Original Research

Surplus Fertilization Effect on Photosynthetic Response of Pea to Cd Stress at Different Growth Stages

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Abstract

Besides being involved in growth and development of plants, the essential macronutrients also affect stress tolerance. The aim of this study was to assess the surplus fertilization effect on photosynthetic parameters and the growth of Cd-treated pea (*Pisum sativum* L.) plants at different growth stages. Surplus fertilizer norm reduced the negative effect of cadmium on the photosynthetic system of pea at leaf development stage, while at lateral shoots stage their influence was rather different. Fertilization had the largest statistically significant effect on all the investigated parameters while the growth stage of plants was also a strong factor, changing cadmium and plant nutrient interactions.

Keywords: net photosynthesis, transpiration, photosynthetic pigments, cadmium, growth stage

Introduction

Fertilizers are added to soil in order to increase crop yields and quality, but they may also affect other soil functions and processes [1]. Protein-based metabolic processes leading to increases in vegetative and reproductive growth and yield are totally dependent upon the adequate supply of nutrients. Under the absence of nutrients, plants weak. Various experiments of fertilization in damaged forests confirm the proposition that additional nutrition increases plant resistance to various stresses [2]. However, excess fertilizer has a negative impact on plants [3].

One of the serious problems is contamination of agricultural soil with cadmium (Cd). Its cumulative properties together with the ability to disrupt a number of biological systems makes it one of the most toxic elements [4]. The concentrations of cadmium in Lithuanian soils are not that high, but there exists another problem: in fields close to highways, the previous main risk factor, lead, has now been replaced by cadmium [5]. According to data by various authors, cadmium suppresses the growth of plants, especially their roots, cell division, decreases biomass, and increases membrane conductance [6-9]. The response to Cd can be different and sometimes opposite, depending on the species. Net photosynthesis is also sensitive to Cd because it directly affects chlorophyll biosynthesis and the proper development of the chloroplast ultrastructure [10]. However, the main targets for the influence of Cd appears to be ribulose 1,5-bisphosphate carboxylase (RuBPC) and phosphoenol pyruvate carboxylase (PEPC). It has been shown that Cd^{2+} ions lower the activity of RuBPC and damage its structure by substituting for Mg^{2+} ions, which are important cofactors of carboxylation reactions, and may also shift RuBPC activity toward oxygenation reactions [11]. A drop in the activities of RuBPC and PEPC was also observed for Cd-treated maize plants by Krantev et al. [12]. It has been demonstrated that Cd^{2+} induces changes in the antioxidant status in plants too [7, 13].

Plants employ various strategies to counteract the inhibitory effect of Cd, and it is thought that nutrient management is a possible way to reduce negative Cd effect. It is well known that Cd greatly affects the uptake, transport

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and use of essential microelements. The results of some previous research shows that proper plant nutrition is a good strategy to alleviate the damaging effects of Cd on plants and to avoid its entry into the food chain.

Additionally, several plant nutrients have many direct as well as indirect effects on Cd availability and toxicity because, besides being involved in growth and development, the essential macronutrients also affect stress tolerance [14, 15]. Direct effects include decreased Cd solubility in soil by favouring precipitation and adsorption [16], competition between Cd and plant nutrients for the same membrane transporters [17], and Cd sequestration in the vegetative parts to avoid its accumulation in the grain/edible parts [18]. It is determined that cadmium is bound to cation exchange sites in mucilage excretions of maize root tips [19]. Binding of Cd to these sites would be expected to reduce Cd transport into the cell. For instance, Cd concentration in wheat grain decreased with increasing N concentration in soil and nutrient solution [20].

Phosphate addition was found to reduce the positive charges of soils and enhance its ability to adsorb metallic ions [21] too. Adding phosphorus significantly lowered the amounts of Cd in the soluble-exchangeable fraction, whereas the specifically adsorbed fraction of Cd was increased [22]. Indirect effects include dilution of Cd concentration by increasing plant biomass and alleviation of physiological stress. For example, sulphur uptake and assimilation are crucial in determining crop yield and resistance to Cd stress [23]. Sulphur is known for its role in the formation of the sulphur containing amino acids cysteine (Cys) and methionine (Met) and in the synthesis of proteins, vitamins, chlorophyll, and glutathione (GSH, -glutamylcysteinylglycine), which is involved in stress tolerance [23]. It has been reported about the involvement of Cd in the up-regulation of various genes involved in S metabolism in *Saccharomyces cerevisiae* [15].

Besides the protective effect of S pretreatment against the Cd toxicity discussed above, it is also determined that additional Mg in the nutrient solution alleviated Cd toxicity and enhanced the growth of Japanese mustard spinach plants suffering from Cd toxicity, resulting in a reduced Cd concentration in the plant [25]. In a transcriptomic study of Mg starvation in *Arabidopsis*, Hermans et al. [26] showed that a Mg pretreatment of 7 days alleviated the bleaching of young leaves caused by Cd. A protective effect of Mg pretreatment was also observed on Fe starvation. According to the authors, the protective effect of Mg against Cd toxicity could be partly attributable to the maintenance of Fe status and also to the increase in antioxidative capacity, detoxification, and/or protection of the photosynthetic apparatus. In other research, it is observed that Cd toxicity was also alleviated by Zn treatment in two rice cultivars differing in Cd tolerance [27], maize plants [28], and wheat plants [29], and also by Si supply in peanut seedlings [30] or by Se in broccoli [31].

In comparison to the large number of studies on the photosynthetic response to different fertilizer supplies and cadmium stress, much less effort has been applied to understanding how this response changes with leaf and plant age. The aim of the experiments was to investigate how surplus fertilization of peas at different growth stages changes their photosynthetic response to cadmium stress.

Experimental Procedures

Pea (*Pisum sativum* L., cv. 'Ilgiai') was chosen for the investigation. Experiments were conducted in a vegetation room with a controlled environment: photoperiod – 14 h, average temperature of night and day was 20 and 25ºC, relative humidity – 60%. "Philips Master Green Power CG T" 600W lamps, light intensity at the level of plants 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, provided light.

The peas (20 seeds per pot) were sown in a neutral (pH 6.0-6.5) peat substrate with standard norm (36 g/m^2) (NF) of "YaraMila NPK (12-11-18)+microelements" (N general 12%; N-NO_3 4.8%; N-NH_4 7.2%; P_2O_5 11%; P_2O_5 7.7%; K_2O 18%; MgO 2.7%; SO_3^- 20%; B 0.015%; Fe 0.2%; Mn 0.02%; Zn 0.02%; (YARA, Finland)) fertilizers in 5L pots (21 cm in diameter and 15 cm in height). The seeds were germinated and grown for nine days. Ten days after germination, the pea plants were divided into eleven groups. In the first – reference treatment group – there were six pots of replication (3 pots for the first stage of the experiment and 3 pots for the second stage) and in others – 3 pots of replication in each. The treatments of the groups were started on the 10th and 17th days of the experiment (Fig. 1):

1. The reference treatment group was watered with distilled water all the time.
2. The growth substrate of peas in the 2nd group was watered with 15 g/l H_2O of NPK (12-11-18) fertilizer solution to achieve 3 times higher (108 g/m^2) surplus fertilization norm (3F) at leaf development (BBCH 14-16) stage [32].
3. The growth substrate of peas in the 3rd group was watered with 3 mM cadmium ($CdSO_4$) concentration solution at leaf development (BBCH 14-16) stage [32].
4. The growth substrate of peas in the 4th group was watered with surplus fertilization norm (3F) and 3 mM cadmium ($CdSO_4$) concentration solution at leaf development (BBCH 14-16) stage [32].
5. The growth substrate of peas in the 5th group was watered with 6 mM Cd at BBCH 14-16 stage [32].
6. The growth substrate of peas in the 6th group was watered with surplus fertilization norm (3F) and 6 mM cadmium ($CdSO_4$) concentration solution at leaf development (BBCH 14-16) stage [32].
7. The growth substrate of peas in the 7th group was watered with surplus fertilization norm (3F) one week later, i.e. at the formation of lateral shoots (BBCH 21-23) stage [32].
8. The growth substrate of peas in the 8th group was watered with 3 mM Cd at the formation of lateral shoots (BBCH 21-23) stage [32].
9. The growth substrate of peas in the 9th group was watered with surplus fertilization norm (3F) and 3 mM cadmium ($CdSO_4$) concentration solution at BBCH 21-23 stage [32].

10. The growth substrate of peas in the 10th group was watered with 6 mM cadmium at BBCH 21-23 stage [32].
11. The growth substrate of peas in the 11th group was watered with surplus fertilization norm (3F) and 6 mM cadmium ($CdSO_4$) concentration solution at BBCH 21-23 stage [32].

Each pot of peat substrate received 1L of solution. According to the results obtained in earlier experiments done at the Environmental Department of Vytautas Magnus University, week 3 mM and medium 6 mM Cd solution treatment variants [9, 33] were chosen. Also, surplus 108 g/m^2 fertilization norm (3F) was chosen according to the earlier experiments done at the department [2, 6]. The duration of experiments was five days.

Gas exchange was measured with portable photosynthesis system LI-6400 (LI-COR, USA) at the end of each stage of the experiment. Net photosynthetic rate (Pn) (µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), transpiration (Tn) (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$), intercellular CO_2 concentration (Ci) (µmol $CO_2 \cdot$mol air^{-1}) and water use efficiency (WUE) (µmol $CO_2 \cdot$mmol H_2O^{-1}) of the second pair of fully expanded leaves were registered every 30 seconds for 30 minutes. The measurements were performed for one randomly selected seedling per pot; from these data a daily mean of measured indices was calculated. The environmental conditions during the experiments were: air flow rate – 400 µmol$\cdot s^{-1}$; block and leaf temperature – 25ºC; CO_2 concentration in sample cell – 300-400 µmol $CO_2 \cdot mol^{-1}$; relative humidity in sample cell – 30%; lightness in quant – 180 µmol$\cdot m^{-2} \cdot s^{-1}$.

The second pair of fully expanded leaves was harvested for the photosynthetic pigments determination at the end of the experiment. The photosynthetic pigments were analyzed using a spectrophotometer (Genesys 6, ThermoSpectronic, USA) and 100% acetone extracts prepared according to Wettstein's method [35]. Photosynthetic pigments were expressed in mg/g of fresh weight.

At the end of the experiment, the plants were harvested. The shoots were dried in an oven at 60ºC until a constant dry shoots biomass was obtained. The shoots biomass was expressed in mg$\cdot$plant^{-1}.

ANOVA were used to determine the effects of cadmium impact and growth stage. For independent variables comparison Student's T (for parametric) and U tests (for non-parametric) were employed. All the analyses were performed by STATISTICA and the results were expressed as mean values and their confidence intervals (CI) ($p < 0.05$).

Results

Gas Exchange Parameters

Photosynthetic rate (Pn) decreased in pea plants at both growth stages treated with cadmium (Fig. 2, A, B), and surplus fertilization had a positive effect on photosynthetic rate of Cd-treated peas, except 6 mM Cd-treated peas at the lateral shoots stage. 3 mM cadmium reduced photosynthetic rate by 43.2% ($p < 0.05$) and 45.6% ($p < 0.05$) at leaf development and lateral shoots stage peas respectively, while under surplus fertilization these losses decreased to 32.4% ($p < 0.05$) and 39.2% ($p < 0.05$), respectively, compared to the reference treatment. 6 mM Cd exposure resulted in high and statistically significant decreases in Pn, i. e. 74.7% ($p < 0.05$) at leaf development and 61.4% ($p < 0.05$) at lateral shoots stage peas compared to the reference treatment. Under surplus fertilization, negative Cd effect was lower only in leaf development stage peas, when photosynthetic rate only decreased by 35.5% ($p < 0.05$), compared to the reference treatment. Pn in lateral shoots stage peas treated with 6 mM Cd and surplus fertilization decreased even more than without fertilization and by 81.1% ($p < 0.05$), compared to the reference treatment.

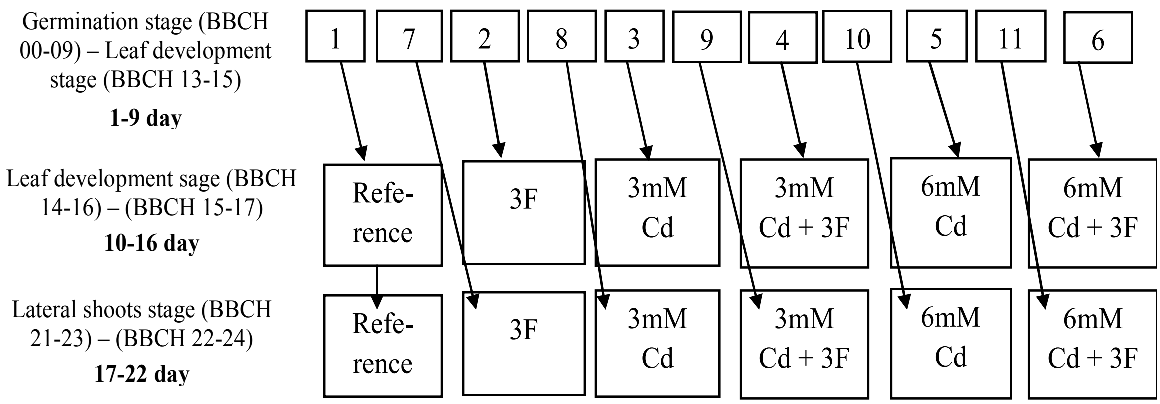

Fig. 1. Experiment scheme.
3F – surplus 108 g/m^2 NPK (12-11-18) fertilization norm added.
3 mM Cd – watered with 3 mM $CdSO_4$ concentration solution.
3 mM Cd+3F – surplus 108 g/m^2 NPK (12-11-18) fertilization norm added and watered with 3 mM $CdSO_4$ concentration solution.
6 mM Cd – watered with 6 mM $CdSO_4$ concentration solution.
6 mM Cd+3F –surplus 108 g/m^2 NPK (12-11-18) fertilization norm added and watered with 6 mM $CdSO_4$ concentration solution.

Intercellular CO_2 concentration (Ci) decreased in pea plants at leaf development stage treated with cadmium, while Ci in peas at lateral shoots stage treated with cadmium increased, in comparison to the reference treatment (Fig. 2, C, D). Surplus fertilization increased Ci in peas treated with 3 mM Cd and 6 mM Cd at leaf development stage by 44.3% ($p < 0.05$) and 32.1% ($p < 0.05$) respectively, compared to normal fertilization, but it was still less than Ci of the reference treatment peas. Changes in Ci in peas at lateral shoots stage were lower and mostly statistically insignificant, except in the peas treated with 3 mM cadmium, when it decreased by 16.6% ($p < 0.05$) compared to the reference treatment.

Transpiration rate (Tn) in peas treated with 3 and 6 mM cadmium decreased by 52.8% ($p < 0.05$) and 86.5% ($p < 0.05$) at leaf development and by 41.1% ($p < 0.05$) and 34.7% ($p < 0.05$) at lateral shoots stage, respectively (Fig. 3, A, B), compared to the reference treatment. Surplus fertilization increased Tn in peas treated with 3 and 6 mM cadmium by 25.4% and 7 times at leaf development stage, and decreased it by 18.6% ($p < 0.05$) and 48.2% ($p < 0.05$) respectively at lateral shoots stage, as compared to normal fertilization.

Under the impact of 3 mM and 6 mM cadmium, water use efficiency (WUE) in peas increased by 13.0% ($p < 0.05$) and 97.3% ($p < 0.05$) at leaf development stage and decreased by 17.7% ($p < 0.05$) and 47.6% ($p < 0.05$) at lateral shoots stages, respectively, compared to the reference treatment (Fig. 3, C, D). Surplus fertilization decreased WUE in all cadmium-treated peas compared to normal fertilization, except for peas at the lateral shoots stage treated with 3 mM Cd, when WUE increased by 36.9% ($p < 0.05$), compared to normal fertilization.

Photosynthetic Pigments Content

Cadmium treatment decreased chlorophyll *a* content in pea leaves at both development stages, but was statistically significant only under the higher impact (Fig. 4 A, B). Under 6 mM cadmium exposure, chlorophyll *a* content decreased by 34.1% ($p < 0.05$) in peas at leaf development stage, and the same impact on ones at lateral shoots stage led to a decrease by 37.5% ($p < 0.05$). Surplus fertilization resulted in higher 6 mM cadmium impact on chlorophyll *a* content and it decreased by 56.6% ($p < 0.05$) in pea leaves at leaf development stage, and by 80.1% ($p < 0.05$) at lateral shoots stage. Chlorophyll *b* decreases under Cd impact in pea leaves at both investigated growth stages were statistically insignificant, except for the peas at lateral shoots stage treated with 6 mM Cd and surplus fertilization (Fig. 4 C, D).

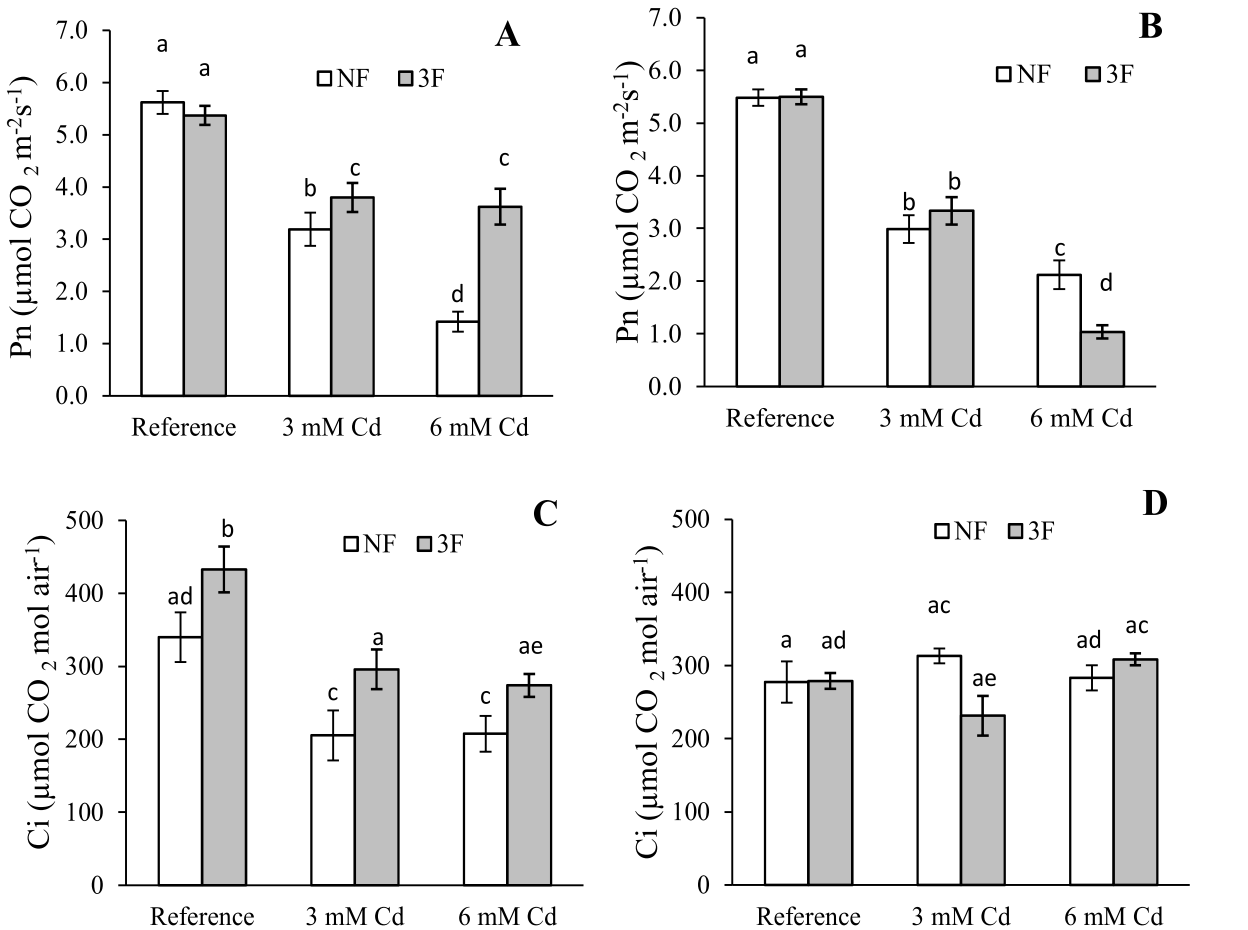

Fig. 2. Changes in photosynthetic rate (Pn) and in intercellular CO_2 concentration (Ci) in pea plants treated with 3 mM and 6 mM Cd under fertilization norm (NF) and surplus fertilization (3F) at leaf development (A, C) and lateral shoots (B, D) stages. The values are means ± $CI_{0.05}$. Significant differences ($p < 0.05$) between treatments are denoted with different letters.

The investigated Cd and surplus fertilization exposure to the content of carotenoids was very low and statistically insignificant (Fig. 4 E, F).

Growth Parameters

Dry shoots biomass of the peas at leaf development stage treated with 3 mM cadmium increased by 18.3% ($p > 0.05$), but after 6 mM Cd treatment, dry biomass of peas at leaf development stage decreased by 54.2% ($p < 0.05$), as compared with the reference treatment (Fig. 5 A). At lateral shoots stage, the rising concentration of cadmium decreased dry biomass of peas too, when it showed approximately 6.2% ($p > 0.05$) and 15.7% ($p > 0.05$) reduction in 3 and 6 mM cadmium-treated plants, respectively. Surplus fertilization decreased dry biomass of all cadmium-treated peas as compared to the normal fertilization, except the peas at leaf development stage treated with 6 mM Cd, when dry biomass increased by 42.7% ($p < 0.05$), as compared to the normal fertilization. If compared to the reference treatment, statistically significant decreases in dry biomass were only observed in peas treated with both cadmium concentrations and surplus fertilization at lateral shoots stage (Fig. 5 A, B).

ANOVA

The investigated factors (growth stage, cadmium and fertilization) influenced the physiological and morphological parameters of the peas (Table 1). Fertilization had the highest statistically significant impact on all eight investigated parameters, cadmium on seven, and growth stage on five, while the combined effect of growth stage, cadmium, and fertilization was statistically significant only for four out of eight investigated parameters.

Discussion of Results

There are many studies on the photosynthetic response of plants to cadmium and different fertilizers supply. However, much less effort has been applied to understanding how these responses change with leaf or plant age. Cadmium can negatively affect the efficiency of photosystem 2 (PSII), photochemistry, and photosynthetic electron transport chain [35]. As reported before, the photosynthetic response of peas to cadmium stress at different growth stages was slightly different [9]. In the present research, the additional effect of surplus fertilization also changed pho-

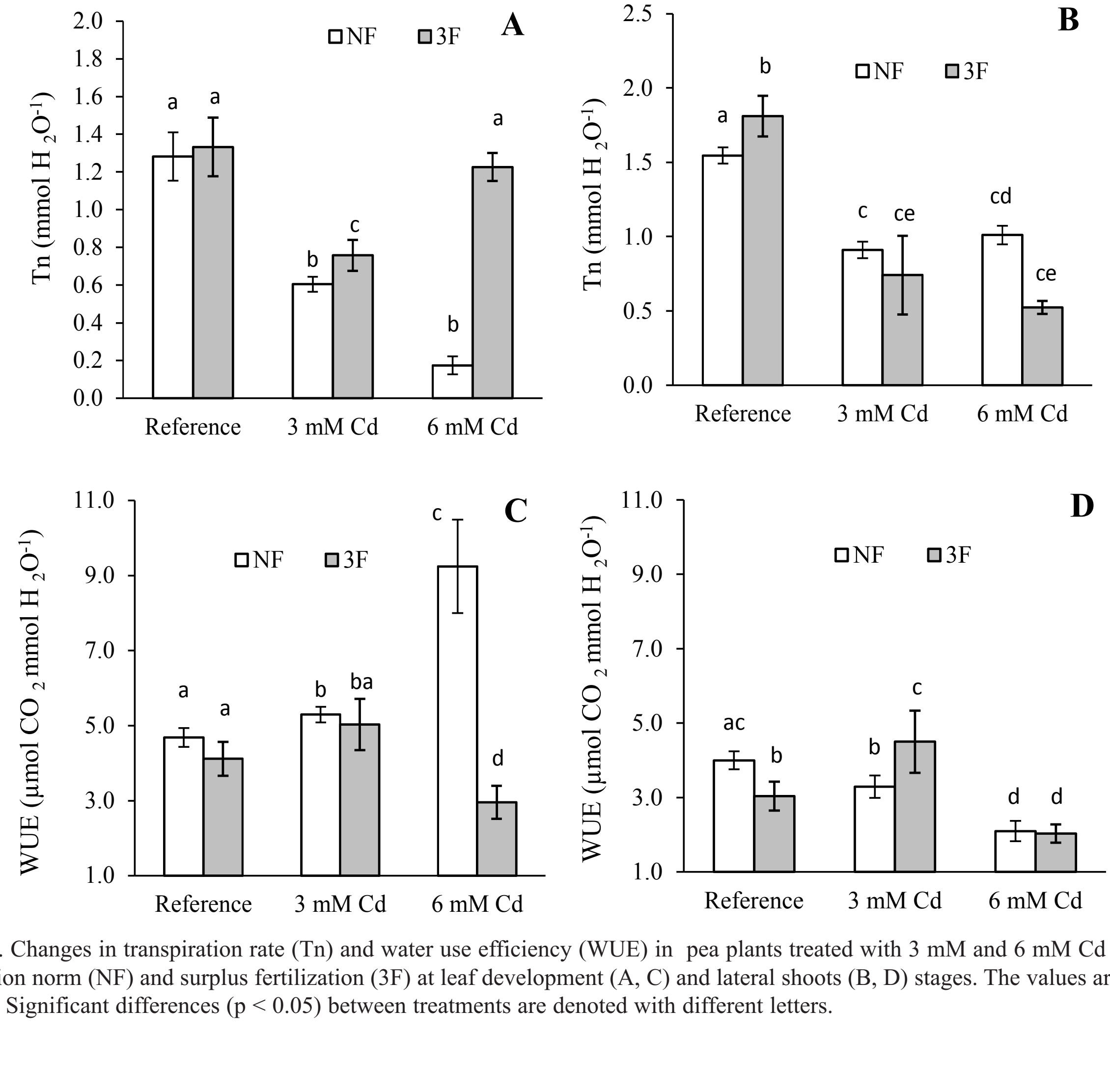

Fig. 3. Changes in transpiration rate (Tn) and water use efficiency (WUE) in pea plants treated with 3 mM and 6 mM Cd under fertilization norm (NF) and surplus fertilization (3F) at leaf development (A, C) and lateral shoots (B, D) stages. The values are means ± $CI_{0.05}$. Significant differences ($p < 0.05$) between treatments are denoted with different letters.

tosynthetic response to cadmium stress in peas at different growth stages.

Surplus fertilization and cadmium effect increased Pn, Ci, and Tn in peas at leaf development stage, compared to normal fertilization and cadmium effects. Transpiration rate of 6 mM Cd-treated peas at leaf development stage was almost the same as in peas under reference treatment (Fig. 3 A). Thus, in comparison to the normal fertilization, surplus (3F) fertilization induced higher transpiration rate in pea leaves treated with Cd and it also caused a statistically significant (68.0%) decrease in WUE in 6 mM Cd-treated peas at leaf development stage. Similarly, fertilization increased transpiration rate in other research [1]. Thus, it is possible that the photosynthetic rate increased because of the opened stomata, when the higher amount of CO_2 reached the cells and the CO_2 reduction processes in pea leaves were intensive, while even increased Ci in Cd-treated and fertilized peas was still lower than Ci in the reference treatment peas at leaf development stage. Also, the higher fertilization norm included the higher amount of sul-

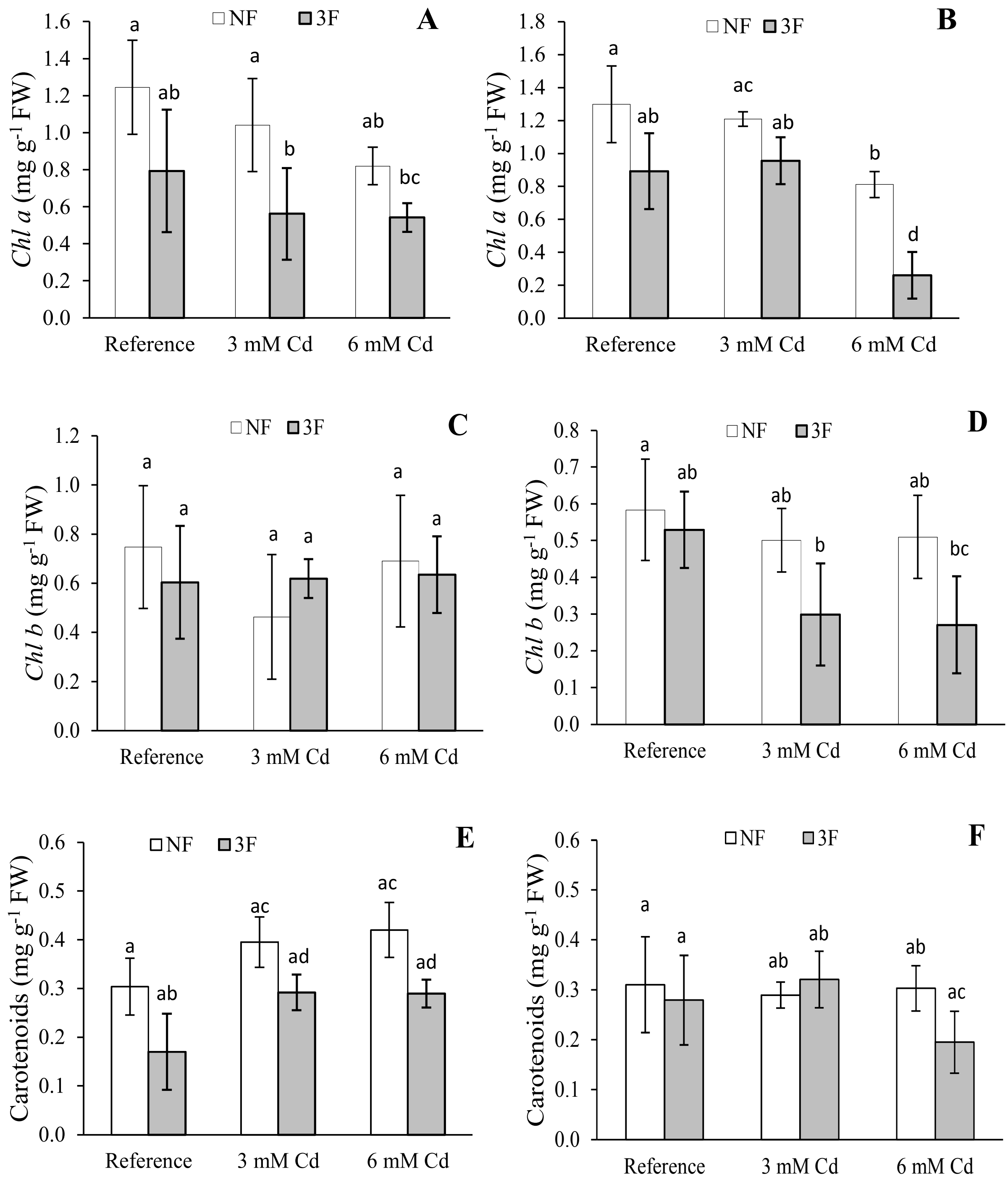

Fig. 4. Changes in the content of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids in pea plants treated with 3 mM and 6 mM Cd under fertilization norm (NF) and surplus fertilization (3F) at leaf development (A, C, E) and lateral shoots (B, D, F) stages. The values are means ± $CI_{0.05}$. Significant differences ($p < 0.05$) between treatments are denoted with different letters.

phur (S) in growth substrate, so the application of S could help in reducing Cd toxicity, as determined by Ernst et al. [23] and Gallego et al. [15], and the increase in photosynthetic rate, as compared to the normal fertilization and Cd effects (Fig. 2 A). Sulphur metabolism tightly regulates biosynthesis of PCs in plants through the regulation of GSH and helps metal sequestration [36]). Anjum et al. [37] also suggest that S may ameliorate Cd toxicity and protect the growth and photosynthesis of plants involving AsA and GSH.

In contrast to peas at leaf development stage, the photosynthetic rate reduction in 3 mM and 6 mM Cd-treated peas at the lateral shoots stage caused the closure of stomata, and under additional surplus fertilization impact it decreased by higher intensity, i.e. by 52.1% and 66.1% respectively, in comparison to the reference treatment (Fig. 2 B). The changes of Ci in peas at lateral shoots stage were lower and mostly statistically insignificant, except in peas treated with 3 mM cadmium and surplus fertilization (3F), when it decreased by 16.6% ($p < 0.05$), compared to the reference treatment. Lower changes in Ci under very low Tn indicates that Cd had a higher deleterious effect on enzymatic reactions of photosynthesis rather than on transpiration, because under low transpiration stomata were closed (Fig. 3 B) and it was possible for Ci to decrease while in fact it increased ($p > 0.05$) (Fig. 2 D).

Not only did cadmium treatments lead to decreases in photosynthetic rate (Fig. 2 A, B), but also induced decreases in the leaf concentrations of chlorophylls, the negative effect being more marked in the 6 mM Cd treatment than in the 3 mM one (Fig. 4 A, B). Surplus fertilization resulted in higher decreases in 6 mM cadmium impact on chlorophyll *a* contents in both growth stages (Fig. 4 A, B). Chlorophyll *a* content decreases were higher in lateral shoots stage pea leaves than in leaf development ones. Cadmium alters chloroplast ultrastructure and reduces net photosynthetic rate, stomatal conductance, and leaf transpiration [38]. Besides, Cd inhibits photosynthesis by decreasing the transcription of the photosynthesis-related genes psbA, psaB and rbcL [39], inactivates enzymes involved in CO_2 fixation [40], induces lipid peroxidation [41], enhances proteolysis [42], and disturbs N and S metabolism and plant antioxidant machinery [14, 15].

The reduction in chlorophyll content as well as the reduction in transpiration and photosynthesis rates, and growth in Cd-treated plants is related with the toxic effects of cadmium on plants [7-9]. In the present research, dry shoots biomass decreased in almost all plants that were treated with cadmium, but statistically significant figures are only under 6 mM Cd treatment (Fig 5 A). Surplus fertilization, in the present research, decreased dry biomass in all cadmium treated peas compared to normal fertilization, except for peas at leaf development stage treated with 6 mM Cd, when dry biomass increased by 42.7% ($p < 0.05$) as compared to normal fertilization. For the younger plants surplus fertilization did not affect as a stressor, while for older ones it became a stressor, because most crops required significant quantities of nutrition elements during the early stages of growth [43, 44]. In addition, rhizosphere composition, root growth, and general crop growth are likely to be affected by the application of fertilizers [20]. Also, as mentioned above, fertilizers can influence Cd speciation and complexation, which affects the movement of Cd to plant roots and perhaps also its absorption into the roots [20]. Moreover, higher levels of mineral N in soil decreases root biomass [45], thus the lower amount of cadmium reaches the plant.

In conclusion, taking into account the results of other studies as well as the present research, it can be suggested that additional supply of nutritional element to plants against Cd toxicity can alleviate its induced damages. Also, the use of plant nutrients to alleviate Cd toxicity in plants is a relatively inexpensive, time saving, and effective approach to avoid Cd contamination of food [46]. However, this research also revealed another important factor: that the plant's ability to cope with stress, respond to additional stimulus and acclimate to the adverse environment is highly dependent on the growth stage of the plant

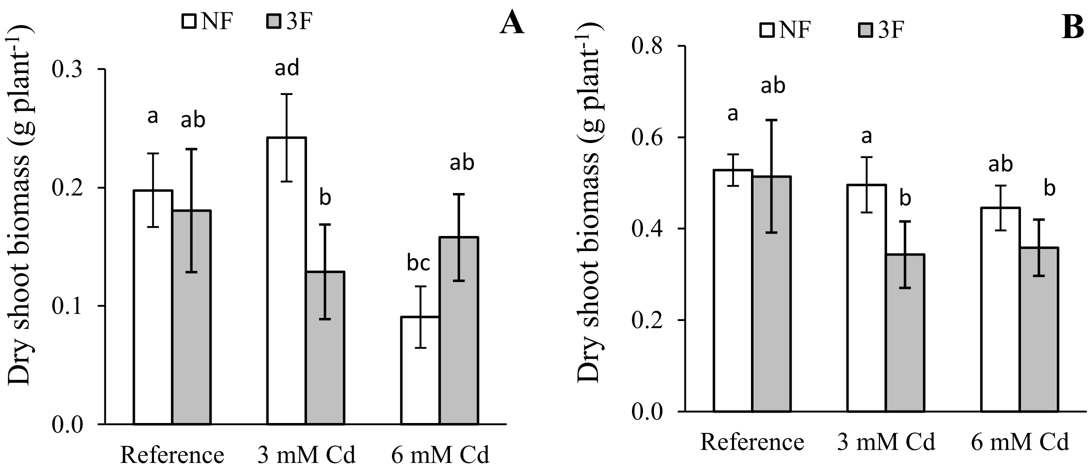

Fig. 5. Changes in dry shoot biomass of pea plants treated with 3 mM and 6 mM Cd under fertilization norm (NF) and surplus fertilization (3F) at leaf development (A) and lateral shoots (B) stages. The values are means ± $CI_{0.05}$. Significant differences ($p < 0.05$) between treatments are denoted with different letters.

Table 1. Analysis of variance for pea plants treated with 3 mM and 6 mM Cd under different fertilization at leaf development and lateral shoots stages. *F* values for growth stage, cadmium and fertilization and their interaction on photosynthetic rate (Pn), intercellular CO_2 concentration (Ci), transpiration (Tn), water use efficiency (WUE), chlorophyll ^{a} (Chl ^{a}), chlorophyll ^{b} (Chl ^{b}), carotenoids, shoots dry biomass.

Source of variation	*F* value							
	Pn	Ci	Tn	WUE	*Chl a*	*Chl b*	Carotenoids	Shoots biomass
Growth stage	64.5***	25.5***	203.0***	719.5***	1.9 N.s.	0.3 N.s.	0.3 N.s.	255.4***
Cd	1,668.5***	653.7***	1,823.5***	25.1***	4.2*	3.6*	0.05 N.s.	9.9***
Fertilization	33.5***	245.2***	105.9***	210.6***	40.5*	40.1***	74.3***	9.7**
Growth stage × Cd × Fertilization	99.2***	69.0***	362.3***	156.2***	1.9 N.s.	0.7 N.s.	1.3 N.s.	1.9 N.s.

*, **, *** Significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.
N.s. – not significant.

when it was exposed to stressful conditions. A higher fertilizer norm decreases the negative effect of cadmium on the photosynthetic system of pea at leaf development stage, while at lateral shoots stage, surplus fertilization negative cadmium effect increases; also, fertilization had the highest statistically significant impact on all eight investigated parameters (Table 1). As a final consideration emerging from the present experiment results, it is noteworthy that the growth stage of a plant is one more important factor in Cd and plant nutrient interaction in the process of protecting against cadmium toxicity.

References

1. MITCHELL L.G., GRANT C.A., RACZ G.J. Effects of nitrogen application on concentration of cadmium and nutrient ions in soil solution and in durum wheat, Can. J. Soil Sci. **80**, 107, **2000**.
2. DUCHOVSKIS P., BRAZAITYTĖ A., JUKNYS R., JANUŠKAITIENĖ I., SLIESARAVIČIUS A., RAMAŠKEVIČIENĖ A., BURBULIS N., ŠIKŠNIANIENĖ J.B., BARANAUSKIS K., DUCHOVSKIENĖ L., STANYS V., BOBINAS Č. Changes of physiological and genetic indices of *Lycopersicon Esculentum* Mill. by cadmium under different acidicity and nutrition. Pol. J. Environ. Stud., **15**, (2), 235, **2006**.
3. OSMAN A.M.H. Influence of nitrogen and potassium fertilization on yield and quality of two sugar beet varieties. Egyptian J. Agricul. Res. **83**, (3), 132, **2005**.
4. SOUSA N.R., RAMOS M.A., MARQUES A.P.G.C., CASTRO P.M.L. A genotype dependent-response to cadmium contamination in soil is displayed by Pinus pinaster in symbiosis with different mycorrhizal fungi. Appl. Soil Ecol. **76**, 7, **2014**.
5. ANTANAITIS A., ANTANAITIS Š., LUBYTĖ J., STAUGAITIS G. The impact of anthropogenic factors on the concentration of heavy metals in cereal grain (summary). Žemdirbystė=Agriculture., **94**, (2), 49, **2007** [In Lithuanian].
6. JANUŠKAITIENĖ I., JUKNYS R., BRAZAITYTĖ A., DUCHOVSKIS P. The fertilization impact on tomato resistance to substrate acidity and heavy metal cadmium. Environmental research engineering and management. **3**, (29), 44, **2004**.
7. SMIRI M., CHAOUI A., ROUHIER N., GELHAYE E., JACQUOT J.P., EL FERJANI E. Effect of cadmium on resumption of respiration in cotyledons of germinating pea seeds. Ecotox. Environ. Safe. **73**, 1246, **2010**.
8. SHAMSI I.H., JIANG L., WEI K., JILANI G., HUA S., ZHANG G.P. Alleviation of cadmium toxicity in soybean by potassium supplementation. J. Plant Nutr. **33**, 1926, **2010**.
9. JANUŠKAITIENĖ I. The effect of cadmium on several photosynthetic parameters of pea (*Pisum sativum* L.) at two growth stages. Žemdirbystė =Agriculture, **99**, (1), 71, **2012**.
10. RASCIO N., VECCHIA F. D., LA ROCCA N., BARBATO R., PAGLIANO C., RAVIOLO M., GONNELLI C., GABBRIELLI R. Metal accumulation and damage in rice (cv. *Vialone nano*) seedlings exposed to cadmium. Environ. Exp. Bot. **62**, (3), 267, **2008**.
11. SIEDLECKA A., KRUPA Z. Cd/Fe interaction in higher plants – its consequences for the photosynthetic apparatus. Photosynthetica. **36**, 321, **1999**.
12. KRANTEV A., YORDANOVA R., JANDA T., SZALAI G., POPOVA L. Treatment with salicilyc acid decreases the effect of cadmium on photosynthesis in maize plants. Journal of Plant Physiology. **165**, 920, **2008**.
13. BALESTRASSE K.B., NORIEGA G.O., BATLLE A., TOMARO M. L. Heme oxygenase activity and oxidative stress signaling in soybean leaves. Plant Science. **170**, 339, **2006**.
14. GILL S.S., TUTEJA N. Cadmium stress tolerance in crop plants. Probing the role of sulphur. Plant Signalling and Behavior. **6**, 1, **2011**.
15. GALLEGO S.M., PENA L.B., BARCIA R.A., AZPILICUETA C.E., IANNONE M.F., ROSALES E.P., ZAWOZNIK M.S., GROPPA M.D., BENAVIDES M.P. Unravelling cadmium toxicity and tolerance in plants: Insight into regulatory mechanisms. Environ. Exp. Bot. **83**, 33, **2012**.
16. MATUSIK J., BAJADA T., MANECKI M. Immobolization of aqueous cadmium by addition of phosphates. J. Hazard. Mater. **152**, 1332, **2008**.
17. ZHAO F.J., JIANG R.F., DUNHAM S.J., MCGRATH S.P. Cadmium uptake, translocation and tolerance in the hyperaccumulator *Arabidopsis Halleri*. New Phytol. **172**, 646, **2006**.
18. HALL J.L. Cellular mechanisms for heavy metal detoxification and tolerance. J. Exp. Bot. **53**, 1, **2002**.
19. MOREL J.L., MENCH M., GUCKER A. Measurement of Pb^{2+}, Cu^{2+} and Cd^{2+} binding with mucilage exudates from maize (*Zea Mays* L.) roots. Biol. Fert. Soils. **2**, 29, **1986**.

20. WANGSTRAND H., ERIKSSON J., OBORN I. Cadmium concentration in winter wheat as affected by nitrogen fertilization. Eur. J Agron. **26**, 209, **2007**.
21. ZHAO Z.Q., ZHU Y.G., SMITH S.E. Cadmium uptake by winter wheat seedlings in response to interactions between phosphorus and zinc supply in soils. J. Plant Nutr. **28**, 1569, **2005**.
22. JIAO W., CHEN W., CHANG A.C., PAGE A.I. Environmental risks of trace elements associated with long-term phosphate fertilizers applications: A review. Environ. Pollut. **168**, 44, **2012**.
23. ERNST W.H.O., KRAUSS G.J., VERKLEIJ J.A.C., WESENBERG D. Interaction of heavy metals with the sulphur metabolism in angiosperms forma n ecological point of view. Plant Cell Environ. **31**, 123, **2008**.
24. RAUSCH T., WACHTER A. Sulfur metabolism: a versatile platform for launching defense operations. Trends Plant Sci. **10**, 503, **2005**.
25. KASHEM M.D.A., KAWAI S. Alleviation of cadmium phytotoxicity by magnesium in Japanese mustard spinach. Soil Sci. Plant Nutr. **53**, 346, **2007**.
26. HERMANS C., CHEN J., COPPENS F., INZE D., VERBRUGGEN N. Low magnesioum status in plants enhances tolerance to cadmium exposure. New Phytol. **192**, 428, **2011**.
27. HASSAN M.J., ZHANG G., WU F., WEI K., CHEN Z. Zinc alleviates growth inhibition and oxidative stress caused by cadmium in rice. J. Plant Nutr. Soil Sc. **168**, 255, **2005**.
28. ADILOGLU A.I., ADILOGU S., GONULSUZ E., ONER N. Effect of zinc application on cadmium uptake of maize grown in zink deficient soil. Pakistan Journal of Biological Sciences. **8**, 10, **2005**.
29. KOLELI N., EKER S., CAKMAK I. Effect of zinc fertilization on cadmium toxicity in durum and bread wheat grown in zinc-deficient soil. Environ. Pollut. **131**, 453, **2004**.
30. SHI G., CAI Q., LIU C., WU L. Silicon alleviates cadmium toxicity in peanut plants in relation to cadmium distribution and stimulation of antioxidative enzymes. Plant Growth Regul. **61**, 45, **2010**.
31. PEDRERO Z., MADRID Y., HARTIKAINEN H., CAMARA C. Protective effect of selenium in broccoli (*Brassica oleracea*) plants subjected to cadmium exposure. J. Agr. Food Chem. **56**, 266, **2007**.
32. Growth stages of mono-and dicotyledonous plants: BBCH monograph, U. Meier (Ed.), Federal Biological Research Centre for Agriculture and Forestry, Braunschweig: Germany, pp. 10, **2001**.
33. JANUŠKAITIENĖ I., JUKNYS R., PIPIRAITĖ A. Spring barely photosynthehetics rate and changes in stress phases under different cadmium impact, Žemdirbystė=Agriculture. **95**, (1), 73, **2008** [In Lithuanian].
34. WETTSTEIN D. Chlorophyll Letale und der submikroskopische Formweschsel der Plastiden. Exp. Cell Res. **12**, 427, **1957** [In German].
35. PAGLIANO C., RAVIOLO M., DALLA VECCHIA F., GABBRIELLI R., GONNELLI C., RASCIO N., BARBATO R., LA ROCCA N. Evidence for PSII donor-side type photoinhibition induced by cadmium treatment of rice (*Oryza sativa*). J. Photoch. Photobio. B. **84**, 70, **2006**.
36. THANGAVEL P., LONG S., MINOCHA R. Changes in phytochelatins and their biosynthetic intermediates a red spruce (*Picea rubens* Sarg.) cell suspention culture under cadmium and zinc stress. Plant Cell Tiss. Org. **88**, 201, **2007**.
37. ANJUM N.A., UMAR S., AHMAD A., IQBAL M., KHAN N.A. Sulphur protects mustard (*Brassica campestirs* L.) from cadmium toxicity by improving leaf ascorbate and glutathione. Plant Growth Regul. **54**, 271, **2008**.
38. SOUZA V.L., DE ALMEIDA A.A., LIMA S.G., DE M CASCARDO J.C., DA C SILVA D., MANGABEIRA P.A., GOMES F.P. Morphophysiological responses and programmed cell death indiced by cadmium in *Genipa Americana* L. (*Rubiaceae*). Biometals. **24**, 59, **2011**.
39. QIAN H.LI., PAN X., JIANG H., SUN L., FU Z. Photoperiod and temperature influence cadmium's effects on photosynthesis-related gene transcription in *Chorella vulgaris*. Ecotox. Environ. Safe. 73, 1202, **2010**.
40. PERFUS-BARBEOCH L., LEONHARDT N., VAVASSEUR A., FORESTIER C. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. Plant J. **32**, 539, **2002**.
41. IANNONE M.F., ROSALES E.P., GROPPA M.D., BENAVIDES M.P. Reactive oxygen species formation and cell death in catalase-deficient tobacco leaf disks exposed to cadmium. Protoplasma. **245**, 15, **2010**.
42. PENA L.B., PASQUINI L.A., TOMARO M.L., GALLEGO S.M. 20S proteasome and accumulation of oxidized and ubiquitinated proteins in maize leaves subjected to cadmium stress. Phytochemistry. **68**, 1139, **2007**.
43. JONES C., OLSON-RUTZ K., DINKINS C.P. Nutrient Uptake Timing by Crops. EB0191. Revision. MSU Extension. **2011**.
44. MCCAULEY A.M., JONES C.A., MILLER P.R., BURGESS M.H., ZABINSKI C.A. Nitrogen fixation by pea and lentil green manures in a semi-arid agroecoregion: effect of planting and termination timing, Nutr. Cycl. Agroecosys. **92**, 305, **2012**. DOI: 10.1007/s10705-012-9491-3.
45. WANG B., LAI T., HUANG Q.W., YANG X.M., SHEN Q.R. Effect of N fertilizers on root growth and endogenous hormones in strawberry. Pedosphere. **19**, (1), 86, **2009**.
46. TRAN T.A, POPOVA L.P. Functions and toxicity of cadmium in plants: recent advances and future prospects. Turkish Journal of Botany. **37**, 1, **2013**.