

## The effect of cadmium and lead pollution on growth and physiological parameters of field beans (*Vicia faba*)

V. Alle<sup>1,\*</sup>, A. Osvalde<sup>2</sup>, M. Vikmane<sup>1</sup> and U. Kondratovics<sup>1</sup>

<sup>1</sup>University of Latvia, Faculty of Biology, Department of Plant Physiology, 1 Jelgavas street, LV-1004 Riga, Latvia

<sup>2</sup>University of Latvia, Institute of Biology, Laboratory of Plant Mineral Nutrition, 3 Miera street, LV-2169 Salaspils, Latvia

\*Correspondence: [vita.alle@lu.lv](mailto:vita.alle@lu.lv)

**Abstract.** Research on the impact of soil contamination on crops is important as plants directly take up heavy metals from the soil through the roots, so heavy metals can enter the food chain. The aim of this study was to investigate the impact of cadmium (Cd) and lead (Pb) pollution on growth and physiological parameters of field beans. Plants in the vegetation experiment were grown under controlled conditions. Changes in growth and physiological parameters were studied at five levels of Cd (0–25 mg L<sup>-1</sup>) and at 6 levels of Pb in substrate: from (0–1,000 mg L<sup>-1</sup>) at the first day of the experiment, to (0–2,000 mg L<sup>-1</sup>) at the end of the experiment after gradual Pb additions after every sample collecting day. Methods used for analysing the plant material: the content of amino acid proline and photosynthetic pigments were determined by spectrophotometry; chlorophyll *a* fluorescence parameters – using continuous excitation chlorophyll fluorimeter. The fresh weight of plant above-ground parts and roots was detected. The growth and development of field beans was slightly influenced by increasing amount of Cd and Pb in substrate only at the end of the experiment. The highest Cd treatments (Cd20 and Cd25) caused 2.5 and 1.3 times increased proline concentration in bean leaves. The chlorophyll *a* + *b* content and chlorophyll *a* fluorescence parameter  $F_v/F_m$  changed differently throughout the experiment. In general, during the experiment, there was a tendency for the content of proline in leaves for Pb treatments to be increased compared to control. At the end of the experiment the content of proline in field bean leaves of the highest Pb treatments (Pb600 + 100 + 400 + 500, Pb800 + 100 + 400 + 500 and Pb 1,000 + 100 + 400 + 500) was 1.66, 1.44 and 1.55 times higher, respectively, than that of the control plant leaves. The negative impact of exposure to Pb on chlorophyll *a* + *b*, chlorophyll *a* fluorescence parameter  $P_{Index}$  and  $F_v/F_m$  in bean leaves was less pronounced compared to Cd. The obtained results confirm that field beans until their flowering stage can grow and develop in the presence of a large amount of Cd and Pb in substrate without significant growth inhibition and detrimental impact on physiological parameters, if optimal cultivation conditions are provided.

**Key words:** heavy metals, *Vicia faba*, photosynthesis parameters, proline.

### INTRODUCTION

With the development of agriculture and industry, research on the impact of soil contamination on crops is of vital importance not only in Europe but throughout the

world (Pourrut et al., 2011; Van Liedekerke et al., 2014, Fu et al., 2017). Plants directly take up heavy metals from the soil through the roots, so heavy metals can enter the food chain, thus affecting human health (Peralta-Videa et al., 2009). In Europe the most frequently occurring contaminant groups in soil are heavy metals and mineral oils – 35% and 24%, respectively (Van Liedekerke et al., 2014). Heavy metals (for example, Cd and Pb) can enter the soil as the result of human activities, for example, using plant fertilizers in agriculture (Grant et al., 1998; Peralta-Videa et al., 2009; Swartjes, 2011). Therefore, it is important to pay attention to the questions of phytoremediation – on how to recover the soil from heavy metal pollution. In this aspect it is relevant to find out plants with high capacity to accumulate heavy metals. Previous studies have shown that hyperaccumulants are able to accumulate up to 100 times more heavy metals in leaves than other plants (Rascio & Navari-Izzo, 2011). On the other hand, studies on tolerance/sensitivity and distribution models of heavy metals in plants are of great value also for crop plants taking into account food safety issues.

Increased amounts of heavy metals in the environment can cause detrimental changes in physiological and biochemical processes in plants, thus affecting growth and productivity (Loi et al., 2012; Loi et al., 2014). The impact of heavy metals on plant growth and development is species-specific and tissue specific, for example, in vegetables heavy metals reduce shoot – root biomass ratio (Martin et al., 2006). It is noteworthy that heavy metal transport from soil to plants are affected by different factors as soil properties (soil pH, organic matter content, granulometric composition etc.), plant species specificity, the physical and chemical properties of the elements, growing conditions, environmental biotic factors and others (Titov et al., 2007; Loi et al., 2014). It is known that under different environmental stress conditions, including heavy metal contamination, the plants may show changes both in the amount of chlorophyll *a + b* and in the chlorophyll *a* fluorescence parameters (Zarco-Tejada et al., 2002; Sayed, 2003; Pourrut et al., 2011). Heavy metal induced alteration in photosynthetic pigments influenced the biosynthesis of photoassimilates which resulted in decreased biomass production and stunted plant growth (Titov et al., 2007; Sengar et al., 2008; Capelo et al., 2012).

The changes in the accumulation of amino acid proline in plants under different biotic and abiotic stresses are widely documented (Hare & Cress, 1997; Kavi Kishor et al., 2005; Verbruggen & Hermans, 2008; Szabados & Savoué, 2010). The increased proline accumulation in plants can be caused by heavy metals, drought, oxidative stress, high light and UV irradiation, high salinity, high/low temperature, atmospheric pollution and also biotic stresses (Schat et al., 1997; Trovato et al., 2008; Verbruggen & Hermans, 2008; Szabados & Savoué, 2010; Karlsons, 2011). Proline accumulation in plants can also be a stress signal that affects plant adaptive responses like as stabilizing the structure of proteins, providing a way to buffer cytosolic pH and balance cell redox status (Verbruggen & Hermans, 2008). Schat et al. (1997) have reported that the plant water balance declines under exposure to heavy metals (especially Cd). In the conditions of osmotic stress the growth of plants is inhibited, stomata closed and the rate of photosynthesis decreased (Trovato et al., 2008). Proline as essential osmolite participates in osmotic stress tolerance protecting plant cells against osmotic stress damage (Hare & Cress, 1997; Trovato et al., 2008). The initial amount of proline in plants can be species specific (Verbruggen & Hermans, 2008).

According to the regulation of the European Union starting from 2015 farmers can get a payment for agricultural practices beneficial for the climate and the environment (called: ‘greening payment’) (Regulation (EU) No 1307/2013, 2013; Hart et al., 2017). Diversification of crops in Latvia is a mandatory requirement from 2015. The aim of diversification of crops is to promote enhanced environmental protection, especially in the area of soil quality improvement. Field beans are one of the crops used for ‘greening’ to improve soil quality in Latvia (The Rural Support Service, 2018). However, so far, there is little research on field bean physiological and adaptive responses to different stress conditions, including soil contamination with heavy metals.

The aim of this study was to investigate the impact of Cd and Pb pollution on growth and physiological parameters of *Vicia faba*, to find out the changes in biomass production, photosynthesis and chlorophyll *a* fluorescence parameters as well as changes in the accumulation of amino acid proline.

## MATERIALS AND METHODS

The vegetation experiment was carried out with the field bean (*Vicia faba*, cv. ‘WITKIEM’) as a model object. Plants were grown in 1 L polyethylene containers from seeds; quartz sand was used as a growing substrate. The following controlled conditions were provided for plant growth: photoperiod light/dark 16/8 h, moisture of substrate 60–65%, day/night temperature + 20/18 °C and a photon flux density of 160  $\mu\text{mol m}^{-2} \text{s}^{-1}$  supplied by fluorescent tubes. To ensure sufficient moisture level in the substrate, it was regularly gravimetrically watered using deionized water.

Changes in growth and physiological parameters were studied at five levels of Cd in substrate: 0, 10, 15, 20, 25  $\text{mg L}^{-1}$ . The experiment was arranged with the following 6 levels of Pb in substrate at the first day of the experiment: 0, 200, 400, 600, 800, 1,000  $\text{mg L}^{-1}$ . To find out changes in growth and physiological parameters under conditions of variable Pb amount in substrate, the amount of Pb in the substrate was gradually added after every sample collecting day during the experiment. Thereby, after the 14th, 21st, and 28th day of the experiment 100  $\text{mg L}^{-1}$  Pb, 400  $\text{mg L}^{-1}$  Pb and 500  $\text{mg L}^{-1}$  Pb, respectively, was added in substrate for every Pb treatment. Abbreviations of treatments for experiment with Pb supply in the substrate are given in the Table 1.

**Table 1.** Abbreviations of treatments for the experiment with Pb supply in the substrate in during the experiment

| The day of sampling   | 14       | 21             | 28                   | 35                         |
|-----------------------|----------|----------------|----------------------|----------------------------|
| Treatment 1 (control) | Pb 0     | Pb 0           | Pb 0                 | Pb 0                       |
| Treatment 2           | Pb 200   | Pb 200 + 100   | Pb 200 + 100 + 400   | Pb 200 + 100 + 400 + 500   |
| Treatment 3           | Pb 400   | Pb 400 + 100   | Pb 400 + 100 + 400   | Pb 400 + 100 + 400 + 500   |
| Treatment 4           | Pb 600   | Pb 600 + 100   | Pb 600 + 100 + 400   | Pb 600 + 100 + 400 + 500   |
| Treatment 5           | Pb 800   | Pb 800 + 100   | Pb 800 + 100 + 400   | Pb 800 + 100 + 400 + 500   |
| Treatment 6           | Pb 1,000 | Pb 1,000 + 100 | Pb 1,000 + 100 + 400 | Pb 1,000 + 100 + 400 + 500 |

Cd was added as  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  solution in substrate and Pb was added as  $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$  solution in substrate. Nutrient solution was added in substrate for control groups and for all treatment groups, it contained optimal concentrations of macronutrients and micronutrients (in  $\text{mg L}^{-1}$ : N 120, P 60, K 150, Ca 800, Mg 50, S 60, Mn 1.5, Zn 1, Cu 0.5, Mo 0.02, B 0.2, Fe 30) (Osvalde, 2011). Ca was added as two grams of  $\text{CaCO}_3$  in substrate of each container at the beginning of the experiment. As the Cd standard solution contains nitrogen, adjustments were done to prepare the complete nutrient solutions for Cd treatments by reducing the content of ammonium nitrate.

The experiment lasted 35 days; plants were collected on the day of 14th, 21st, 28th and 35th of the experiment. Field bean fresh weight was determined for all sampling dates. The fresh weight of plant above-ground parts and root system was separately detected.

The content of chlorophyll  $a + b$  was determined in the first fully expanded leaves of field beans. Photosynthetic pigments were determined by spectrophotometry method using JENWAY 6300 Spectrophotometer (JENWAY, UK). Pigments were extracted with 20 mL of 96% ethanol, extracts were centrifuged and absorbances were measured at 470, 649 and 664 nm. Lichtenthaler (1987) equation was used to calculate amount of chlorophyll  $a + b$ .

Chlorophyll  $a$  fluorescence parameters such as the variable fluorescence ( $F_v/F_m$ ) and Performance Index ( $P_{Index}$ ) were determined with continuous excitation chlorophyll fluorimeter Handy PEA system (Hansatech, UK). *Vicia faba* leaves were dark-adapted with leaf clips for 20 minutes.

The content of amino acid proline was determined by spectrophotometry method, adjusting the method with ninhydrin (Bates et al., 1973). Fresh plant material was homogenized in sulfosalicylic acid. Two milliliters of acid ninhydrin and glacial acetic acid was added to the extract, it was heated for 1 h at 100 °C and then the mixture was extracted into a toluol (Karlsons, 2011). Absorbances were measured at 520 nm using JENWAY 6300 Spectrophotometer (JENWAY, UK).

The statistical analysis of results was done using MS Excel 2013. Standard errors (SE) were calculated in order to reflect the mean of the results.

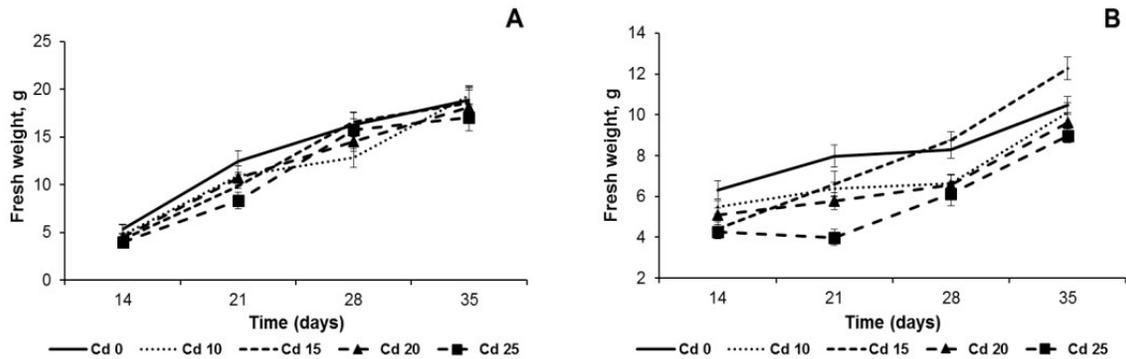
## RESULTS AND DISCUSSION

Accumulation of biomass is the key factor for evaluating plant responses to various environmental stresses. There were different changes in the fresh weight of the above-ground parts and roots of the *V. faba* under exposure to increasing Cd and Pb levels in sand substrate. Laboratory experiments showed that the fresh weight of the above-ground parts of field beans cultivated under different Cd treatments varied similarly to the control variant throughout the experiment (Fig. 1, A). In general, the effect of the Cd contamination in substrate on the fresh weight of the above-ground parts of field beans was found to be insignificant ( $p < 0.1$ ). Thus, on the last day of the experiment, the fresh weight of bean leaves and stems at the highest contamination level (Cd 25  $\text{mg L}^{-1}$ ) was only 1.81 g lower (i.e., 9.58% lower) than that of the control plants (Cd0).

Unlike to the above-ground parts, significant effect of Cd pollution in substrate on the fresh weight of roots was found ( $p < 0.05$ ). Field bean exposure to Cd10, Cd20 and Cd25 treatments lead to an increasing reduction in the fresh weight of roots throughout

the experiment (Fig. 1, B). The treatment of Cd 25 mg L<sup>-1</sup> resulted in the 1.51 g lower (i.e., 14.5% lower) fresh weight of roots than that of the control level at the last sampling time (the 35th day).

More pronounced inhibition on the fresh weight of roots under heavy metal pollution could be mainly explained by root direct contact with the contaminated substrate (Pourrut et al., 2011). Thus, the negative effects of Cd could first affect plant roots.



**Figure 1.** Fresh weight of the above-ground parts (A) and roots (B) of *V. faba* at five levels of Cd added in substrate,  $\pm$ SE.

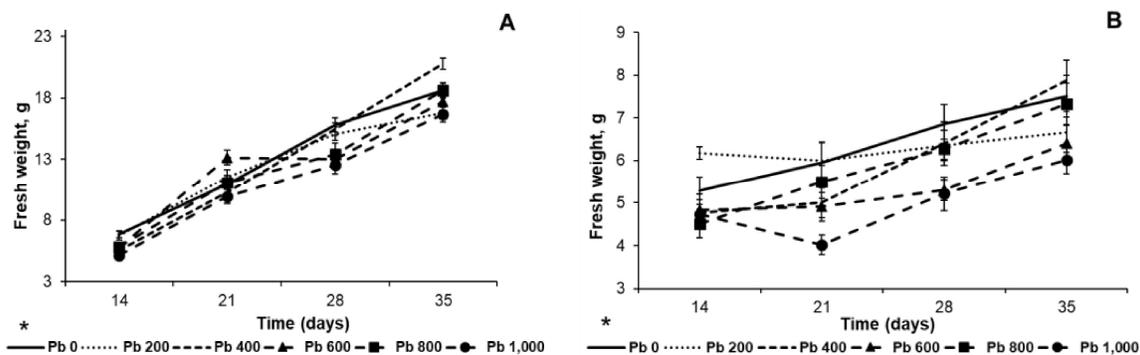
There was similar pattern of Pb impact on the fresh weight of above-ground parts and roots of *V. faba* as described for Cd treatments (Fig. 2, A, B) despite the fact that the level of Pb in the substrate during the experiment was gradually increased for all treatment variants.

Overall, a significant effect of Pb in substrate on fresh weight of field bean roots ( $p < 0.05$ ) was observed.

On the last day of the experiment, fresh weight of the above-ground parts was 10.44% lower than that of the control plants at the highest contamination level (Pb 1,000 + 100 + 400 + 500 mg L<sup>-1</sup>) in the substrate (Fig. 2, A). For roots this reduction reached 20% (Fig. 2, B).

Thus, no significant inhibitory effect of Cd and Pb on the fresh weight of the above-ground parts of *V. faba* was found until flowering stage. Only a slight trend of reduction was stated. In general, there is contradictory evidence about the effect of Cd on field bean weight. Sajwani et al. (1996) have found that Cd, Ni and Se did not affect the biomass on *Phaseolus vulgaris*. Likewise, Loi et al. (2014) have reported no significant Cd impact on the *Faba vulgaris* dry mass for treatments with Cd 0, 25, 50 and 75 mg L<sup>-1</sup> in substrate. Similar conclusion is made by Jin et al. (2017) in the study on effect of Cd stress on broad bean. In contrast, Simek & Tuma (2016) reported about stimulating impact of Cd (Cd 2 and 20 mg kg<sup>-1</sup>) on the production of *Phaseolus vulgaris* biomass.

The content of chlorophyll *a* + *b* in *V. faba* leaves varied throughout the experiment, both in treatment with Cd and Pb (Fig. 3, A, B). The negative impact of Cd on chlorophyll *a* + *b* in bean leaves was more pronounced compared to Pb.



\* Abbreviations;

Days

| 14       | 21             | 28                   | 35                         |
|----------|----------------|----------------------|----------------------------|
| Pb 0     | Pb 0           | Pb 0                 | Pb 0                       |
| Pb 200   | Pb 200 + 100   | Pb 200 + 100 + 400   | Pb 200 + 100 + 400 + 500   |
| Pb 400   | Pb 400 + 100   | Pb 400 + 100 + 400   | Pb 400 + 100 + 400 + 500   |
| Pb 600   | Pb 600 + 100   | Pb 600 + 100 + 400   | Pb 600 + 100 + 400 + 500   |
| Pb 800   | Pb 800 + 100   | Pb 800 + 100 + 400   | Pb 800 + 100 + 400 + 500   |
| Pb 1,000 | Pb 1,000 + 100 | Pb 1,000 + 100 + 400 | Pb 1,000 + 100 + 400 + 500 |

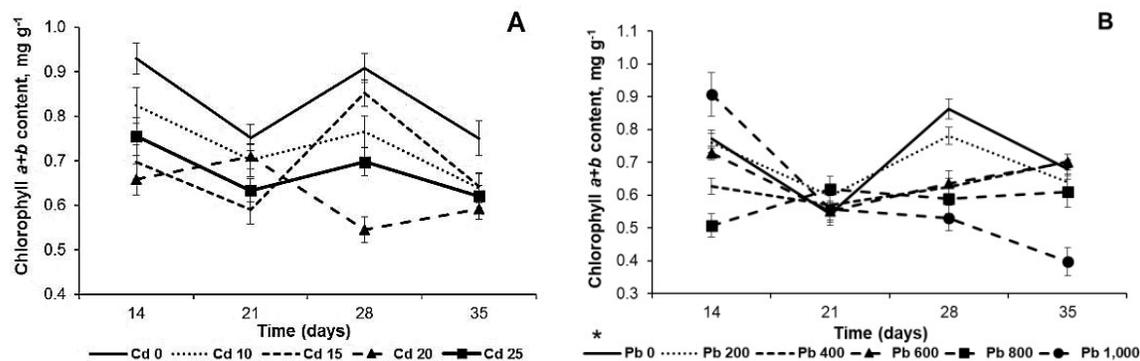
**Figure 2.** Fresh weight of the above-ground parts (A) and roots (B) of *V. faba* at 6 levels of Pb added in substrate,  $\pm$ SE.

In general, a significant effect of Cd pollution in the substrate on chlorophyll *a* + *b* content in leaves of *V. faba* ( $p < 0.05$ ) was observed. During the experiment, the chlorophyll *a* + *b* content in plant leaves for all Cd treatments was lower than that in control plants (Fig. 3, A).

Unlike to Cd, the content of the chlorophyll *a* + *b* in bean leaves for Pb treatments was lower than that of the control plants mainly in the second half of the experiment, that is, from the 28th to 35th day of the experiment (Fig. 3, B). It should be noted that, the chlorophyll *a* + *b* content in plants in the variants Pb400 + 100 and Pb600 + 100 changed uniformly from day 21 to day 35 of the experiment.

On the 21st day of the experiment, a decrease in the content of chlorophyll *a* + *b* was observed for almost all treatments (except only: Cd20, Pb800 + 100), including control. This may be due to a certain stage of plant development or probably due to the impact of environmental factors. After this point, the decrease in chlorophyll content was mainly found in the conditions of higher pollution doses.

According to Masarovičová et al. (2010), heavy metal toxicity resulted in reduction of total chlorophyll content and, consequently, in photosynthesis inhibition in plants. Pb and Cd was reported to disrupt photosynthesis by changed chloroplast ultrastructure, reduced synthesis of chlorophyll, plastoquinone, carotenoids, disturbed electron transport (Seregin & Ivanov, 2001; Cheng, 2003; Titov et al., 2007). In general, various stress conditions can also cause chlorophyll hydrolysis to chlorophyllides and phytol (Pshibytko et al., 2004). Loi et al. (2012) studies have shown that Cd in the substrate has a negligible effect on the photosynthesis process in the *Faba vulgaris*, indicating on the tolerance of beans to heavy metal pollution.



\* Abbreviations;

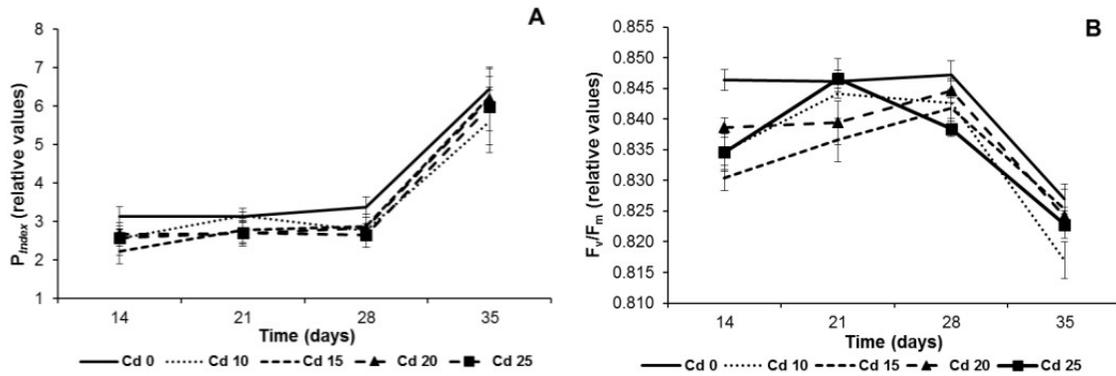
Days

| 14       | 21             | 28                   | 35                         |
|----------|----------------|----------------------|----------------------------|
| Pb 0     | Pb 0           | Pb 0                 | Pb 0                       |
| Pb 200   | Pb 200 + 100   | Pb 200 + 100 + 400   | Pb 200 + 100 + 400 + 500   |
| Pb 400   | Pb 400 + 100   | Pb 400 + 100 + 400   | Pb 400 + 100 + 400 + 500   |
| Pb 600   | Pb 600 + 100   | Pb 600 + 100 + 400   | Pb 600 + 100 + 400 + 500   |
| Pb 800   | Pb 800 + 100   | Pb 800 + 100 + 400   | Pb 800 + 100 + 400 + 500   |
| Pb 1,000 | Pb 1,000 + 100 | Pb 1,000 + 100 + 400 | Pb 1,000 + 100 + 400 + 500 |

**Figure 3.** Chlorophyll *a* + *b* content ( $\text{mg g}^{-1}$ ) in *V. faba* leaves at five levels of Cd (A) and at 6 levels of Pb (B) added in substrate,  $\pm$ SE.

Chlorophyll *a* fluorescence parameters are sensitive to changes in photosynthesis process depending on the environmental impact, so they are used to describe photosynthesis process of plants (Sayed, 2003; Kalaji et al., 2014). Chlorophyll *a* fluorescence parameter  $P_{Index}$  and  $F_v/F_m$  during the experiment changed differently depending on Cd level in the substrate (Fig. 4, A, B). The relative values of the parameter  $P_{Index}$  for all treatments (Cd10, Cd15, Cd20 and Cd25) were lower than that of the control variant. A similar situation was found for the relative values of the parameter  $F_v/F_m$ . On the 35th day of the experiment, there was a significant increase of the relative values of  $P_{Index}$  for all variants compared to the 28th day of the experiment:  $P_{Index}$  values increased 1.9 times for Cd0 plants, 2.0 times for Cd10, 2.2 times for Cd15 and Cd20, and 2.3 times for Cd25 (Fig. 4, A). Conversely, on the last day of the experiment, rapidly reduced values of  $F_v/F_m$  for plants of all variants were found: for control variant plants the  $F_v/F_m$  value decreased to 0.827, while for Cd treatments they ranged from 0.817 to 0.823 (Fig. 4, B). It is assumed that 0.8 is the limit of  $F_v/F_m$  value between optimal and stress conditions (Gailite, 2012). Since none of the treatments had a  $F_v/F_m$  value lower than 0.8, Cd as a stress factor probably did not cause photo-inhibition for field beans.

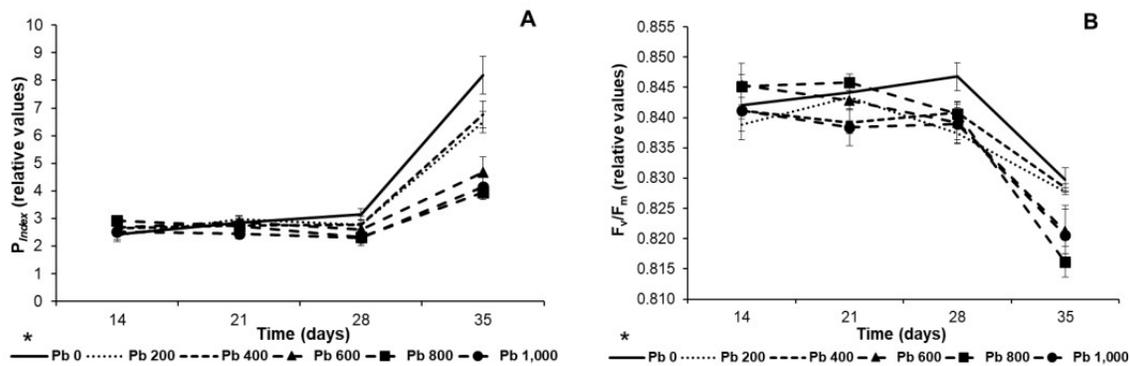
In general, a significant effect of Cd on  $P_{Index}$  values was observed during the experiment ( $p < 0.05$ ). As  $P_{Index}$  represents vitality of plants, our results showed that at the flowering phase (end of the experiment) vitality of the field beans increased for all Cd treatments, indicating possible adaptation to stress conditions.



**Figure 4.** Chlorophyll *a* fluorescence parameter  $P_{Index}$  (relative values) (A) and  $F_v/F_m$  (relative values) (B) of *V. faba* at five levels of Cd added in substrate,  $\pm$ SE.

It was found that the relative values of  $P_{Index}$  and  $F_v/F_m$  in the experiment with Pb pollution in the substrate changed similarly to those stated in the Cd experiment (Fig. 5, A, B). In general, the effect of Pb on changes in chlorophyll *a* fluorescence parameters were less pronounced than they were in the Cd variants even under conditions when Pb level of pollution in the substrate, gradually increased during the experiment, was very high reaching 1,200, 1,400, 1,600, 1,800, 2,000 mg L<sup>-1</sup> at the end of the experiment.

Thereby, field bean as an experimental model object showed high adaptation potential to stress conditions in the case of Cd and Pb contamination in the growing medium.



\* Abbreviations;

Days

| 14       | 21             | 28                   | 35                         |
|----------|----------------|----------------------|----------------------------|
| Pb 0     | Pb 0           | Pb 0                 | Pb 0                       |
| Pb 200   | Pb 200 + 100   | Pb 200 + 100 + 400   | Pb 200 + 100 + 400 + 500   |
| Pb 400   | Pb 400 + 100   | Pb 400 + 100 + 400   | Pb 400 + 100 + 400 + 500   |
| Pb 600   | Pb 600 + 100   | Pb 600 + 100 + 400   | Pb 600 + 100 + 400 + 500   |
| Pb 800   | Pb 800 + 100   | Pb 800 + 100 + 400   | Pb 800 + 100 + 400 + 500   |
| Pb 1,000 | Pb 1,000 + 100 | Pb 1,000 + 100 + 400 | Pb 1,000 + 100 + 400 + 500 |

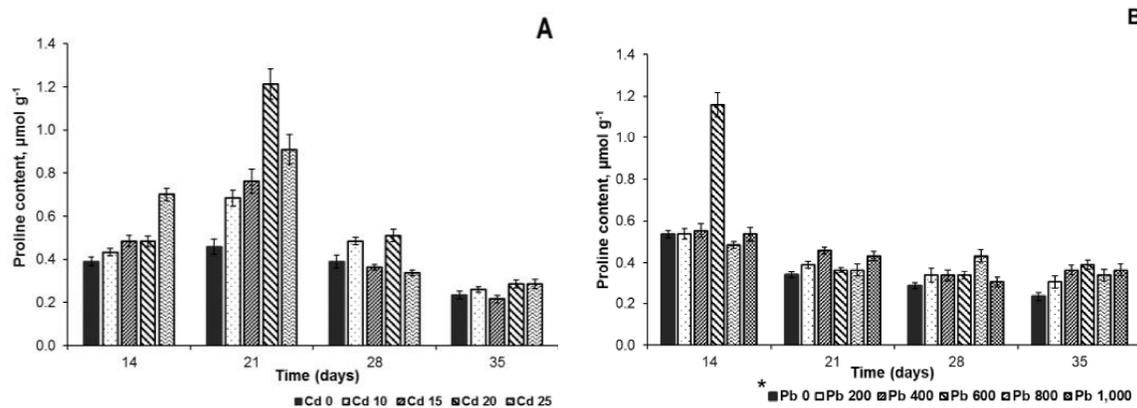
**Figure 5.** Chlorophyll *a* fluorescence parameter  $P_{Index}$  (relative values) (A) and  $F_v/F_m$  (relative values) (B) of *V. faba* at 6 levels of Pb added in substrate,  $\pm$ SE.

The research revealed a significant increase in the content of amino acid proline in the field bean leaves with increase of Cd in the substrate till the 21st day of the experiment (Fig. 6, A).

The highest Cd treatments Cd20 and Cd25 caused 2.5 and 1.3 times, respectively, increased proline concentration in *V. faba* leaves. Further in the course of the experiment, this plant biochemical response significantly decreased. Nevertheless, on the last day of the experiment, the content of proline in the treatments Cd20 and Cd25 was 22% higher than that in the control variant plants.

There are evidences in the literature (Loi et al., 2014) that the content of proline in field beans in Cd treatments (Cd 2, 5, 10, 20 and 50 mg kg<sup>-1</sup> in soil) is similar to the content of proline in the control variant plants on the 21st day of the experiment.

In experiment with Pb pollution, only one treatment (Pb600) induced massive accumulation of free proline in field bean leaves on the 14th day of the experiment. It is notable that in the conditions of Pb800 this impact was lost and the content of proline in the leaves was even 9.68% lower than that of the control plants (Fig. 6, B).



\* Abbreviations;

Days

| 14       | 21             | 28                   | 35                         |
|----------|----------------|----------------------|----------------------------|
| Pb 0     | Pb 0           | Pb 0                 | Pb 0                       |
| Pb 200   | Pb 200 + 100   | Pb 200 + 100 + 400   | Pb 200 + 100 + 400 + 500   |
| Pb 400   | Pb 400 + 100   | Pb 400 + 100 + 400   | Pb 400 + 100 + 400 + 500   |
| Pb 600   | Pb 600 + 100   | Pb 600 + 100 + 400   | Pb 600 + 100 + 400 + 500   |
| Pb 800   | Pb 800 + 100   | Pb 800 + 100 + 400   | Pb 800 + 100 + 400 + 500   |
| Pb 1,000 | Pb 1,000 + 100 | Pb 1,000 + 100 + 400 | Pb 1,000 + 100 + 400 + 500 |

**Figure 6.** Proline content ( $\mu\text{mol g}^{-1}$ ) in *V. faba* leaves at five levels of Cd (A) and at six levels of Pb (B) added in substrate,  $\pm$ SE.

On the 35th day of the experiment the content of proline in the field bean leaves of the highest treatments: Pb600 + 100 + 400 + 500, Pb800 + 100 + 400 + 500 and Pb 1,000 + 100 + 400 + 500 was 66.67%, 44.44% and 55.56%, respectively, higher than that of the control plant leaves. In general, during the experiment, there was a tendency for the content of proline in the leaves for Pb treatment variants to be increased compared to control. Thus, Cd and Pb showed impact on proline biosynthesis in *V. faba* leaves. Increased proline accumulation could be regarded as *V. faba* adaptive response to Cd and Pb pollution in growing medium. Pb induced increase in proline content was also reported for cowpea (*Vigna unguiculata*) (Krishnaveni et al., 2015).

## CONCLUSIONS

Summarizing the results, it can be concluded that in laboratory experiment in sand substrate cultivated *V. faba* showed different changes in the fresh weight of the above-ground parts and roots under increasing Cd and Pb levels in substrate. It is evident that both Cd and Pb significantly decreased root fresh weight, while Cd pollution had negative effect also on the fresh weight of above-ground parts. Increase of Cd and Pb in the substrate showed slight impact on the photosynthetic performance of *V. faba*. The negative impact of exposure to Cd on chlorophyll *a + b*, chlorophyll *a* fluorescence parameter  $P_{Index}$  and  $F_v/F_m$  in bean leaves was more pronounced compared to Pb. In general, chlorophyll *a* fluorescence parameter  $P_{Index}$  and  $F_v/F_m$  during the experiment changed differently depending on Cd and Pb level in the substrate and plant development stage. Since none of the treatments had a  $F_v/F_m$  value lower than 0.8, Cd and Pb as a stress factor probably did not cause photo-inhibition for field beans. According to the results, Cd and Pb showed stimulative impact on proline biosynthesis in *V. faba* leaves. Increased proline accumulation could be regarded as *V. faba* adaptive response to Cd and Pb pollution in growing medium. Thereby, field beans showed high adaptation potential to stress conditions in the case of Cd and Pb contamination in the growing medium.

As the results on the content of Cd and Pb in the above-ground parts and roots of the field beans is still in the process of determining, discussion about possible use of field beans for forage or in phytoremediation from/of polluted territories is the subject of next paper.

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## REFERENCES

- Bates, L.S., Waldren, R.P. & Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil* **39**(1), 205–207.
- Capelo, A., Santos, C., Loureiro, S. & Pedrosa, M.A. 2012. Phytotoxicity of lead on *Lactuca sativa*: Effects On growth, mineral nutrition, photosynthetic activity and oxidant metabolism. *Fresenius Environmental Bulletin* **21**(2a), 450–459.
- Cheng, S. 2003. Effect of Heavy Metals on Plants and Resistance Mechanisms. *Environmental Science and Pollution Research* **10**(4), 256–264.
- Fu, W., Huang, K., Cai, H.H., Li, J., Zhai, D.L., Dai, Z.C. & Du, D.L. 2017. Exploring the Potential of Naturalized Plants for Phytoremediation of Heavy Metal Contamination. *International Journal of Environmental Research* **11**(4), 515–521.
- Gailite, A. 2012. Physiological and genetic aspects of conservation of Estonian Saw-wort (*Saussurea esthonica*). Riga, 93 pp. (in Latvian).
- Grant, C.A., Buckley, E.T., Bailey, L.D. & Selles, F. 1998. Cadmium accumulation in crops. *Canadian Journal of Plant Science* **78**(1), 1–17.
- Hare, P. & Cress, W. 1997. Metabolic implications of stress induced proline accumulation in plants. *Plant Growth Regulation* **21**(2), 79–102.
- Hart, K., Mottershead, D., Tucker, G., Underwood, E. & Maréchal, A. 2017. *Evaluation study of the payment for agricultural practices beneficial for the climate and the environment. Final Report*. European Commission. Alliance Environnement and the Thünen Institute, Luxembourg: Publications Office of the European Union, 248 pp.

- Jin, C., Nan, Z., Wang, H., Li, X., Zhou, J., Yao, X. & Jin, P. 2018. Effect of Cd stress on the bioavailability of Cd and other mineral nutrition elements in broad bean grown in a loess subsoil amended with municipal sludge compost. *Environmental Science and Pollution Research* **25**(8), 7418–7432.
- Kalaji, H.M., Schansker, G., Ladle, R.J., Goltsev, V., Bosa, K., Allakhverdiev, S.I., Brestic, M., Bussotti, F., Calatayud, A., Dąbrowski, P., Elsheery, N.I., Ferroni, L., Guidi, L., Hogewoning, S.W., Jajoo, A., Misra, A.N., Nebauer, S.G., Pancaldi, S., Penella, C., Poli, D., Pollastrini, M., Romanowska-Duda, Z.B., Rutkowska, B., Serôdio, J., Suresh, K., Szulc, W., Tambussi, E., Yanniccari, M. & Zivcak, M. 2014. Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. *Photosynthesis Research* **122**, 121–158.
- Karlsons, A. 2011. *Adaptive mechanisms of mineral nutrition and peculiarities of supply of mineral elements to marine coastal plants*. Riga, 128 pp. (in Latvian).
- Kavi Kishor, P.B., Sangam, S., Amrutha, R.N., Sri Laxmi, P., Naidu, K.R., Rao, K.R.S.S., Sreenath Rao, Reddy, K.J., Theriappan, P. & Sreenivasulu, N. 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Current Science* **88**(2), 424–438.
- Krishnaveni, M., Kumar, J.S. & Sharvanan, P.S. 2015. Influence of lead on biochemicals and proline contents of *Vigna unguiculata* (L.) Walp. *International Journal of Plant Sciences* **10**(2), 142–151.
- Lichtenthaler, H.K. 1987. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Methods in Enzymology* **148**, 350–382.
- Loi, N.N., Gubareva, O.S., Stepanchikova, N.S & Sanzharova, N.I. 2012. Effect of Cadmium Pollution of Sod-Podzolic Soil on Growth and Development of Broad Beans. *Russian Agricultural Sciences* **38**(5–6), 374–376.
- Loi, N.N, Sanzharova, N.I. & Gubareva, O.S. 2014. Effects of Cadmium Pollution of Chernozemic Soils on the Growth and Development of Field Beans. *Russian Agricultural Sciences* **40**(3), 198–201.
- Martin, D., Vollenweider, P., Buttler, A. & Günthardt-Goerg, M.S. 2006. Bioindication of heavy metal contamination in vegetable gardens. *Forest Snow and Landscape Research* **80**(2), 169–180.
- Masarovičová, E., Cicák, A. & Štefančík, I. 1999. Plant Responses to Air Pollution and Heavy Metal Stresses. In Pessaranli, M. (ed.): *Handbook of Plant and Crop Stress*. Marcel Dekker, Inc., New York, pp. 569–598.
- Osvalde, A. 2011. Optimization of plant mineral nutrition revisited: the roles of plant requirements, nutrient interactions, and soil properties in fertilization management. *Environmental and Experimental Biology* **9**, 1–8.
- Peralta-Videa, J.R., Lopez, M.L., Narayan, M., Saupe, G. & Gardea-Torresdey, J. 2009. The biochemistry of environmental heavy metal uptake by plants: Implications for the food chain. *The International Journal of Biochemistry & Cell Biology* **41**, 1665–1677.
- Pourrut, B., Shahid, M., Dumat, C., Winterton, P. & Pinelli, E. 2011. *Lead uptake, toxicity, and detoxification in plants*. Reviews of Environmental Contamination and Toxicology. Springer Verlag, **213**, pp. 113–136.
- Pshibytko, L.N., Kalitukho, N.L., Zhavoronkova, B.N. & Kabashnikova, F.L. 2004. The pool of chlorophyllous pigments in barley seedlings of different ages under heat shock and water deficit. *Russian Journal of Plant physiology* **51**(1), 15–20.
- Rascio, N. & Navari-Izzo, F. 2011. Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Science* **180**, 169–181.

- Regulation (EU) No 1307/2013 of the European Parliament and of the Council of 17 December 2013 establishing rules for direct payments to farmers under support schemes within the framework of the common agricultural policy and repealing Council Regulation (EC) No 637/2008 and Council Regulation (EC) No 73/2009. *Official Journal of the European Union* **L 347**, 20.12.2013, 608–670.
- Sajwani, K.S., Ornes, W.H., Youngblood, T.V. & Alva, A.K. 1996. Uptake of soil applied cadmium, nickel and selenium by bush beans. *Water, Air, and Soil Pollution* **91**(3-4), 209–217.
- Sayed, O.H. 2003. Chlorophyll fluorescence as a tool in cereal crop research. *Photosynthetica* **41**, 321–330.
- Schat, H., Sharma, S.S. & Vooijs, R. 1997. Heavy metal-induced accumulation of free proline in a metal-tolerant and a nontolerant ecotype of *Silene vulgaris*. *Physiologia Plantarum* **101**, 477–482.
- Sengar, R.S., Gautman, M., Sengar, R.S., Garg, S.K., Sengar, K., Chaudhary, Y.R. 2008. *Lead Stress Effects on Physiobiochemical Activities of Higher Plants*. In Whitacre, D.M. (ed.) *Reviews of Environmental Contamination and Toxicology*. Springer Science + Business Media, **196**, pp.73–87.
- Seregin, I.V. & Ivanov, V.B. 2001. Physiological Aspects of Cadmium and Lead Toxic Effects on Higher Plants. *Russian Journal of Plant Physiology* **48**(4), 523–544.
- Simek, J. & Tuma, J. 2016. Response of *Phaseolus vulgaris* plants to cadmium with different accompanying anions exposure. *Fresenius Environmental Bulletin* **25**(9), 3781–3788.
- Swartjes, F.A. 2011. *Introduction to Contaminated Site Management*. In Swartjes, F.A. (ed.): *Dealing with Contaminated Sites: From Theory towards Practical Application*. Springer, pp. 3–90.
- Szabados, L. & Savoué, A. 2010. Proline: a multifunctional amino acid. *Trends in Plant Science* **15**(2), 89–97.
- Titov, A.F., Talanova, V.V., Kaznina, N.M. & Laidinen, G.F. 2007. *Resistance of plants to heavy metals*. Institute of Biology Karelian Research Center of the Russian Academy of Sciences. Petrozavodsk: Karelian Research Center of the Russian Academy of Sciences, 172 pp. (in Russian).
- The Rural Support Service 2018. Facility for receiving area payments. [http://www.lad.gov.lv/files/2018\\_info\\_materials\\_11aprilis2018.pdf](http://www.lad.gov.lv/files/2018_info_materials_11aprilis2018.pdf) Accessed 13.01.2019. (in Latvian).
- Trovato, M., Mattioli, R. & Costantino, P. 2008. Multiple Roles of Proline in Plant Stress Tolerance and Development. *Rendiconti Lincei* **19**, 325–346.
- Van Liedekerke, M., Prokop, G., Rabl-Berger, S., Kibblewhite, M. & Louwagie, G. 2014. *Progress in the Management of Contaminated Sites in Europe*. European Environmental Agency, Luxembourg, 68 pp.
- Verbruggen, N. & Hermans, C. 2008. Proline accumulation in plants: a review. *Amino Acids* **35**(4), 753–759.
- Zarco-Tejada, P.J., Miller, J.R., Mohammed, G.H., Noland, T.L. & Sampson, P.H. 2002. Vegetation Stress Detection through Chlorophyll *a + b* Estimation and Fluorescence Effects on Hyperspectral Imagery. *Journal of Environmental Quality* **31**, 1433–1441.