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# IMPACT OF SHORT-TERM EXPOSURE TO LEAD AND CADMIUM OF COMMON BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH) SEEDLINGS GROWN IN HYDROPONIC CULTURE\*

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## ABSTRACT

In a model hydroponic experiments, the impact of short-term exposure to lead ( $Pb^{2+}$ ) and cadmium ( $Cd^{2+}$ ) in a nutrient medium on seedlings of two cultivars of common buckwheat (*Fagopyrum esculentum* Moench) has been examined. Cadmium and lead were added separately into the nutrient medium (Hoagland solution) in concentrations of 0 (control), 0.01, 0.10 or 1.00 mM. To evaluate the effect of the examined metal ions, the elongation of shoots and primary root of buckwheat seedlings, proline and malonic dialdehyde (MDA) content, as well as the level of photosynthetic pigments were measured. This study concludes that the presence of Pb and Cd in a nutrient medium causes reduction in biochemical activities and distinctly inhibits the growth of buckwheat seedlings. Cadmium and lead ions had a more inhibitory effect on the growth of roots than shoots of buckwheat seedlings. Increasing the concentration of both metals caused an increase in the content of malonic dialdehyde and proline in all examined organs of seedlings. Short-term exposure of seedlings to cadmium and lead ions stimulated the accumulation of *a* and *b* chlorophylls, and carotenoids in buckwheat cotyledons. Highly significant correlation between elongation of primary root and proline level, and elongation and MDA content indicate that roots are particularly sensitive to the presence of lead and cadmium in nutrient medium. Common buckwheat is known to be a lead hyper-accumulator, and the results obtained in this study confirm it. Buckwheat seedlings show higher resistance to lead in a nutrient medium than to cadmium.

**Keywords:** common buckwheat, growth, lead, cadmium, proline, MDA, chlorophyll.

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## INTRODUCTION

Among the heavy metals, lead (Pb) and cadmium (Cd) belong to the most important pollutants (PAGE, FELLER 2015). Soil contamination with Pb and Cd can result in loss of vegetation, and toxicity to microorganisms, animals and humans (GUPTA et al. 2013). Soils along roads are particularly contaminated with lead, because for many years it was added as tetraethyllead (TEL) to gasoline as an antiknock agent. The addition of TEL to gasoline has been banned in most countries since 2005, although environmental pollution with lead is still high. Other important sources of lead pollution are industrial processing, leaching of metals from solid and animal waste, as well as municipal sewage. Lead contaminated soil causes a decrease in crop yields, thus being a serious problem for agriculture (GHANI 2010, NAGAJYOTI et al. 2010). The presence of cadmium (Cd) in the environment is associated with its use in industry and agriculture. Cadmium is one of the most phytotoxic heavy metals due to its high water solubility and ease of uptake by plants (DALCORSO et al. 2008). Even low concentrations have a negative effect on metabolism, growth and development of plants (DALCORSO et al. 2010, NAGAJYOTI et al. 2010).

Soil is the major source of Cd and Pb reaching the plants. Plants take them up and then dispose or store the pollutants in their tissues (SEREGIN, IVANOV 2001). The heavy metals favor the production of free radicals and reactive oxygen species in the plant, which leads to oxidative stress (SYTAR et al. 2013, EMAMVERDIAN et al. 2015). This causes lipid peroxidation, and malonic dialdehyde (MDA) is formed during this process (SKÓRZYŃSKA-POLIT 2007). MDA affects the properties of cell membranes and impairs their permeability and transport. As a result, it interferes with the proper functioning of the cell (VERMA, DUBEY 2003, SKÓRZYŃSKA-POLIT 2007).

Proline is secreted under stress, and may be involved in the neutralization of heavy metals. It is recognized as a general indicator that allows to determine the impact of adverse factors on plant development. This amino acid has defensive functions and allows plants to adapt to adverse environmental conditions (LEHMANN et al. 2010).

Common buckwheat is recognized as a hyperaccumulator of lead, and therefore when grown in contaminated soil it can accumulate a large amount of Pb without significant changes in physiological functions (TAMURA et al. 2005, NIKOLIC et al. 2010). Thus, this species is a suitable candidate for cultivation on heavy metals polluted soils (FRANZARING et al. 2018). Certainly, the efficient antioxidant system, of which the flavonoids present there are an important part, is responsible for such properties of this species. During our previous study, it was found that the presence of low concentrations of  $Pb^{2+}$  and  $Cd^{2+}$  (0.1 mM) in a nutrient medium resulted in slight stimulation of the growth of seedlings of three buckwheat cultivars, while high doses (1mM) inhibited root growth and, to a much lesser extent, shoots of the seedlings

(HORBOWICZ et al. 2013). Overall, cadmium was more harmful for the growth of buckwheat seedlings than lead.

The present study aimed to identify the extent of changes in root and shoot elongation as well as content of proline, MDA and photosynthetic pigments in tissues of common buckwheat seedlings due to lead and cadmium presence in a nutrient medium.

## MATERIAL AND METHODS

### Plant material

Seedlings of common buckwheat (*Fagopyrum esculentum* Moench) cv. Kora and Panda were used in this study. Germination was carried out for four days in darkness at  $24\pm 1^\circ\text{C}$  by placing buckwheat seeds between two layers of wet filter paper, as was described earlier (HORBOWICZ et al. 2008). Four-day seedlings were standardized with respect to size, transplanted to the Hoagland's nutrient solution and exposed for 7 days to various concentrations of  $\text{Pb}^{2+}$  (as  $\text{Pb}(\text{NO}_3)_2$ ) added to this medium. One-fifth Hoagland's nutrient solution was used to limit the interaction between them and lead ions, which could affect the availability of  $\text{Pb}^{2+}$  for the plants. Phosphorus ions ( $\text{KH}_2\text{PO}_4$ ) were excluded from the nutrient medium in all Pb treatments to prevent the precipitation of lead phosphate. In the experiment with cadmium ( $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ ), the Hoagland's nutrient solution included  $\text{KH}_2\text{PO}_4$ . The experiments were conducted in controlled conditions at a temp.  $24\pm 2^\circ\text{C}$  during days (16 h) and  $18\pm 2^\circ\text{C}$  during nights (8 h). Photosynthetically active radiation ( $100 - 120 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was provided by high-pressure sodium lamps (400 W, Plantaster, Osram, Germany). Before and after 7 days of growth of buckwheat seedlings under these conditions, the lengths of the shoot and the primary root were measured. Shoot and primary root elongation was calculated for each buckwheat seedling as the difference between plant length before and after the experiment. Elongation measurements were carried out for each seedling. The mean results of 5 replicates (one replicate was mean of 10 seedlings) for each concentration of Pb and Cd in the medium were statistically elaborated.

### Chemical analyses

The rate of the lipid peroxidation process was analyzed on the basis of measurements of the malondialdehyde (MDA) in freeze dried plant tissues, after homogenization with 80% ethanol. The reaction with thiobarbituric acid (TBA) was carried out by heating in  $95^\circ\text{C}$  for 0.5 h. After cooling, the samples were centrifuged (0.5 h, 3,000 g), absorbance at 440 nm, 532 and 600 nm was measured, and appropriate formulas were used to the calculation of MDA (HODGES et al. 1999).

For determination of the free proline content, a ninhydrin-based standard method was applied (BATES 1973). Proline was extracted with 3% sulphosalicylic acid and the product reaction with ninhydrine was determined spectrophotometrically at 520 nm.

Chlorophyll *a* and *b*, and total carotenoid content were analyzed in 80% acetone extracts of freeze dried buckwheat cotyledons. Samples were extracted for 24 h at room temperature in darkness with occasional shaking, and after filtration were quantified on a spectrophotometer (645 nm, 883 nm and 470 nm) using the formulas published by LICHTENTHALER and WELBURN (1983). All spectrophotometric analyses were performed in five replicates using a spectrophotometer UV-1800 UV/Vis (Rayleigh).

### Statistical analyses

Biochemical analyses were performed in five replicates. One-way analysis of variance (ANOVA) and the Tukey's post hoc test were used to check the significance of differences between treated and untreated fruit tissues. We also calculated the linear correlation coefficient between the elongation of seedlings' organs and the proline and MDA content in the hypocotyl and primary root, and also between the proline and MDA content in cotyledons, hypocotyl and primary root of buckwheat seedlings.

## RESULTS AND DISCUSSION

The presence of cadmium and lead in soil is of concern because it inhibits plant growth, thus leading to lower yield, and also causing a decline in crop quality (GHANI 2010, NAGAJYOTI et al. 2010). NATARAJAN et al. (2018) noted that the exposure to cadmium inhibited the growth of roots and shoots of tomato seedlings, but the effect on the shoot length was stronger than on the root length. The results obtained in our hydroponic experiment confirm previous reports. However, a low concentration of  $Pb^{2+}$  (0.01 mM) in the nutrient medium slightly stimulated the growth of shoots and roots of seedlings of both buckwheat cultivars (Figure 1). A similar concentration of  $Cd^{2+}$  clearly inhibited the growth of these parts of buckwheat seedling (Figure 2), although not statistically significantly. In the case of cadmium, no such effect was observed.

Particularly significant influence of  $Pb^{2+}$  and  $Cd^{2+}$  was found on the root growth. The highest dose of Pb (1.00 mM) inhibited elongation of the primary root in both cultivars by over 60%, relative to control plants, and in the case of Cd the reduction reached over 70%. Under the same concentration, Pb reduced the shoot growth by 10% (statistically not significant). In contrast, 1 mM of Cd inhibited elongation of the shoot by 40%.

Common buckwheat is recognized as a hyperaccumulator of lead (TAMURA et al. 2005, FRANZARING et al. 2018). Proline is a component of non-specific

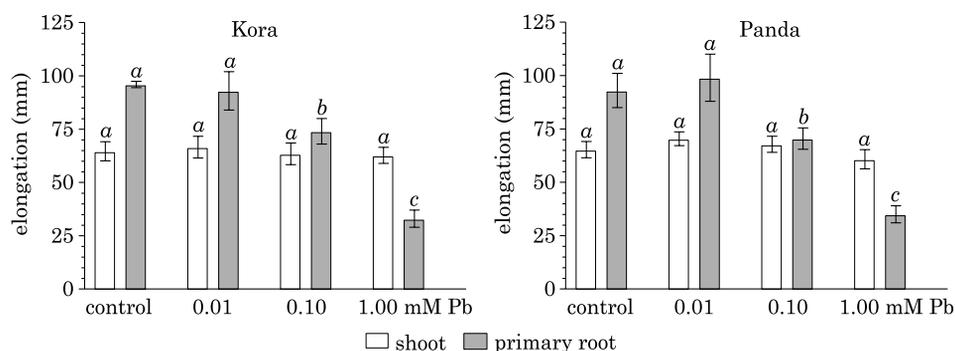


Fig. 1. Effect of lead concentrations in a nutrient medium on elongation of the shoot and primary root of common buckwheat seedlings. Results marked with the same letter were not significantly different at  $P < 0.05$  (Tukey's post hoc test). Comparisons were made within both parts of buckwheat seedling and within each cultivar separately

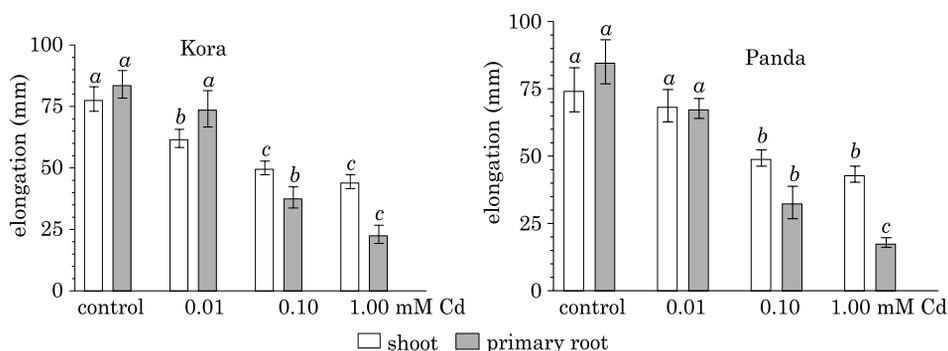


Fig. 2. Effect of cadmium concentrations in a nutrient medium on elongation of the shoot and primary root of common buckwheat seedlings. Results marked with the same letter were not significantly different at  $P < 0.05$  (Tukey's post hoc test). Comparisons were made within both parts of buckwheat seedling and within each cultivar separately

defense systems towards various plant stresses, including heavy metals' toxicity. HAYAT et al. (2013) showed that exogenous application of proline alleviates the damaging effects of Cd in plants, thereby enhancing the growth and photosynthesis. In our study, lead and cadmium ions in the nutrient medium affected proline levels in all tissues of buckwheat seedlings (Table 1). The highest increase in proline under the influence of Pb occurred in cotyledons, and the lowest was in hypocotyls. At the highest concentration of lead, proline levels increased by 89% and by 66% in the Kora and Panda cotyledons, respectively. But the same concentration caused a smaller increase in the level of proline in hypocotyls, raising it by about 30%. The highest concentration of lead in the nutrient medium caused an increase in the proline content by 50% - 55% in the roots of the evaluated cultivars. Similarly, the proline content was increased under the influence of Pb in *Vigna mungo* L. seedlings (SINGH et al. 2012), and of many other plant species (SEREGIN, IVANOV 2001, ZENGIN, MUNZUROGLU 2005).

Table 1

Effect of lead and cadmium concentrations in a nutrient medium on the content of proline in tissues of common buckwheat seedlings (mg g<sup>-1</sup> FW).

Cultivar	Tissue analysed	Control	0.01 mM	0.10 mM	1.00 mM
Lead (Pb <sup>2+</sup> )					
Kora	cotyledons	1.42 ± 0.18 <sup>c*</sup>	1.53 ± 0.18 <sup>c</sup>	2.08 ± 0.22 <sup>b</sup>	2.66 ± 0.18 <sup>a</sup>
	hypocotyl	1.18 ± 0.11 <sup>a</sup>	1.25 ± 0.13 <sup>a</sup>	1.27 ± 0.11 <sup>a</sup>	1.41 ± 0.11 <sup>a</sup>
	primary root	1.63 ± 0.09 <sup>c</sup>	1.88 ± 0.10 <sup>bc</sup>	2.15 ± 0.14 <sup>ab</sup>	2.43 ± 0.10 <sup>a</sup>
Panda	cotyledons	2.23 ± 0.15 <sup>c</sup>	2.48 ± 0.22 <sup>bc</sup>	2.85 ± 0.18 <sup>b</sup>	3.69 ± 0.22 <sup>a</sup>
	hypocotyl	1.32 ± 0.13 <sup>b</sup>	1.43 ± 0.18 <sup>b</sup>	1.27 ± 0.11 <sup>b</sup>	1.71 ± 0.15 <sup>a</sup>
	primary root	1.67 ± 0.10 <sup>b</sup>	1.87 ± 0.17 <sup>b</sup>	2.22 ± 0.10 <sup>a</sup>	2.57 ± 0.10 <sup>a</sup>
Cadmium (Cd <sup>2+</sup> )					
Kora	cotyledons	1.26 ± 0.09 <sup>b</sup>	1.60 ± 0.06 <sup>b</sup>	2.83 ± 0.31 <sup>a</sup>	3.59 ± 0.25 <sup>a</sup>
	hypocotyl	0.96 ± 0.06 <sup>b</sup>	0.94 ± 0.09 <sup>b</sup>	1.34 ± 0.34 <sup>ab</sup>	1.56 ± 0.24 <sup>a</sup>
	primary root	1.49 ± 0.13 <sup>d</sup>	1.88 ± 0.09 <sup>c</sup>	2.28 ± 0.13 <sup>b</sup>	2.67 ± 0.15 <sup>a</sup>
Panda	cotyledons	1.98 ± 0.10 <sup>d</sup>	2.92 ± 0.25 <sup>c</sup>	3.42 ± 0.06 <sup>b</sup>	4.19 ± 0.09 <sup>a</sup>
	hypocotyl	1.09 ± 0.07 <sup>b</sup>	0.96 ± 0.09 <sup>b</sup>	1.50 ± 0.10 <sup>a</sup>	1.59 ± 0.18 <sup>a</sup>
	primary root	1.75 ± 0.09 <sup>d</sup>	2.19 ± 0.13 <sup>c</sup>	2.60 ± 0.13 <sup>b</sup>	3.02 ± 0.11 <sup>a</sup>

\* Results marked with the same letter were not significantly different at  $P < 0.05$  (Tukey's post hoc test). Comparisons were made within each part of buckwheat seedling and within each cultivar separately.

Proline levels in cotyledons of buckwheat seedlings growing on a medium containing 1 mM Cd<sup>2+</sup> increased by 184% in cv. Kora, and by 198% in cv. Panda (Table 1). The same cadmium concentration in the medium increased the proline level by 64% in the hypocotyl of cv. Kora, and by 46% in cv. Pand. The lowest Cd<sup>2+</sup> concentration (0.01 mM) slightly stimulated proline accumulation in seedling roots, while the highest one (1 mM) caused an increase in the proline content by about 75-80%. Cotyledons turned out to be the most sensitive to Cd ions, and hypocotyls were the least vulnerable. The results obtained by ZENGİN and MUNZUROĞLU (2005) also showed that lead, copper, cadmium and mercury caused the proline accumulation in leaves of bean seedlings. Our results achieved for common buckwheat confirm previous reports. However, the stimulating effect of cadmium ions on proline accumulation is much higher than that of lead. NATARAJAN et al. (2018) found that the proline content in roots of tomato plants exposed to Cd was higher than in the shoots. In our study, the highest increase in the proline content occurred in cotyledons, slightly lower was in the roots and the lowest rise was noted in the hypocotyl of buckwheat seedlings.

Lead and cadmium ions are known to induce lipid peroxidation in several plant species (ZHANG et al. 2007, SINGH et al. 2010, SYTAR et al. 2013). Lipid

peroxidation causes disturbance in the efficiency of biological membranes, and also increases their permeability. The results obtained in our study indicated that as the concentrations of Pb and Cd increased, the MDA content in all buckwheat organs showed a rising trend. Lead ions at a concentration higher than 0.1 mM induced a significant increase in the malondialdehyde (MDA) content in all examined vegetative organs of buckwheat seedlings (Table 2). The highest concentration of Pb and Cd (1mM) caused about a 2-fold increase in the MDA content in the cotyledons and roots of buckwheat seedlings, and about a 1.5-fold rise in the hypocotyl. However, in the case of Cd, significant stimulation of the MDA formation in cotyledons and roots was found even at the lowest concentration of this element. These results confirm previous data obtained in studies of other species (NAGAJYOTI et al. 2010, SYTAR et al. 2013, EMAMVERDIAN et al. 2018). It should only be noted that hypocotyl tissues accumulated much less MDA than other organs. The phenomenon of greater accumulation of stress factors in cotyledons and roots than in the hypocotyl probably has the same or similar causes. The reason is probably that the physiological and metabolic role of leaves and roots is certainly greater than that of the hypocotyl. A similar conclusion was drawn in our previous study regarding the effect of Pb and Cd on the flavonoid content in buckwheat tissues (HORBOWICZ et al. 2013)

Table 2

Effect of lead and cadmium concentrations in a nutrient medium on the content of malonic diladehyde (MDA) in tissues of common buckwheat seedlings (nmol g<sup>-1</sup> FW).

Cultivar	Tissue analysed	Control	0.01 mM	0.10 mM	1.00 mM
Lead (Pb <sup>2+</sup> )					
Kora	cotyledons	2.95 ± 0.42 <sup>bc*</sup>	3.30 ± 0.42 <sup>b</sup>	4.91 ± 0.35 <sup>a</sup>	6.11 ± 0.21 <sup>a</sup>
	hypocotyl	2.31 ± 0.38 <sup>c</sup>	2.65 ± 0.25 <sup>bc</sup>	3.45 ± 0.42 <sup>ab</sup>	3.66 ± 0.34 <sup>a</sup>
	primary root	2.78 ± 0.15 <sup>c</sup>	3.09 ± 0.41 <sup>c</sup>	4.58 ± 0.26 <sup>b</sup>	5.66 ± 0.36 <sup>a</sup>
Panda	cotyledons	3.16 ± 0.28 <sup>c</sup>	3.65 ± 0.28 <sup>bc</sup>	4.70 ± 0.35 <sup>b</sup>	5.75 ± 0.49 <sup>a</sup>
	hypocotyl	1.89 ± 0.28 <sup>b</sup>	2.44 ± 0.33 <sup>ab</sup>	2.69 ± 0.38 <sup>ab</sup>	3.15 ± 0.21 <sup>a</sup>
	primary root	2.93 ± 0.21 <sup>c</sup>	3.40 ± 0.31 <sup>c</sup>	4.38 ± 0.38 <sup>b</sup>	5.30 ± 0.15 <sup>a</sup>
Cadmium (Cd <sup>2+</sup> )					
Kora	cotyledons	4.24 ± 0.28 <sup>d</sup>	5.44 ± 0.30 <sup>c</sup>	6.62 ± 0.36 <sup>b</sup>	7.84 ± 0.45 <sup>a</sup>
	hypocotyl	1.89 ± 0.28 <sup>b</sup>	2.44 ± 0.33 <sup>ab</sup>	2.69 ± 0.38 <sup>a</sup>	3.15 ± 0.21 <sup>a</sup>
	primary root	2.75 ± 0.16 <sup>d</sup>	3.67 ± 0.13 <sup>c</sup>	4.52 ± 0.26 <sup>b</sup>	5.77 ± 0.33 <sup>a</sup>
Panda	cotyledons	2.89 ± 0.35 <sup>d</sup>	3.86 ± 0.26 <sup>c</sup>	4.99 ± 0.32 <sup>b</sup>	6.62 ± 0.39 <sup>a</sup>
	hypocotyl	1.88 ± 0.28 <sup>b</sup>	2.21 ± 0.50 <sup>ab</sup>	2.76 ± 0.25 <sup>a</sup>	3.14 ± 0.55 <sup>a</sup>
	primary root	3.08 ± 0.33 <sup>d</sup>	4.07 ± 0.20 <sup>c</sup>	5.77 ± 0.33 <sup>b</sup>	7.07 ± 0.39 <sup>a</sup>

\* Results marked with the same letter were not significantly different at  $P < 0.05$  (Tukey's post hoc test). Comparisons were made within each part of buckwheat seedling and within each cultivar.

The content of chlorophylls in plants is often measured in order to assess the impact of heavy metals in soil, and in most of papers a decrease in chlorophyll in photosynthetic tissues has been noted (ZENGİN, MUNZUROĞLU 2005, SINGH et al. 2012). In contrast, the results of our research indicate that short-term exposure of buckwheat seedlings to Pb in the medium stimulates the accumulation of chlorophylls *a* and *b*, and to a lesser extent carotenoids (Table 3). Even the lowest concentration (0.01 mM) of lead or cadmium

Table 3

Effect of lead and cadmium concentrations in a nutrient medium on the content of photosynthetic pigments in cotyledons of common buckwheat seedlings ( $\mu\text{g}^{-1}$  FW).

Cultivar	Pigment analysed	Control	0.01 mM	0.10 mM	1.00 mM
Lead ( $\text{Pb}^{2+}$ )					
Kora	Chlorophyll <i>a</i>	1041 $\pm$ 31 <sup>a*</sup>	1278 $\pm$ 31 <sup>c</sup>	1402 $\pm$ 41 <sup>ab</sup>	1495 $\pm$ 103 <sup>a</sup>
	Chlorophyll <i>b</i>	378.6 $\pm$ 14.3 <sup>c</sup>	446.4 $\pm$ 10.7 <sup>b</sup>	489.3 $\pm$ 17.9 <sup>ab</sup>	525.0 $\pm$ 32.1 <sup>a</sup>
	Carotenoids	219.4 $\pm$ 27.4 <sup>c</sup>	285.7 $\pm$ 5.5 <sup>b</sup>	313.1 $\pm$ 13.7 <sup>ab</sup>	322.3 $\pm$ 20.6 <sup>a</sup>
Panda	Chlorophyll <i>a</i>	1093 $\pm$ 82 <sup>b</sup>	1237 $\pm$ 41 <sup>a</sup>	1258 $\pm$ 51 <sup>a</sup>	1278 $\pm$ 62 <sup>a</sup>
	Chlorophyll <i>b</i>	417.9 $\pm$ 32.1 <sup>b</sup>	457.1 $\pm$ 17.9 <sup>ab</sup>	460.7 $\pm$ 28.6 <sup>ab</sup>	471.4 $\pm$ 28.6 <sup>a</sup>
	Carotenoids	253.7 $\pm$ 16.0 <sup>b</sup>	283.4 $\pm$ 5.5 <sup>ab</sup>	292.6 $\pm$ 11.4 <sup>a</sup>	297.1 $\pm$ 18.3 <sup>a</sup>
Cadmium ( $\text{Cd}^{2+}$ )					
Kora	Chlorophyll <i>a</i>	1011 $\pm$ 85 <sup>c</sup>	1234 $\pm$ 53 <sup>b</sup>	1468 $\pm$ 85 <sup>a</sup>	1479 $\pm$ 64 <sup>a</sup>
	Chlorophyll <i>b</i>	436.9 $\pm$ 72.8 <sup>b</sup>	534.0 $\pm$ 48.5 <sup>ab</sup>	621.4 $\pm$ 68.0 <sup>a</sup>	626.2 $\pm$ 58.3 <sup>a</sup>
	Carotenoids	178.5 $\pm$ 36.7 <sup>b</sup>	261.7 $\pm$ 26.9 <sup>ab</sup>	322.8 $\pm$ 19.6 <sup>a</sup>	322.8 $\pm$ 22.0 <sup>a</sup>
Panda	Chlorophyll <i>a</i>	1170 $\pm$ 74 <sup>c</sup>	1372 $\pm$ 53 <sup>b</sup>	1425 $\pm$ 96 <sup>b</sup>	1649 $\pm$ 127 <sup>a</sup>
	Chlorophyll <i>b</i>	504.9 $\pm$ 43.7 <sup>b</sup>	568.0 $\pm$ 43.7 <sup>ab</sup>	601.9 $\pm$ 53.3 <sup>ab</sup>	699.0 $\pm$ 77.7 <sup>a</sup>
	Carotenoids	254.3 $\pm$ 39.1 <sup>b</sup>	283.7 $\pm$ 14.7 <sup>b</sup>	300.8 $\pm$ 44.0 <sup>ab</sup>	357.1 $\pm$ 24.4 <sup>a</sup>

\* Results marked with the same letter were not significantly different at  $P < 0.05$  (Tukey's post hoc test). Comparisons were made within each pigment and within each cultivar.

in the medium resulted in a significant increase in the level of this pigment. A similar relationship was found for chlorophyll *b*: as the concentration of metals in the medium increased, the level of chlorophyll *b* also increased. A high lead concentration (1 mM) increased the chlorophyll *b* content in cotyledons by 42% and 17% in the cultivars Kora and Panda, respectively. A similar relationship, although less distinct, was observed regarding the levels of total carotenoids. The phenomenon of increasing the content of these photosynthetic pigments under the influence of Pb and Cd was more pronounced in cv. Kora than cv. Panda.

These results seem surprising, but they should be combined with information about possible accumulation of large amounts of Pb in buckwheat tissues (TAMURA et al. 2005). However, a new finding in our research is that cadmium also stimulates the content of photosynthetic pigments in buck-

wheat cotyledons, and that this species may have the ability to accumulate of Cd as well.

There are known plant species which are hyperaccumulators of Pb and Cd (TANG et al. 2009). Under the hydroponic culture, the chlorophyll concentrations in *Arabis paniculata* were stimulated up to moderate levels of Pb (97  $\mu\text{M}$ ), and high levels of Cd (up to 267  $\mu\text{M}$ ). The authors suggest that the increase in the chlorophyll content was partially due to the increase of the Mg uptake. Cd treatments were demonstrated to result in an increase of the chlorophyll content in *Sedum alfredii* (ZHOU, QIU 2005). Recently YAN et al. (2019) found that *Microsorium fortunei* could accumulate Cd up to 2249  $\mu\text{g g}^{-1}$  DM in roots under a 15-day treatment, with little Cd translocation into leaves. The authors also noted that in such conditions leaves of *M. fortunei* could maintain their normal physiological functions with no impairment of photosynthesis. Considering the results obtained by us and published scientific data, it should be assumed that the species *Fagopyrum esculentum* probably had broader phytoremediation properties, thus confirming an earlier report (FRANZARING et al. 2018).

Table 4 summarizes the results of calculations of the significance of correlation between the growth of roots and shoots of buckwheat seedlings,

Table 4

Linear correlation coefficient between the elongation of seedlings' organs and proline, elongation of seedlings' organs and MDA and between proline and MDA in cotyledons, hypocotyl and primary root of buckwheat seedlings ( $N=5$ )

	Proline	MDA	Proline	MDA
	Pb		Cd	
Shoot elongation	-0.3921 <sup>NS</sup>	-0.5412 <sup>NS</sup>	-0.8976*	-0.9829**
Primary root elongation	-0.9278**	-0.9500**	-0.9515**	-0.9809**
MDA, cotyledons	0.6420 <sup>NS</sup>	-	0.5867 <sup>NS</sup>	-
MDA, hypocotyl	0.3339 <sup>NS</sup>	-	0.8743*	-
MDA, root	0.9657**	-	0.9879**	-

NS – non-significant ( $P>0.05$ ), \* significant ( $P\leq 0.05$ ), \*\* highly significant ( $P\leq 0.01$ )

and some indicators of stress associated with the presence of Pb and Cd in the nutrient medium. The results indicate a close negative association of the growth of the primary root with the content of MDA and proline. Concerning the hypocotyl elongation, a significant negative relationship occurred only under the influence of cadmium. Furthermore, there was a significant positive correlation between MDA and proline levels in the roots, both under the influence of Pb and Cd.

Highly significant ( $P\leq 0.01$ ) correlation coefficients between the elongation of the primary root and the proline and MDA content indicate that roots are particularly sensitive to the presence of lead and cadmium in a nutrient

medium. This is confirmed by the results showing stronger inhibition of the root than shoot growth by Cd and Pb. Therefore, plant stress due to exposure to both heavy metals seems to be the most severe in roots of common buckwheat (SYTAR et al. 2013, EMAMVERDIAN et al. 2015).

## CONCLUSIONS

This study concludes that presence of  $Pb^{2+}$  and  $Cd^{2+}$  in a nutrient medium causes reduction in biochemical activities, and inhibits growth of common buckwheat seedlings. Cd and Pb ions had a more inhibitory effect on the root than on shoot growth of buckwheat seedlings. Increasing the concentration of both metals caused an increase in the content of malonic dialdehyde and proline in all examined organs of seedling. Short-term exposure of seedlings to cadmium and lead ions stimulated the accumulation of *a* and *b* chlorophylls and carotenoids in buckwheat cotyledons. Highly significant correlations between the elongation of the primary root and the proline level, or the elongation and the MDA content indicate that roots are particularly sensitive to the presence of lead and cadmium in a nutrient medium. Common buckwheat is known to be a lead hyperaccumulator, and the results obtained in this experiment confirm it. Buckwheat seedlings show higher resistance to lead in a nutrient medium than to cadmium.

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