cdot kg^{-1}$). Studies on seed germination and root growth of flax, vetch, charlock and pea revealed the high toxicity of cadmium even at the lowest concentration (Baran *et al.* 2008). We observed an inhibition of fresh and dry weight gain in *L. luteus* organs, while in the case of *L. angustifolius* we observed both the inhibitory and stimulating effect of cadmium (Tabs. 2, 3). Moreover, we noticed the increase of water content in tested underground and aboveground organs compared to the control sample (Tab. 4). The plants in response to heavy metals can not only limit metal absorption but also defend the cell metabolism against the toxic influence of metals (Baranowska-Morek 2003). According to Jing *et al.* (2007) the excessive accumulation of heavy metals in plant organs can cause the inhibition of various physiological and biochemical processes, especially photosynthesis and respiration. It can lead to degradation of main cell organelles and finally to death of the plant.

Conclusions

According to obtained results we can conclude, that intensity of morphological changes in plants depends not only on species and variety but also on the concentration of stressful factor, exposure time and resistance of the organism.

References

Baran A., Jasiewicz C., Klimek A. 2008. Reakcja roślin na toksyczną zawartość cynku i kadmu w glebie. *Proceedings of ECOpole* **2 (2)**: 417–422. (In Polish)

Baranowska-Morek A. 2003. Roślinne mechanizmy tolerancji na toksyczne działanie metali ciężkich *Kosmos – Problemy Nauk Biologicznych* **52 (2-3)**: 283–298. (In Polish)

Chang L., Meier J.R., Smith M.K. 1997. Application of plant and earthworm bioassays to evaluate remediation of a lead-contaminated soil. A*rch. Environ. Contam. Toxicol.* **32**: 166–171.

Ciećko Z., Rzoska R., Rolka E., Harnisz M. 2000. Wpływ zanieczyszczenia gleby kadmem na plonowanie i skład chemiczny marchwi. *Zeszyty Problemowe Postępów Nauk Rolniczych* **471**: 889–894. (In Polish)

Drab M., Greinert A., Kostecki J., Grzechnik M. 2011. Seed germination of selected plants underthe influence of heavy metals. *Civil Environ. Eng. Rep.* **7**: 47–57.

Jasiewicz C.Z. 1993. Wpływ wzrastających dawek Cd na plon i zawartość tego metalu w niektórych warzywach. *Acta Agraria et Silvestria. Ser. Agraria* **31**: 64–69. (In Polish)

Jing Y., He Z., Yang X. 2007. Role of soil rhizobacteria in phytoremiediation of heavy metal contaminated soils. *J. Zhejiang Univ. Sci. Ser. B* **8 (3)**: 192–207.

Kabata-Pendias A., Pendias H. 1999. Biogeochemia pierwiastków śladowych: 156–170 Wydawnictwo PWN, Warszawa. (In Polish)

Kosynets O., Szatanik-Kloc A., Szerement J. 2012. Zmiany pozornej powierzchni właściwej korzeni życicy wielokwiatowej (*Lolium multiflorum* L.) determinowane toksycznością kadmu. *Acta Agrophysica* **19 (3)**: 587–595. (In Polish)

Lewicki P. 2010. Kiełki nasion jako źródło cennych składników odżywczych. *ŻYWNOŚĆ. Nauka. Technologia. Jakość* **6 (73)**: 18–33. (In Polish)

Luo L.X., Sun T.H., Jin Y.H. 1998. Accumulation of superoxide radical in wheat leaves under cadmium stress. *Acta Sci. Circum.* **18**: 495–499.

Ociepa-Kubicka A., Ociepa E. 2012. Toksyczne oddziaływanie metali ciężkich na rośliny, zwierzęta i ludzi. *Inżynieria i Ochrona Środowiska* **15 (2)**: 169–180. (In Polish)

Wang X.F., Zhou Q.X. 2005. Ecotoxicological effects of cadmium on three ornamental plants. *Chemosphere* **60**: 16–21.