

Activity of the Photosynthetic Apparatus in *Phaseolus vulgaris* L. Leaves Under the Cadmium Stress

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Abstract

The development of civilisation, urbanisation and industrialisation cause to ever increasing contamination of the natural environment. Cadmium belongs to this group of chemical elements, which represent a serious threat, not only for the proper development of plants but also for the health of humans and animals. Hence, the interest of scientists in the toxic effects of this kind chemical elements on the various life functions of organisms. The aim of this study was to investigate the effect of cadmium nitrate aqueous solutions on photosynthetic activity and degree of disorganisation of cell membranes in leaves of common bean (*Phaseolus vulgaris* L. cv. 'Laurina'). A fully formed second leaves were wetted with cadmium solutions nitrate ($\text{Cd}(\text{NO}_3)_2$) at concentrations of 5 and 10 mM. With the increasing of cadmium concentration and time of its application (20, 40, 60 min and 24 h), the changes in the fluorescence of bean leaves were observed. Under the influence of cadmium ions, damage occurred both on the edge and the central part of the leaves, and then the whole surface. The highest changes of the chlorophyll fluorescence values were observed after 24 h of application the aqueous cadmium solutions. Use of SPAD chlorophyll Meter, the reduction of chlorophyll content was noticed over time. The flow of electrolytes from the bean leaves was increased with the time and concentration of applied cadmium compounds.

Keywords: chlorophyll; common bean; electrolyte leakage; environmental stress; PSII

Introduction

Heavy metals are polluting of environment and their phytotoxicity affects changes of metabolic processes, anatomy and morphology of plants. In literature are known results of research concerning, among others, accumulation of heavy metals in the soil, in the water, in the transport to the individual organs, and influence on the functioning of the whole plant organism (Aziz *et al.*, 2015). Heavy metals ion uptakes by plants are dependent on many environmental factors. These include soil pH, temperature,

oxygen availability, and presence of other chemical elements in the soil (Solymosi and Bert, 2012). Unfortunately, the development of civilisation contributes to the fact that year by year the heavy metals concentration increase in the soil, in the water, also in the air, which are harmful not only to plants but also to all living organisms (Możdziej *et al.*, 2014).

The cadmium is one of the most toxic heavy metal occurs in the environment (Nazar *et al.*, 2012; Clemens *et al.*, 2013). This chemical element is released in the metallurgical industry, waste incineration plants, urban traffic, cement mill, mining, phosphate fertilizers and others (Khan *et al.*, 2015). In atmospheric air, the cadmium is occurred as cadmium oxide (CdO), which is easily soluble in water, which determines its mobility and bioavailability

in the environment (Ostrowska, 2008). Cadmium has similar physical and chemical properties to iron (Fe), copper (Cu) and zinc (Zn) cations. It is bound by the components of cell walls in the root system (Khan *et al.*, 2015). Excessive quantity of cadmium in plants cause, among others, growth of cellular respiration, transpiration and metabolism of nitrogen compounds, enzyme activity, and changes in permeability of cell membranes and DNA structure (Xue *et al.*, 2013).

The response of different plants on stress factors depends on the organism resistance, duration of action and type of stress. The potential of cadmium in phytoremediation of the other heavy metals is underlining in literature. There are a lot of researches on plant growth under conditions of cadmium stress (da Silva *et al.*, 2012; Zhang *et al.*, 2015). In highly industrialised regions, the high cadmium accumulation is noted both roots and leaves vegetables (Czeczot and Skrzycki, 2010). However, the information about effect of cadmium on plant physiology is not fully documented yet and further detailed investigations are needed.

Commonly cultivated in temperate climates, the common bean (*Phaseolus vulgaris* L.) is a food crop mainly in the form of seeds, which contain large amounts of protein, phytin acid, potassium, phosphorus, and iron compounds. It has a diuretic effect, lowers blood sugar and cholesterol, inhibits the development of colon cancer and has antioxidant properties. Therefore, in a many variants it is a very valuable useful plant. Depending on the variety, it is characterised by different resistance to diseases and pests, but generally quite easy to cultivation (Szafirowska and Kaniszewski, 2014).

The aim of the study was to early monitoring the physiological state of common bean leaves (*Phaseolus vulgaris* L.), which were wetted of cadmium nitrate solution ($\text{Cd}(\text{NO}_3)_2$) at 5 and 10 mM concentrations. It was realised on base of measurement of the chlorophyll *a* fluorescence parameters, chlorophyll content and term the level of disorganisation of cell membranes by determined of the electrolyte leakage.

Materials and Methods

Plant material

After 72 h germinated on distilled water common bean (*Phaseolus vulgaris* L. cv. 'Laurina') seeds were planted in pots with rinsed river sand. The seedlings thus obtained were grown under greenhouse conditions for 30 days. Once a week they were watered with Steiner's standard medium and every other day with distilled water. The aqueous solutions of cadmium nitrate ($\text{Cd}(\text{NO}_3)_2$) (Chempur, Poland) were prepared in two molar concentrations of 5 and 10 mM. The control group was leaves of plants, were leaves of plants watered with distilled water and Steiner's medium, and untreated of cadmium impact.

Measurement of chlorophyll *a* fluorescence, chlorophyll

Chlorophyll fluorescence measurements were performed on 10 different, fully developed second from the top, bean leaves. During the study, the upper surfaces of leaf bean were wetted with aqueous solutions of 5 and 10 mM

cadmium nitrate and immediately analysed. Leaves of bean wetted in a suitable cadmium nitrate solutions were decomposed on the filter paper with distilled water and adapted in the dark by 20 min in a closed FluorCam FC 800C (Photon Systems Instruments, Czech Republic). According to method used by Synowiec *et al.* (2015), on the same leaf 4 measurements were made: 20 min after application of the cadmium solutions, after 40 min, followed after 60 min and 24 h. Among the results obtained, the following parameters were analysed: the zero fluorescence (F_0), the maximum fluorescence (F_m), the stationary fluorescence (F_s), the maximum efficiency of the photochemical PSII (F_v/F_m), the non-photochemical quenching (NPQ) and the vitality of PSII (Rfd). The chlorophyll content was measured after 20, 40, 60 min and 24 h with the use of SPAD-502 Plus chlorophyll Meter (Konica Minolta, Japan).

Electrolyte leakage

Leaf samples, placed in vials containing 10 ml of deionised water, were incubated on a shaker for 3 h at 25 °C to determine the initial electrolyte conductivity (E1). Then they were frozen at -80 °C. After thawing, the samples were shaken for 3 h to measure the final conductivity (E2). The percentage of electrolyte leakage (EL) was calculated according to the formula: $EL = (E1 / E2) \times 100$.

Statistical analysis

In results include the arithmetic average of five independent replicates ($n = 5$) for each cadmium solution concentration and control group (distilled water). Besides average values, the standard deviations (\pm SD) were also given. Statistical analysis was performed using non-parametric Kruskal-Wallis test after the Levene's test of variance homogeneity, wherein $\alpha = 0.05$. The software *Statistica 10.0* for *Windows* was applied to perform calculations.

Results and Discussion

Heavy metals cause changes in the metabolic processes of plants, inducing a number of different biochemical processes in the cells. They affect changes in cytoplasm pH, electrostatic potential of cell membranes and ion flow. They contribute to changes in metabolic and transport processes leading to the loss of cellular homeostasis and even to the death of plants (Oves *et al.*, 2016). Cadmium, through high mobility, changes the physiology and morphology of the aboveground organs of plants (Chen *et al.*, 2011). High concentration of cadmium inhibits the access of iron, manganese and zinc, which function as enzyme cofactors, pigments and as structural members of the photosynthetic apparatus (Ahammed *et al.*, 2012). This chemical element affects the amount of electrons in the active centre of the photosynthesis reaction. It inhibits the transport of electrons in the photosynthetic reaction chain by changing the conditions of absorption, conversion and dissipation of heat energy. It leads to the formation of excessive reactive forms of oxygen, affects the function and biological activity of macromolecules, among others proteins and nucleic acids (Emamverdian *et al.*, 2015; Oves *et al.*, 2016). Symptoms of

toxic cadmium include: chlorotic (yellow) and necrotic (brown) spots, reddening of the veins, twists and necrosis on the leaves (Czeczot and Skrzycki, 2010; Xue *et al.*, 2013).

In recent years, an instrument useful for early monitoring of plant metabolism changes is FluorCam. This fluorimeter allows to accurately characterise the optically invisible changes that occur in the photosynthetic apparatus. Chlorophyll fluorescence is a factor that characterises the photosynthetic activity of plants. Reflects the basic reactions of light energy absorption, as a result of stress factors effect on plants. It illustrates the relationship between photosynthesis and the environment (Baker and Rosenqvist, 2004).

In the experiment, when the cadmium nitrate concentration increased and its application time, changes in bean leaves fluorescence were observed (Figs. 1-3). Under the influence of cadmium ions, damage occurred both on the periphery and the central part of the leaves, and then on the whole of their surface. Probably, changes in the values of the fluorescence parameters were caused by disorganisation and PSII damage (Baszyński, 2014). Along with the time of cadmium exposure, the zero fluorescence (F_0) values increased on the surface of the whole leaf bean regardless of the cadmium solution concentration (Fig. 1). After 20 and 40 min of application the highest values of F_0 were recorded and after 24 h those values, mainly at the edge of the leaves were reduced. The high values of this parameter are

indicative of a lower energy transfer capacity between pigment molecules to the active PSII centre. They indicate on damage of thylakoids and the dissociation of LHCII, which in leads to PSII inactivation (Hansi *et al.*, 2015).

Compared to the control, after 40 min of application, the maximum fluorescence (F_m) values were higher at 5 mM concentration of cadmium nitrate on all surface of the bean leaves. With the increase in cadmium solution application time – after 60 min and after 24 h, a decrease in the value of this parameter was observed. For 10 mM cadmium nitrate concentration, F_m values were higher after 20 min of application, compared to the control. At subsequent of time intervals, after 40 and 60 min and after 24 h, values of F_m were decreased, especially in innervation of leaves (Fig. 1). Changes in the values of this parameter indicate that not all electron acceptors in PSII are completely reduced, which signals the stress response in the photosynthetic apparatus. Maximum fluorescence yields at high values are quickly lowered to stationary fluorescence (F_t) values that are close to zero fluorescence (F_0).

The F_t values depend on the physiological state of the plant and the efficiency of its photosynthesis (Li *et al.*, 2015). In conducted studies, only after 20 min an increase of F_t values were observed, and along with the prolonged application time of cadmium compounds in bean leaves decrease of values of this parameter was noticed (Fig. 2).

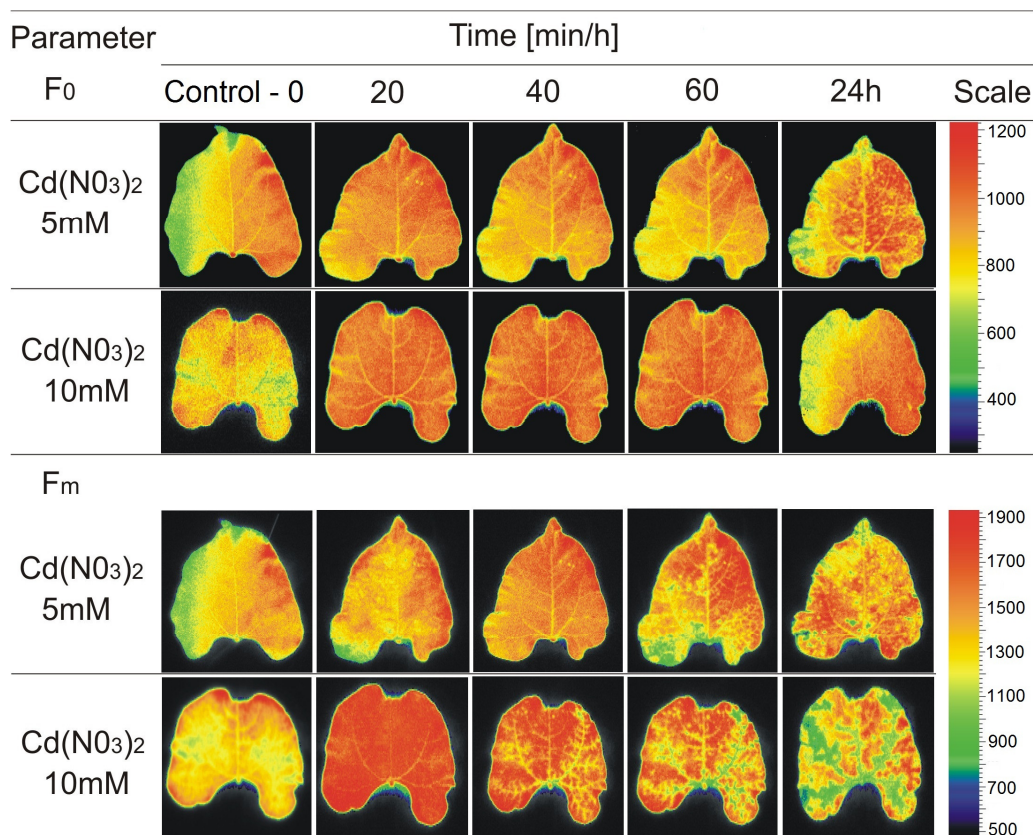


Fig. 1. Imaging chlorophyll *a* fluorescence in the common bean (*Phaseolus vulgaris* L.) leaves in cadmium nitrate stress; F_0 – the zero fluorescence, F_m – the maximum fluorescence

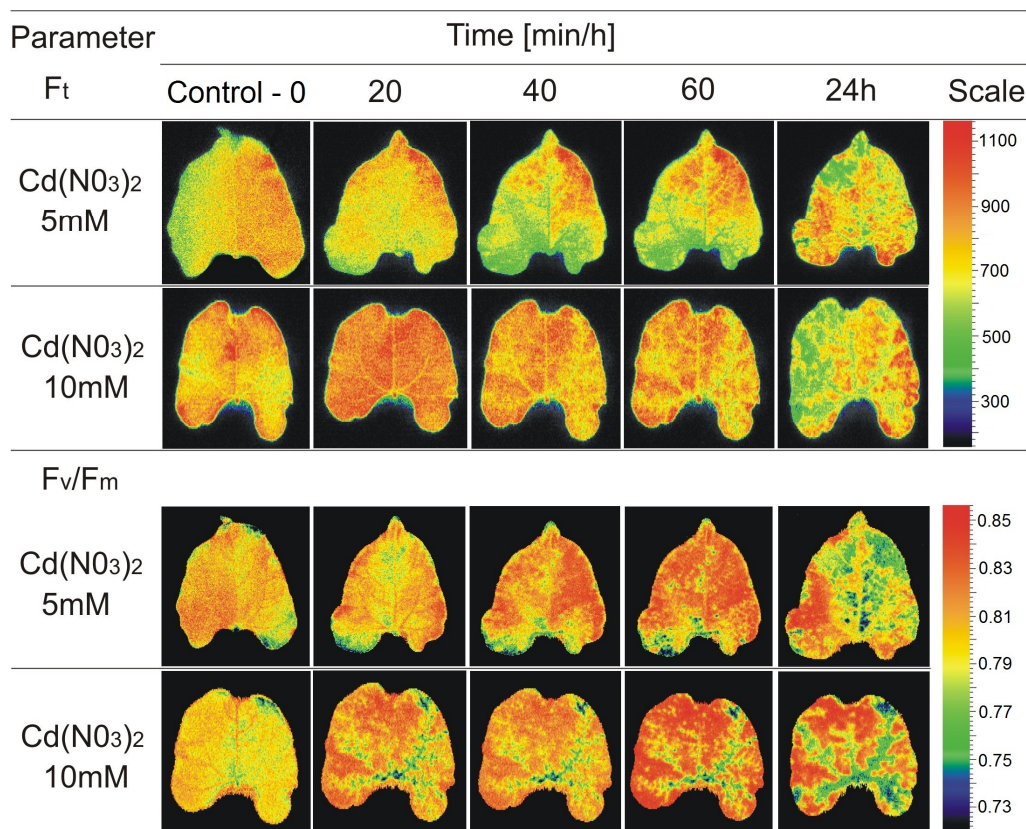


Fig. 2. Imaging chlorophyll *a* fluorescence in the common bean (*Phaseolus vulgaris* L.) leaves in cadmium nitrate stress; F_t – the stationary fluorescence, F_v/F_m – the maximum efficiency of the photochemical PSII

After 20 min of 5 mM cadmium nitrate application, the F_v/F_m parameter was significantly lower than the control group. With the extension of application time – after 40 min and 60 min, its value was increased on the surface of the whole bean leaves. However, after 24 h the value of this parameter was significantly lower, compared to the control sample.

In the case of 10 mM cadmium nitrate concentration, along with the extension of the application time, F_v/F_m reduction at the leaves innervation sites was observed, while on the remaining leaf surface the value of this parameter was higher than that of the control leaves (Fig. 2). The F_v/F_m parameter as a measure of plant vitality indicates the functioning of PSII. Changes in F_v/F_m values may be result of the substitution of cadmium (Cd^{2+}) for calcium (Ca^{2+}) in the PSII catalytic centre during photoactivation. As a result of the activity of cadmium, can decrease of the ability of electron transport in PSII, it may increase of fluidity thylakoid membranes, cause damage of proteins, which are manifested of photochemical disturbances activity of the photosynthetic apparatus (Solymosi and Bert, 2012; Dezhban *et al.*, 2015).

Non-photochemical quenching (NPQ) is associated with energy losses, which may be caused by F_v/F_m changes (Baker, 2008; Sofo *et al.*, 2009). In the case of NPQ parameter, up to 60 min of cadmium compounds application, regardless of their concentration, values of this parameter decreased, generally on the whole surface of

leaves. After 24 h, a high “pointwise” NPQ values were observed on the surface of bean leaves (Fig. 3). Plants under stress conditions can dissipate a lot of their thermal energy with using the xanthophyll cycle (Jahns and Holzwarth, 2012). With longer application of cadmium compounds, the vitality of PSII (Rfd) values decreased on the bean leaves surface. After 24 h application of 10 mM cadmium nitrate the lowest values were recorded (Fig. 3). Such reactions indicate photochemical disturbances in chloroplast thylakoids. In a stroma of cell, they indicate of disorganisation of the course of enzymatic reactions and changes of the assimilation process of carbon dioxide (Solymosi and Bert, 2012).

Toxic effects of cadmium ions induce a progressive reduction in the amount of chlorophyll (Qiu *et al.*, 2013). In the experiment with bean, along with the passage of time the reduction of chlorophyll content was observed. After 24 h application of cadmium nitrate solutions the highest changes were demonstrated (Fig. 4). In chloroplasts, chlorophyll is stable in compounds, among other with specific proteins, phospholipids and sulpholipids. Disturbance of these relationships accelerates the conversion of chlorophyll and causes loss of green colour (Hu *et al.*, 2014). Cadmium replacing magnesium in the chlorophyll leads to its destruction (Chou *et al.*, 2011). According to Emamverdian *et al.* (2015) the sensitivity of cadmium increases with age of plants, and it is easier for younger plants to adapting on cadmium stress.

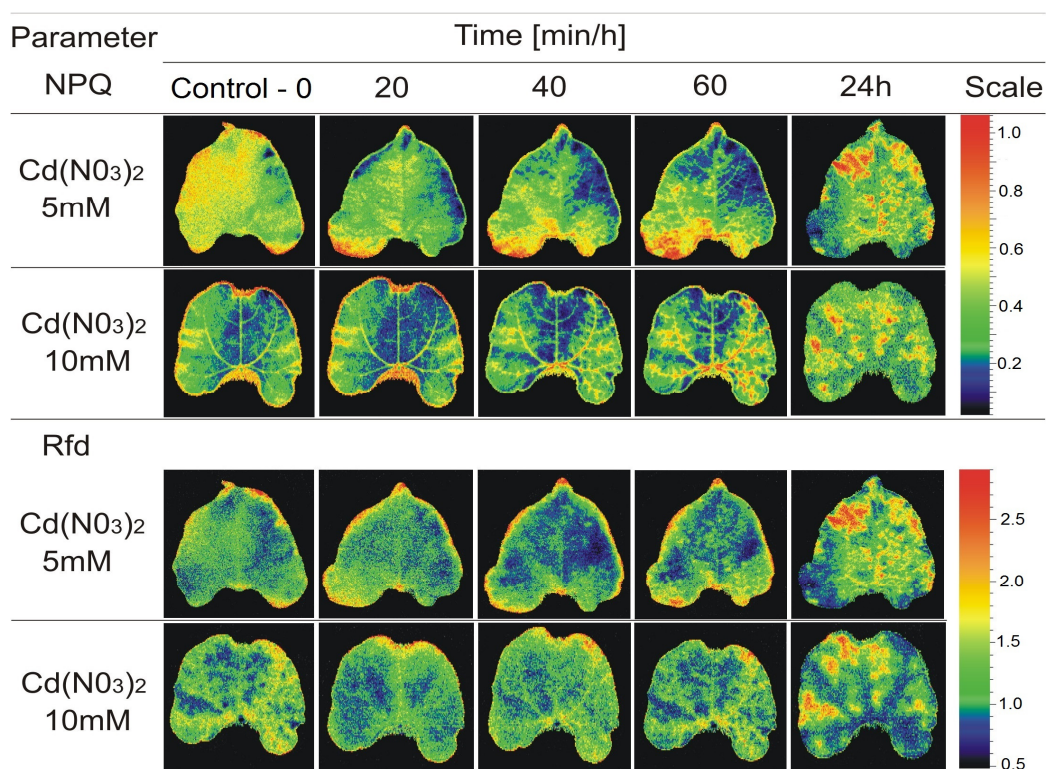


Fig. 3. Imaging chlorophyll *a* fluorescence in the common bean (*Phaseolus vulgaris* L.) leaves in cadmium nitrate stress; NPQ – the non-photochemical quenching, Rfd – the vitality of PSII

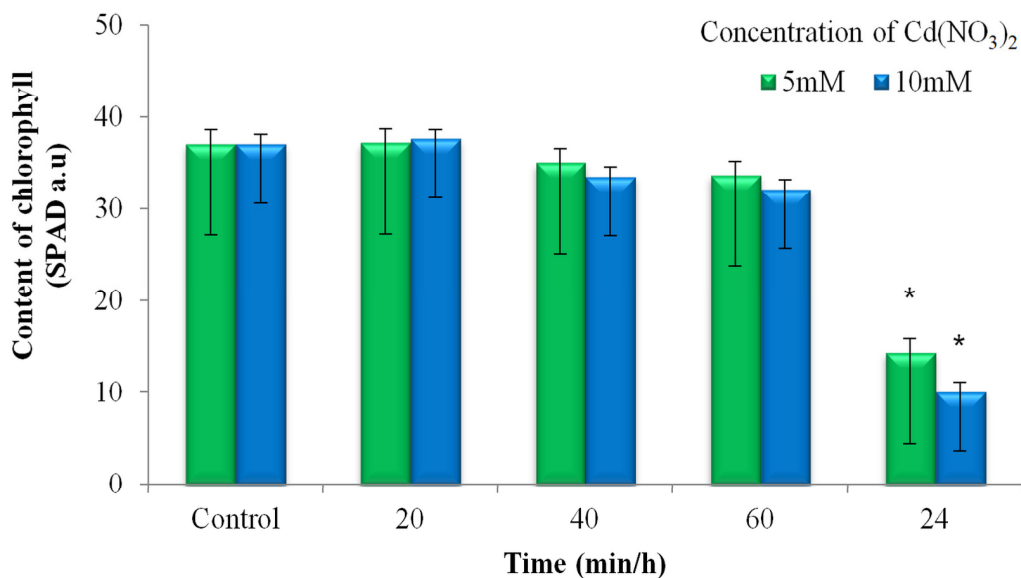


Fig. 4. Chlorophyll content in common bean (*Phaseolus vulgaris* L.) leaves in cadmium nitrate of 5 and 10 mM concentration after time applications: 20, 40, 60 min and 24 h and control group; means ± SD (n = 5) with * differ significance with Kruskal-Wallis test at *p* < 0.05

Changes in photosynthetic activity are due to many other metabolic disturbances that occur in plants under stressful conditions. One of them is disturbance of water management. Cadmium disrupts of water relations in plants by negatively affecting water transport (De Maria *et al.*, 2013; Metwali *et al.*, 2013). It leads to the production of

free radicals that contribute to the breakdown of ion channels in cell membranes (Ahmad *et al.*, 2011; Anjum *et al.*, 2015). In studies with bean leaves, compared to the control group the electrolyte leakages were increased with the time of application of cadmium solutions (Fig. 5).

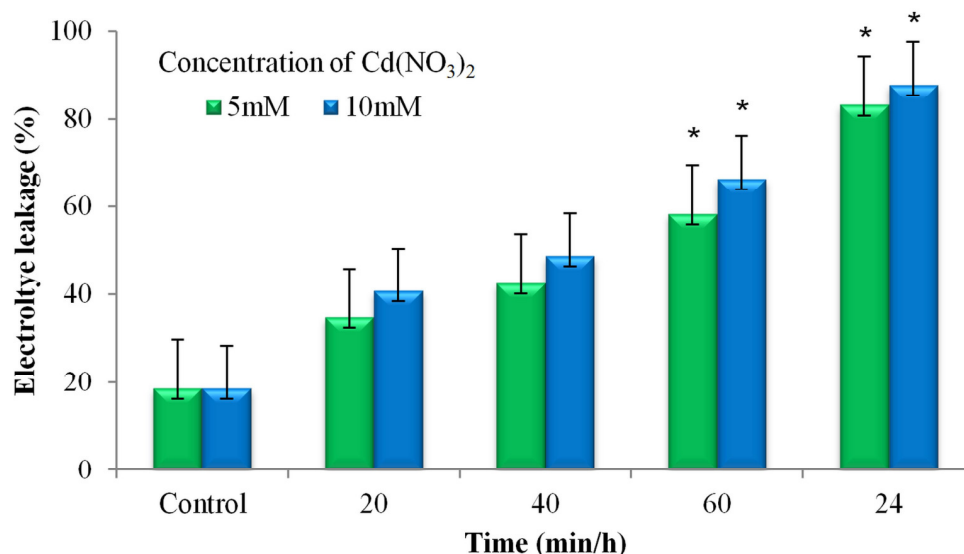


Fig. 5. Electrolyte leakage (%) from common bean (*Phaseolus vulgaris* L.) leaves in cadmium nitrate of 5 and 10 mM concentration after time applications: 20, 40, 60 min and 24 h and control group; means \pm SD (n = 5) with * differ significance with Kruskal-Wallis test at $p < 0.05$

Probably, these changes are due to the different concentration and time of the cadmium compounds application. Also they depend on the species and variety, the part of plant and the stage of development. However, regardless of age, the plants have some protection against the negative effects of cadmium and other heavy metals. The activity of antioxidant enzymes and the ability to synthesize cadmium binding proteins are one of many defence mechanisms activated in response to high concentrations of cadmium (Czeczot and Skrzycki, 2010; Anjum *et al.*, 2015). This kind of defence for plant organisms is not enough. Therefore, in order to prevent the destructive effect of heavy metals, their content must already be controlled in the structures of plant propagation, e.g., in seeds, tubers and roots. The use of cadmium and other heavy metals in industry should be to eliminate by modifying production processes. It is necessary to regulate and coordinate the production of organic compounds, water and ion collection and their distribution.

Conclusions

Under the influence of cadmium ions, at first the damage to either the edge, at the second the central part of the leaves of beans, and then the whole surface were occurred. Along with the prolongation of time exposure to cadmium, the zero fluorescence (F_0) values were increased on the surface of the whole leaves of bean, regardless of the concentration of the solution. Compared to control, after 40 min of application of 5 mM cadmium nitrate and already after 20 min of application of 10 mM concentration this compound, the maximum fluorescence (F_m) values were higher on the whole leaves surface of bean. After 20 min of application of 5 mM cadmium nitrate, the F_v/F_m parameter significantly decreased compared to the control; extension of the application time (40 and 60 min), caused these values increased. After 60 min, regardless of their concentration,

the non-phytochemical quenching values (NPQ) were decreased, generally on the surface of the whole leaves. The value of the PSII vitality (Rfd) decreased with the extension of the cadmium compounds application time. Along with the prolongation of time cadmium application, the reduction of chlorophyll content and the increase of electrolyte leakage from cell membranes were observed; after 24 h, the highest changes of chlorophyll were demonstrated.

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