



Impact of Low Concentration of Cadmium on Photosynthesis and Growth of Pea and Barley

Irena Januškaitienė

Department of Environmental Science, Vytautas Magnus University

(received in July, 2010, accepted in September, 2010)

Photosynthetic gas exchange and growth characteristics were examined in pea and barley plants using 1 mM Cd treatment. Plants were sown into neutral peat substrate and at a leaf development stage were treated with 1 mM cadmium concentration solution. Gas exchange parameters (photosynthetic rate; intercellular CO₂ concentration; transpiration rate; water use efficiency) were measured with portable photosynthesis system LI-6400 on the fifth day after Cd treatment. Under Cd stress the photosynthetic rate of pea and barley plants decreased by 16.7 % ($p < 0.05$) and 12.8 % ($p < 0.05$), respectively, compared to the reference treatments. Photosynthetic response of pea leaves to cadmium stress was slightly different from that of barley. Intercellular CO₂ concentration decreased by 27.4 % ($p < 0.05$) in pea leaves but it increased by 33.5 % ($p < 0.05$) in barley leaves. Cadmium treatment had no statistical significant effect on the transpiration rate of pea plants, while the transpiration rate of Cd treated barley decreased by 23.1 % ($p < 0.05$), compared to the reference treatment. CO₂ reduction processes of Cd treated pea leaves increased (because intercellular CO₂ concentration decreased), but that had no positive effect on a photosynthetic rate, and the photosynthetic rate of pea decreased by 4 % more than that of barley. The changes of dry biomass of cadmium treated plants were weak and statistically insignificant.

Key words: cadmium, photosynthesis, transpiration, water use efficiency, biomass.

1. Introduction

Contamination of heavy metals such as cadmium (Cd) through human activities has increased since the last century. In urban areas or agricultural land with a long history of crop production, the concentrations of trace elements, especially heavy metals, in soil can be higher than those found in parent materials (Senesi et al. 1999). Heavy metal inputs include those from commercial fertilizers, liming materials, and agrochemicals, sewage sludge and other waste used as soil amendments, irrigation waters and atmospheric deposition (Senesi et al. 1999). Highly polluted soils containing over 100 mg kg⁻¹ Cd are reported in China, France and some other countries (Goncalves et al. 2009).

Cadmium, a non-essential element that can be highly phytotoxic at low concentrations, has been ranked No. 7 among the top 20 toxins (Yang et al. 2004). In plants, exposure to Cd causes reductions in biomass production and nutritional quality, and inhibition of photosynthesis, stomatal conductance, transpiration rate (Shi and Cai 2008), saccharide metabolism (Moya et al. 1993), and other metabolic activities (Sharma et al. 1998). Cadmium is easily taken up by plant roots and can be loaded into the xylem for its transport into leaves. A number of studies have demonstrated a toxic effect of Cd on plant metabolism, such as decrease in the uptake of

nutrient elements (Sandalo et al. 2001) and changes in nitrogen metabolism (Shi and Cai 2008).

Cd stress affects photosynthesis in various ways. It inhibits the synthesis of chlorophylls (Chl) (Azevedo et al. 2005, Shukla et al. 2008) and their stable binding to proteins (Horváth et al. 1996), thereby decreasing the accumulation of pigment–lipoprotein complexes, particularly photosystem (PS) I (Wang et al. 2009). The primary targets of Cd toxicity are also PSII and an enzymatic phase of photosynthesis, particularly ribulose-1, 5-bisphosphate carboxylase/oxygenase (Krantev et al. 2008, Popova et al. 2008). However, in other researches no direct Cd effects on the photosystem are found in *Brassica juncea* (Haag-Kerwer et al. 1999) or *Arabidopsis thaliana* (Perfus-Barbeoch et al. 2002). Toxicity of Cd has been related to an increase in lipid peroxidation and alterations in the antioxidant system (Romero-Puertas et al., 2004). Cadmium also produces alterations in functionality of membranes by inducing changes in their lipid composition and this can affect some enzymatic activities associated with membranes such as H⁺-ATPase (Fodor et al. 1995).

Plants have to regulate the protection of their photosynthetic active tissue when exogenous factors or other conditions change in the environment. Stress responses are determined by a stress type and intensity (Ernst 1996) while plant tissue and species are affected (Chen et al. 2007). Differences in stress tolerance among plant species and genotypes within a species may be associated intrinsically with the genetic basis (He et al. 2008). The aim of this work is to study the impact of 1 mM cadmium concentration on photosynthetic gas exchange and growth of pea (*Pisum sativum* L.) and barley (*Hordeum vulgare* L.) plants.

2. Methodology

Pea (*Pisum sativum* L.; cv. ‘Ilgiai’) and barley (*Hordeum vulgare* L. cv. ‘Aura’) plants have been chosen for this study. Experiments were carried out in a vegetation room in the controlled environment: photoperiod – 14 h, average temperature - 20–25 °C, relative humidity – 60 %. “Philips Master Green Power CG T” 600W lamps, with light intensity at the level of plants 14000 Lx, provided the light.

Plants (20 per pot) were sown in a neutral (pH 6.0–6.5) peat substrate in 5-L pots (21 cm in diameter) and at a leaf development stage (Growth stages... 2001) were treated with 1 mM cadmium concentration solution. Reference treatment was watered with distilled water. In each treatment there were three pots of replication. Duration of the experiment was 5 days.

The following indices were determined on the fifth day after Cd treatment: net photosynthetic rate (Pn), intercellular CO₂ concentration (Ci), transpiration rate (Tn), water use efficiency (WUE) and dry over-ground biomass.

Gas exchange was measured with portable photosynthesis system LI-6400 (LI-COR, USA). Net photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), intercellular CO₂ concentration ($\mu\text{mol CO}_2 \text{ mol air}^{-1}$) and water use efficiency ($\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$) of a second pair of fully expanded leaves of pea and second fully expanded leaves of barley were registered every 30 seconds for 40 minutes; from these data there were calculated a mean of day of measured indices. Environment conditions during experiments were: air flow rate – 400 $\mu\text{mol s}^{-1}$; block and leaf temperature – 25 °C; CO₂ concentration in a sample cell – 300–400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$; relative humidity in a sample cell – 30 %; lightness in quant – 180 $\mu\text{mol m}^{-2} \text{ s}^{-1}$.

The samples were dried in an oven at 60°C until constant dry over-ground biomass was obtained. The over-ground biomass was expressed in mg plant⁻¹.

To compare independent variables Student’s t and U tests were used. All analyses were performed by STATISTICA and the results were expressed as the mean values and their confidence intervals (CI) ($p < 0.05$).

3. Research results

It was found that Cd toxicity caused a notable reduction of a photosynthetic rate in different plant species (Krantev et al. 2008). In this study there has been also observed that a significant decrease in a photosynthetic rate (Pn) occurred in pea and barley treated with 1 mM cadmium (Fig. 1, A). Cadmium exposure resulted in 16.7 % and 12.8 % decrease in a photosynthetic rate of pea and barley plants, respectively, compared to reference treatments. Regarding Cd toxicity to PSII activities in plants, some researchers have suggested that Cd binds in the sites of both the acceptor and the donor sides of PSII (Sigfridsson et al. 2004). On the donor side, the presence of Cd²⁺ exchanges, with high affinity in a slow reaction, the Ca²⁺ cofactor in the Ca/Mn cluster that constitutes the oxygen-evolving centre (Sigfridsson et al. 2004, Faller et al. 2005), which results in inhibition of photosynthetic oxygen evolution. Cd also inhibits electron transfer from redox-active tyrosine residues D1-161 (Sigfridsson et al. 2004, Wang et al. 2009).

Whereas in other researches no direct Cd effects on the photosystem are found in either *Brassica juncea* (Haag-Kerwer et al. 1999) or *Arabidopsis thaliana* (Perfus-Barbeoch et al. 2002). Toxicity of Cd has been related to an increase in lipid peroxidation and alterations in the antioxidant system (Romero-Puertas et al. 2004). A photosynthetic response of pea leaves to cadmium stress was also slightly different from that of barley.

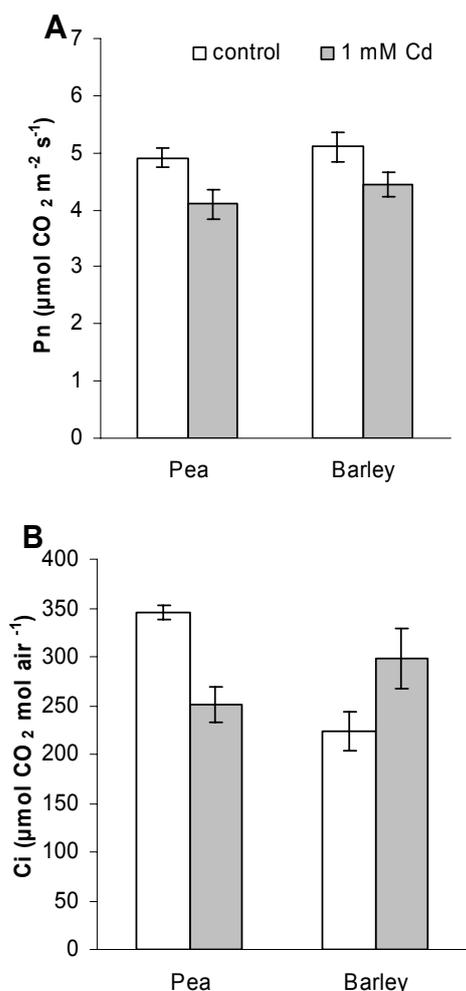


Fig.1. Changes in net photosynthetic rate, Pn (A) and intercellular CO_2 concentration, Ci (B) in pea and barley plants treated with 1 mM cadmium. Values are the means of $\pm CI_{0.05}$.

Intercellular CO_2 concentration (Ci) of peas decreased by 27.4 % under 1 mM cadmium impact (Fig. 1, B), on the contrary, Ci of barley increased by 33.5 % under the mentioned impact, compared to the reference treatment. An increase in an intercellular CO_2 level (Ci) indicated that Cd reduced net photosynthesis (Pn) by reducing CO_2 fixation by Rubisco (Wahid et al. 2007). A high intercellular CO_2 concentration decrease shows that despite the decrease in the photosynthetic rate by 17 % the reduction processes of CO_2 in pea leaves were very intensive. Ci and Pn reduction also could cause the closure of stomata (Musyimi et al. 2007; Wang et al. 2009), but as it is presented in Fig. 2 (A) the transpiration rate (Tn) of peas treated with 1 mM cadmium increased even by 13.3 % ($p > 0.05$). The reason of inhibition of the photosynthetic rate of peas is not the closure of stomata. On the contrary, 1 mM cadmium exposure reduced a transpiration rate of barley by 23.1 % compared to the reference treatment (Fig. 2, A).

Cadmium stress also produced disturbances in water balance and thus reduction of water use efficiency (WUE) was observed with Cd treatments.

This might be due to the inhibition of absorption and translocation of water, as previously observed by Barcelo and Poschenrieder (1990) and Singh et al. (2008). Under an impact of 1 mM cadmium WUE of peas decreased by 26.5 % ($p < 0.05$) (Fig. 2, B), while that change had also affected photosynthetic rate losses. Meanwhile, 1 mM cadmium exposure had a different impact on WUE of barley, i.e. WUE of those plants increased by 14.7 % ($p < 0.05$). An increase in WUE of barley was caused by a higher decrease in the transpiration rate compared to the photosynthetic rate of those plants (Musyimi et al. 2007).

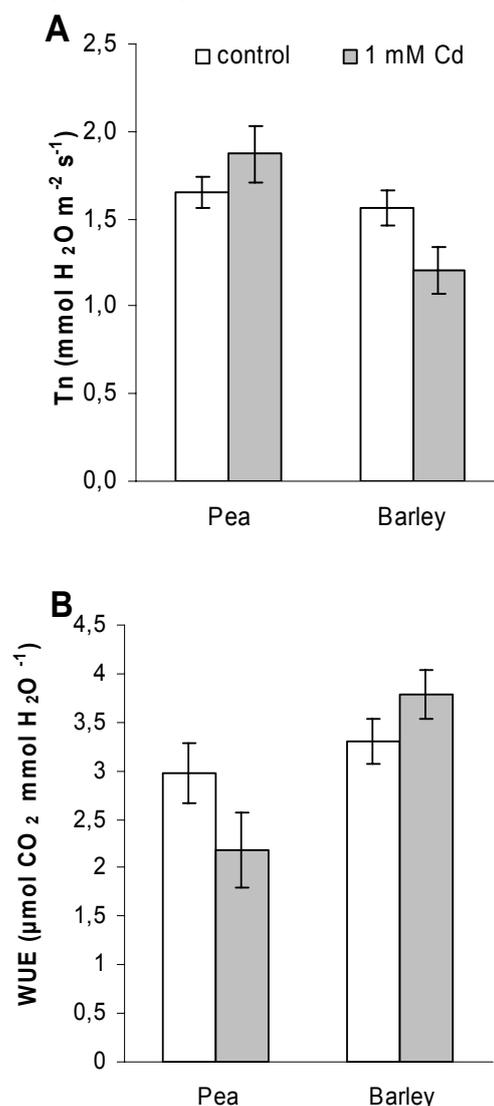


Fig. 2. Changes in transpiration rate, Tn (A) and water use efficiency, WUE (B) in pea and barley plants treated with 1 mM cadmium. Values are the means of $\pm CI_{0.05}$.

Heavy metals disturb the uptake and distribution of essential nutrients including the uptake and transport of Fe to leaves (Krupa et al. 2002). Consequently, contact of plants with Cd leads to reduction in the total photosynthetic area and plant biomass (Lopez-Millan et al., 2009). However, there are studies about a stimulating effect of low concentrations of cadmium on the plant root system, biomass accumulation and leaf area (Ivanov et al.

2001, Wu and Zhang 2002; Wu et al. 2003). Peas treated with 1 mM Cd have shown a slight ($p > 0.05$) increase in dry biomass accumulation (Fig. 3), supporting the findings of other researches that there is some potentially positive impact of cadmium on the

plant growth at its lower concentration (Wu and Zhang 2002, Wu et al. 2003). The same exposure of dry over-ground biomass of barley has decreased by 5 % compared to the reference treatment, but it is statistically insignificant.

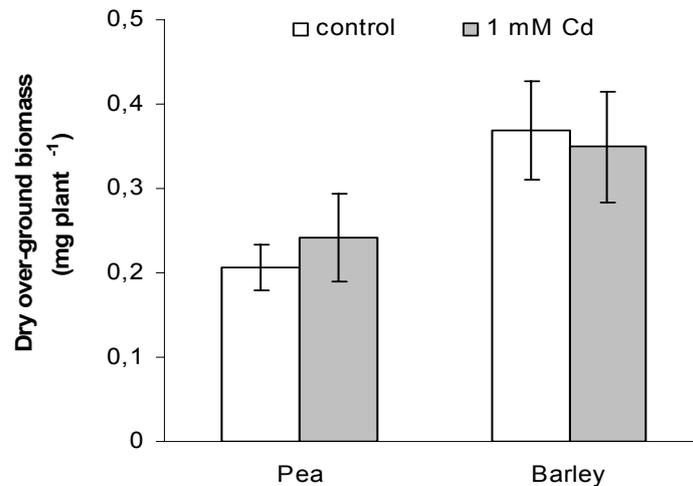


Fig. 3. Changes in dry over-ground biomass of pea and barley plants treated with 1 mM cadmium. Values are the means of $\pm CI_{0.05}$.

4. Conclusions

Cadmium stress reduced the photosynthetic rate of pea and barley plants by 16.7 % ($p < 0.05$) and 12.8 % ($p < 0.05$), respectively, compared to the reference treatments. A photosynthetic response of pea leaves to cadmium stress was slightly different from that of barley.

CO₂ reduction processes of Cd treated pea leaves increased because intercellular CO₂ concentration decreased by 27.4 % ($p < 0.05$), but that had no positive effect on a photosynthetic rate. Whereas cadmium stress produced disturbances in water balance and thus reduction of water use efficiency arose by 26.5 % ($p < 0.05$).

Increase in intercellular CO₂ concentration of barley by 33.5 % ($p < 0.05$) suggests that enzymatic dark reaction of photosynthesis was strongly affected. But the parallel change in photosynthetic and transpiration rates of barley indicated that the photosynthetic response to Cd stress could also be attributed to the alteration of a photosystem and a transpiration rate.

The changes in dry biomass of cadmium treated plants were weak and statistically insignificant.

References

- Azevedo, H., Pinto, C. G. G., Fernandes, J., Loureiro, S., Santos, C. Cadmium effects on sunflower growth and photosynthesis. *Journal of Plant Nutrition*, 2005, Vol. 28, pp. 2211-2220.
- Barcelo, J., Poschenrieder, C. Plant water relation as affected by heavy metal stress: a review. *Journal of Plant Nutrition*, 1990, Vol.13, pp. 1-17.
- Chen, J., Zhu, C., Lin, D., Sun, Z.X. The effects of Cd on lipid peroxidation, hydrogen peroxide content and

antioxidant enzyme activities in Cd-sensitive mutant rice seedlings. *Canadian Journal of Plant Science*, 2007, Vol. 87, pp. 49-57.

Ernst, W.H.O. Bioavailability of heavy metals and decontamination of soils by plants. *Applied Geochemistry*, 1996, Vol. 11, pp. 163-167.

Faller, P., Kienzler, K., Krieger-Liszkay, A. Mechanism of Cd²⁺ toxicity: Cd²⁺ inhibits photoactivation of Photosystem II by competitive binding to the essential Ca²⁺ site. *Biochim. Biophys. Acta-Bioenergetics*, 2005, Vol. 1706, pp. 158-164.

Fodor, A., Szabo-Nagy, A., Erdei, L. The effects of cadmium on the fluidity and H-ATPase activity of plasma membrane from sunflower and wheat roots. *Journal Plant Physiology*. 1995, Vol. 14, pp. 787-792.

Goncalves, J.F., Tabaldi, L.A., Cargnelutti, D., Pereira, L.B., Maldaner, J., Becker, A.G., Rossato, V., Rauber, R., Bagatini, M.D., Bisognin, D.A., Schetinger, M.R.C. Nicoloso, F.T., Cadmium-induced oxidative stress in two potato cultivars. *Biometals*, 2009, Vol. 22, pp. 779-792.

Growth stages of mono- and dicotyledonous plants. 2001. In: U. Meier (Ed.), *BBCB monograph*, German federal Biological Research Centre for Agriculture and Forests, pp. 10-11.

Haag-Kerwer, A., Schafe, H.J., Heiss, S., Walter, C.T. Rausch Cadmium exposure in *Brassica juncea* causes a decline in transpiration rate and leaf expansion without effect on photosynthesis. *Journal of Experimental Botany*, 1999, Vol. 341, pp. 1827-1835.

He, J.Y., Ren, Y.F., Zhu, C., Yan, Y.P., Jiang, D.A. Effect of Cd on growth, photosynthetic gas exchange, and chlorophyll fluorescence of wild and Cd-sensitive mutant rice. *Photosynthetica*, 2008, Vol. 46, Iss. 3, pp. 466-470.

Horváth, G., Droppa M., Oravec, A., Raskin, V.I., Marder, J.B. Formation of the photosynthetic apparatus during greening of cadmium-poisoned barley leaves. *Planta*, 1996, Vol. 199, pp. 238-244.

Ivanov, V.B., Bystrova, E.I., Seregin, I.V. 2001. Selectivity and specificity of heavy metal toxic effect on root growth. *Plant under Environmental stress*. International symposium. Moscow. pp. 104-105.

- Krantev, A., Yordanova, R., Janda, T., Szalai, G., Popova, L. Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. *Journal Plant Physiology*, 2008, Vol. 165, pp. 920–931.
- Krupa, Z., Siedlecka, A., Skórzynska-Polit, E., Maksymiec, W. 2002. Heavy metal influence on the light phase of photosynthesis. In: Prasad, M.N.V., Strzałka, K., (Ed.), *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*, Kluwer Academic Publishers, Dordrecht, pp. 287–303.
- Lopez-Millan, A.F., Sagardoy, R., Solanas, M., Abadia, A., Abadia, J. Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in Hydroponics. *Environmental and Experimental Botany*, 2009, Vol. 65, pp. 376–385.
- Moya, J.L., Ros, R., Picazo, I. Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. *Photosynth. Res.*, 1993, Vol. 36, pp. 75–80.
- Musyimi, D.M., Netondo, G.W. Ouma, G. Effects of salinity on growth and photosynthesis of avocado seedlings. *International journal of Botany*, 2007, Vol. 3. Iss. 1, pp. 78–84.
- Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A., Forestier, C. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. *Plant Journal*. 2002, Vol. 32, pp. 539–548.
- Popova, L. P., Maslenkova, L.T., Yordanova, R.Y., Ivanova, A.P., Krantev, A.P., Szalai, G., Janda, T. Exogenous treatment with salicylic acid attenuates cadmium toxicity in pea seedlings. *Plant Physiology and Biochemistry*, 2008, Vol. 47. Iss. 3, pp. 224–231.
- Romero-Puertas, M.C., Rodríguez-Serrano, M., Corpas, F.J., Gómez, M., del Río, L.A., Sandalio, L.M. Cadmium-induced subcellular accumulation of O₂^{•-} and H₂O₂ in pea leaves. *Plant Cell Environ.*, 2004, Vol. 27, pp. 1122–1134.
- Sandalio, L.M., Dalurzo, H.C., Gomes, M., Romero-Puertas, M., del Río, L.A. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *Journal of Experimental Botany*, 2001, Vol. 52, pp. 2115–2126.
- Senesi, G.S., Baldassarre, G., Senesi, N. Trace element inputs into soils by anthropogenic activities and implications for human health. *Chemosphere*, 1999, Vol. 39, pp. 343–377.
- Sharma, S.S., Schat, H., Vooijs, R. In vitro alleviation of heavy metal-induced enzyme inhibition by proline. *Phytochemistry*, 1998, Vol. 49, pp. 1531–1535.
- Shi, G.R., Cai, Q.S. Photosynthetic and anatomic responses of peanut leaves to cadmium stress. *Photosynthetica*, 2008, Vol. 46. Iss. 4, pp. 627–630.
- Shukla, U.C., Murthy, R.C., Kakkar, P. Combined effect of ultraviolet-B radiation and cadmium contamination on nutrient uptake and photosynthetic pigments in *Brassica campestris* L. seedlings. *Environ. Toxicol.*, 2008, Vol. 23, pp. 712–719.
- Sigfridsson, K.G.V., Bernát, G., Mamedov, F., Styring, S. Molecular interference of Cd²⁺ with Photosystem II. *Biochim. Biophys. Acta.*, 2004, Vol. 1659, pp. 19–31.
- Singh, S., Nafees, A.K., Rahat, N., Naser, A.A. Photosynthetic Traits and activities of antioxidant enzymes in blackgram (*Vigna mungo* L. Hepper) under cadmium stress. *American Journal of Plant Physiology*, 2008, Vol. 3, Iss. 1, pp. 25–32.
- Wahid, A., Ghani, A., Ali, I., Ashraf, M.Y., Effects of cadmium on carbon and nitrogen assimilation in shoots of mungbean [*Vigna radiata* (L.) Wilczek] seedlings. *Journal Agronomy & Crop Science*, 2007, Vol. 193, pp. 357–365.
- Wang, H., Zhao, S.C., Liu, R.L., Zhou, W., Jin, J.Y. Changes of photosynthetic activities of maize (*Zea mays* L.) seedlings in response to cadmium stress. *Photosynthetica*, 2009, Vol. 47, Iss. 2, pp. 277–283.
- Wu, F.B., Zhang, G.P. Genotypic differences in effect of Cd on growth and mineral concentrations in barley seedling. *Bulleten of Environmental Contamination and Toxicology*, 2002, Vol. 69, pp. 219–227.
- Wu, F., Zhang, G., Dominy, P. Four barely genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. *Environmental and Experimental Botany*, 2003, Vol. 50, Iss. 1, pp. 67–78.
- Yang, X.E., Long, X.X., Ye, H.B., He, Z.L., Calvert, D.V., Stoffella, P.J. Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (*Sedum alfredii* Hance). *Plant Soil*, 2004, Vol. 259, pp. 181–189.

Dr. Irena Januškaitienė, Assoc. Prof. and Researcher at the Department of Environmental Science, Vytautas Magnus University, Lithuania.
Main research areas: single and integrated impact of natural and anthropogenic factors on vegetation.
Address: Vileikos 8 - 125,
LT-44404 Kaunas,
Lithuania
Tel./fax.: +370 7 32 79 04
E-mail: I.Januskaitiene@gmf.vdu.lt

Silpnos kadmio koncentracijos poveikis žirnių ir miežių fotosintezei ir augimui

Irena Januškaitienė

Aplinkotyros katedra, Vytauto Didžiojo universitetas

(gauta 2010 m. liepos mėn.; atiduota spaudai 2010 m. rugsėjo mėn.)

Tyrimo tikslas – ištirti 1 mM Cd koncentracijos poveikį žirnių ir miežių fotosintezėje dalyvaujančių dujų ir augimo parametrų pokyčiams. Augalai buvo sėjami į vegetacinius indus su paruoštu neutralaus rūgštumo durpių substratu, o jiems pasiekus lapų vystymosi tarpsnį buvo palaistyti tirtos koncentracijos kadmio tirpalu. Praėjus 5 dienoms po poveikio, naudojant nešiojamą fotosintezės sistemą LI-6400, augalų lapų paviršiuje buvo matuojami dujų santykių pokyčiai: fotosintezės intensyvumas, viduląstelinis CO₂ kiekis, transpiracijos intensyvumas ir vandens naudojimo efektyvumas. Kadmio sumažino žirnių ir miežių fotosintezės intensyvumą atitinkamai 16,7 % ir 12,8 %, palyginti su kontroliniais augalais. Fotosintetinis žirnių atsakas į Cd keliamą stresą šiek tiek skyrėsi nuo miežių: viduląstelinis CO₂ kiekis žirnių lapuose sumažėjo 27,4 % ($p < 0,05$), o miežių – padidėjo 33,5 % ($p < 0,05$). Taip pat kadmio nedarė statistiškai reikšmingos įtakos ir žirnių transpiracijos intensyvumui, o miežių transpiracijos intensyvumas sumažėjo 23,1 % ($p < 0,05$), palyginti su neveiktais augalais. Paveiktų kadmio žirnių lapuose CO₂ redukcijos procesai suintensyvėjo (kadangi viduląstelinis CO₂ kiekis sumažėjo), palyginti su kontroliniais augalais, bet tai nedarė teigiamos įtakos fotosintezės intensyvumui, kuris buvo net 4 % mažesnis nei miežių. Kadmio paveiktų tirtų augalų sausos biomasės pokyčiai buvo maži ir statistiškai nereikšmingi.

DOI: 10.5755/j01.erep.53.3.85