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**ORIGINAL PAPER** 

# EFFECTS OF PRE-SOWING TREATMENT WITH SYNTHETIC AND NATURAL PLANT GROWTH REGULATORS ON THE RESPONSE OF MAIZE (ZEA MAYS L.) TO LEAD STRESS\*

Dmitry I. Bashmakov<sup>1</sup>, Alexandr S. Lukatkin<sup>1</sup>, Jurga Miliauskienė<sup>2</sup>, Giedrė Samuolienė<sup>2</sup> Laisvūnė Duchovskienė<sup>2</sup>, Arkadiusz Stępień<sup>1</sup>, Pavelas Duchovskis<sup>2</sup>

 <sup>1</sup> Department of Botany, Physiology and Ecology of Plants Mordovia State University, Saransk, Russia
<sup>2</sup> Institute of Horticulture
Lithuanian Research Centre for Agriculture and Forestry, Babtai, Kaunas distr., Lithuania
<sup>3</sup> Department of Agroecosystems and Horticulture
University of Warmia and Mazury in Olsztyn, Poland

#### Abstract

Effects of the pre-treatment of maize seeds with synthetic (Thidiazuron (TDZ), Cytodef, Kinetin, Epin-extra) and natural (Ribav-extra) plant growth regulators (PGRs) on maize (*Zea mays* L. cultivar Tzaritza) seedlings exposed to lead (Pb<sup>2+</sup>) were investigated in this research. The influence of PGRs on Pb<sup>2+</sup> allocation in maize root tissues, the seedlings' axial growth, and the activity of catalase (CAT) in leaves was determined. The seeds of maize were treated with different PGRs and grown for 7 days on 1  $\mu$ M, 10  $\mu$ M, 0.1 mM, 1 mM Pb<sup>2+</sup> solutions. At the end of the experiment, the growth of shoot and root, and the activity of catalase (CAT) in maize leaves were recorded. To detect the effect of PGRs on Pb<sup>2+</sup> allocation in maize root tissues, the seeds were treated with solutions containing different PGRs, then 5-day-old seedlings were treated with 1 mM Pb<sup>2+</sup> for 3 days. Transverse root slices were stained with dithizone and observed under a microscope. The results showed that the pre-treatment of maize seeds with solutions containing different PGRs of Pb<sup>2+</sup> in root tissues. The effect of the pre-sowing treatment of seeds with each PGR on seedlings growth depended on the concentra-

Arkadiusz Stępień, PhD DSC, prof. UWM, Department of Agroecosystems and Horticulture, University of Warmia and Mazury in Olsztyn, Pl. Łódzki 3, 10-718 Olsztyn, Poland, phone: 48 89 523 32 66, e-mail: arkadiusz.stepien@uwm.edu.pl

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tion of  $Pb^{2+}$  in the growth medium. The pre-sowing treatment of seeds with Epin-extra, Ribav-extra and Kinetin was the most effective for axial growth of maize under a high (0.1 and 1 mM)  $Pb^{2+}$  concentration, whereas the pre-sowing treatment of seeds with Epin-extra was effective for CAT activity in maize leaves under higher  $Pb^{2+}$  exposure. The efficiency of PGRs against high  $Pb^{2+}$  concentrations decreased as follows: Epin-extra  $\approx$  Ribav-extra > Kinetin > Cytodef > TDZ.

Keywords: axial organs, catalase, growth, pre-treatment, root, Zea mays L.

## INTRODUCTION

Heavy metal pollution is a very serious problem because of the increasing anthropogenic interference with the environment (Duchovskis et al. 2006, ANJUM et al. 2015). Many plant species, including agricultural crops, are able to accumulate larger amounts of heavy metals, resulting in their accumulation in the food chain (ALI, KHAN 2019). Many of them are indispensable microelements for plants, since they participate in a wide range of enzymatic redox reactions. Lead  $(Pb^{2+})$  is a non-essential element that is unnecessary or even toxic for plants but is actively involved in cell metabolism (KUMAR et al. 2017).  $Pb^{2+}$  is known to interact with proteins, to inhibit plant growth, to impact water regime and metabolism, etc. (KAUR et al. 2012, GUPTA et al. 2013, KUMAR et al. 2017). The main detrimental functional change in plants under  $Pb^{2+}$  stress is alteration in the balance of reactive oxygen species (ROS), like superoxide anion  $(O_{2})$ , hydroxyl radical (OH), hydrogen peroxide  $(H_{a}O_{a})$ , and antioxidants including enzymatic (catalase (CAT), peroxidase (POD), superoxide dismutase (SOD)) and non-enzymatic ones (glutathione, ascorbic acid etc.) in plant cells. Under normal conditions, oxidative processes occur in cellular compartments, but the activity of the antioxidant system and the amount of ROS are balanced. The interrelation between prooxidants and antioxidants varies depending on stressful environmental factors and a change in this ratio triggers plant cell damage responses. In plants, the enzymatic and non-enzymatic defence system due to a response to Pb<sup>2+</sup> stress is being actively studied (MAŁKOWSKI et al. 2020).

Nowadays, there are several methods how to improve a deleterious environment. Different solutions, including biologically active substances such as plant growth regulators (PGRs) are extensively used in modern agriculture to neutralize the harmful effects of environmental stresses on plants (LUKATKIN et al. 2003, LUKATKIN et al. 2007, SYTAR et al. 2019). Biologically active substances are able of modify the response of plants to abiotic stressors. There are numerous studies investigating the modification of heavy metal absorption using biologically active substances in order to improve phytoremediation of contaminated soils. With these objectives, ethylenediaminetetraacetic acid and other chelating agents (TASSI et al. 2008, HADI et al. 2010), plant hormones (SYTAR et al. 2019), natural regulators in rhizobacteria (DENTON 2007) or synthetic PGRs (LOPEZ et al. 2009) have been applied.

Synthetic PGRs usually used for plant stress resistance are cytokinin--like substances and brassinolide-based PGRs. Among the cytokinins, Kinetin was the first to be discovered and has been widely used in plants for its growth-promoting and antiaging properties as well as the ability to promote cell division and differentiation. It can confer resistance to plants against various abiotic stresses, including the toxicity of heavy metals. PGR thidiazuron (TDZ) was originally used as a defoliant, but this phenyl-urea also presents a cytokinin activity, commonly used in tissue culture to promote plant organogenesis and regeneration. Another PGR Cytodef is a synthetic derivative of the natural cytokinin diphenylurea, which is highly effective against plant stress. Its effectiveness is manifested by reducing the effect of salinity, low temperatures, drought, and heavy metal stress on plants. There are a few research papers dealing with the application of cytokinin-like PGRs as an agent strengthening the resistance of plants to heavy metals. Treatment of maize (Zea mays L.) seeds with Kinetin alleviated the negative symptoms of heavy metals (zinc and nickel): improved root and shoot growth, decreased electrolyte leakage from leaf discs, and increased activity of ascorbate peroxidase (LUKATKIN et al. 2007). Preliminary treatment of cucumber (Cucumis sativus L.) and winter wheat (Triticum aestivum L.) seedlings with synthetic TDZ completely or partially prevented Pb- and Cu-induced stimulation of electrolyte leakage from cotyledon segments (LUKATKIN et al. 2003, SAZANOVA et al. 2012). Synthetic brassinolide-based PGRs, such as Epin-extra (contains 50 µM of 24-epibrassinolide), control plant growth and development, have a wide range of stimulant and protective properties, can increase resistance to adverse effects and promote productivity of crops. Natural PGR Ribay-Extra is a product of the metabolism of mycorrhizal fungi isolated from the roots of ginseng (Panax ginseng C.A. Mey); it stimulates the synthesis of phytohormones, growth processes, and can increase plant resistance to stressors (GRUZNOVA et al. 2017, 2018). Thus, based on the properties of PGRs and previous studies (GRUZNOVA et al. 2017, 2018), increased resistance of plants to heavy metals can be expected in the treatment of seeds and/or seedlings by PGRs.

Maize (Zea mays L.) is one of the oldest plants cultivated in the world; and ranks third after wheat and rice. It is one of the most popular foods in the world and widely used in the industry. This study aimed to evaluate the efficiency of a pre-sowing maize seed treatment with synthetic and natural PGRs on the Pb<sup>2+</sup> allocation in root tissues, CAT activity and growth of maize seedlings exposed to Pb<sup>2+</sup> stress, as well as to determine the efficiency of PGRs against Pb<sup>2+</sup> concentrations by calculating the PGRs efficiency index. In general, we hypothesized that PGRs may alter Pb<sup>2+</sup> absorption and distribution in the maize roots, thereby altering the effect of Pb<sup>2+</sup> on the antioxidant effects in maize cells and on the growth of maize roots and shoots; and the efficiency of different PGRs can be calculated based on a previously developed universal index of effectiveness (IE).

## MATERIALS AND METHODS

Maize (*Zea mays* L.) of the cultivar Tzaritza was used in this experiment. It is a medium-late cultivar with the following traits:190-210 cm in height, inferior corncob height 70 cm, 1000 grains mass 180-200 g, and crop yield 17 t ha<sup>-1</sup>. The seeds were purchased commercially.

### **Experimental design**

Maize seeds were treated with 0.5%  $\text{KMnO}_4$  for 5 min to sterilize the surface, thereafter with solutions containing PGRs for 4-8 h (the optimum treatment time and concentration of PGR were matched in preliminary calibration experiments) – Table 1. Control seeds were treated with distilled Table 1

Plant growth substances	Chemical formula	Effective concen- tration for maize	Treatment time (h)
Synthetic plant growth regulators:			
Thidiazuron (TDZ)	N-phenyl-N-1,2,3-thiadiazol-5-ylurea	10 nM	8
Cytodef	N-(1,2,4-triazol-4-yl)-i"-phenylurea	0.1 µM	8
Kinetin	i-(2-furanylmethyl)-1H-purin-6-amine	1 μΜ	6
Epin-extra (24-EB)	(1S,2R,4R,5S,7S,11S,12S,15R,16S)-15-[(2S,3R,4R,5R)- 3,4-dihydroxy-5,6-dimethylheptan-2-yl]-4,5-dihy- droxy-2,16-dimethyl-9-oxatetracyclo[9.7.0.02,7.012,16] octadecan-8-one	1 µM	6
Natural plant growth regulator:			
Ribav-extra	metabolic product of ginseng roots mycorrhizal fungus	10 ppm	4

Plant growth regulators (PGRs) used in the research

water. Seeds were germinated and grew in plastic pots (50 seeds per pot) in water (50 ml per pot) or Pb(NO<sub>3</sub>)<sub>2</sub> solutions (from 1  $\mu$ M to 1 mM, 50 ml per pot), at temp. 22-24°C, photoperiod 16/8 h (day/night), photosynthetic photon flux density 80  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> for 7 days. On the 7th day, the length of axial organs of seedlings and CAT activity in leaves were measured. To detect the effects of the PGRs pre-treatment of seeds on Pb allocation in root tissues, the seeds were treated with solutions containing PGRs for 4-8 h and germinated on Petri dishes (10 seeds per pot) in water. Half of 5-day seedlings were exposed to 1 mM Pb<sup>2+</sup> for 72 h.

### Histochemistry

After 5-day exposure to 1 mM Pb<sup>2+</sup>, transverse root slices were prepared and stained with diphenyltiocarbazone (dithizone) (SEREGIN, IVANOV 1997).

To prepare the dithizone solution, just prior to slicing, 3 mg of diphenyltiocarbazone was dissolved in 6 ml of acetone adding 2 ml distilled water and 0.2 ml ice-cold acetic acid. Cross-slices of maize roots in different zones were prepared manually using a razor blade. Cross-slices were maintained in the dithizone solution for 3 minutes, then rinsed well in distilled water for the same time and observed under a microscope Lumam R8 (Lomo, Russia) at ×300 magnification. Some slices were photographed with a digital camera connected to the microscope. Localization of Pb ions (Pb<sup>2+</sup>) was identified by red-coloured tissues. Stronger colour intensity shows more intense accumulation of Pb<sup>2+</sup>.

#### **Detection of catalase (CAT) activity**

Leaf disks (1 g) were homogenized in 10 ml of 50 mM phosphate buffer (pH 7.0). The homogenate was filtered and centrifuged for 10 min at 8000 g, and the supernatant was collected and used as enzyme extract. Then, 2.9 ml of phosphate buffer (pH 7.0) was added to 25 µl of enzyme extract. Shortly before the measurement, 90 µl of 3% hydrogen peroxide was added to the solution. The optical density decrease during 1 min was measured using a spectrophotometer UV-mini1240 (Shimadzu, Japan) at  $\lambda = 240$  nm. The activity of CAT was calculated by adopting a molar extinction coefficient ( $\varepsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$ ) in µM g<sup>-1</sup> min<sup>-1</sup> (LUKATKIN 2002).

### **Effectiveness of PGRs**

To find out the most efficient PGR, the previously developed universal index of effectiveness (IE) was applied (GRUZNOVA et al. 2017). IE was calculated according to the formula:  $IE = (\pm |P1 - 100| \pm |P2 - 100| \pm ... \pm |Pn - 100|) / n$ , where P1, P2, etc., are the measured parameters (% compared to untreated plants that are considered 100%); n is the number of parameters considered in the calculation of IE. We marked the difference with a "+" sign if the PGR effect was positive and a "-" sign if the last one was negative. The following gradation was used to indicate the PGRs efficiency: 1) 0 < IE < 20 - very low (vl), 2) 21 < IE < 40 - low (l), 3) 41 < IE < 60 - moderate (m), 4) 61 < IE < 80 above moderate (am), 5) 81 < IE < 100 - high (h), 6) IE > 100 - very high (vh).

#### Statistical analysis

All experiments were conducted in triplicate, and each experiment consisted of 150 seeds or seedlings. All biochemical analyses were performed in 3 biological and 3 analytical replications. For all measurements, the averages and standard errors were calculated in Microsoft Excel (Microsoft Inc., USA). Differences between means were assessed by ANOVA by the Duncan's multiple range test at  $P \leq 0.05$  using the software MS Excel Analysis Tool-Pak and Statistica, version 12 (StatSoft Inc., USA).

## RESULTS

### Allocation of Pb<sup>2+</sup> in root tissues

In this research, the effects of plant growth regulators (PGRs) on the Pb<sup>2+</sup> distribution in root tissues of maize seedlings exposed to a high Pb<sup>2+</sup> concentration (1 mM) for 72 h were observed. After 3 days of exposure to 1 mM Pb<sup>2+</sup>, Pb ions deposited into the 4-5 layers of the exoderm (both cell wall and cytoplasm). The strong saturation was observed in the cytoplasm of some mesoderm and endoderm cells. Also, the cells of the pericycle, phloem, xylem, and stele parenchyma coloured, indicating the presence of significant amounts of Pb<sup>2+</sup> (Figure 1). Pre-treatment of maize seeds with solutions containing different PGRs caused significant modifications in the allocation of Pb<sup>2+</sup>

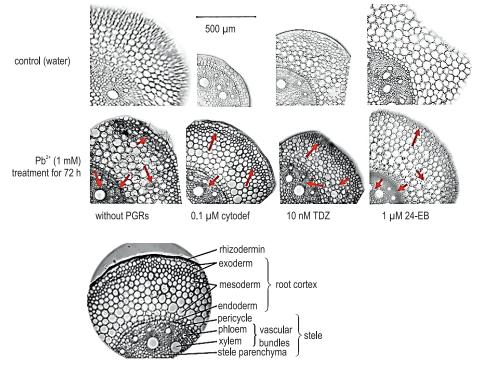


Fig. 1. Allocation of lead ions (Pb<sup>2+</sup>) in maize root tissues in relation to plant growth regulators (PGRs). Pb-accumulating cells and tissues are indicated by red arrows; TDZ – Thidiazuron, 24-EB – Epin-extra

in root tissues. In the plants whose seeds had been treated with 0.1  $\mu$ M Cytodef, Pb<sup>2+</sup> accumulated into the 2–3 layers of the ectoderm and in some cells of the mesoderm (into the cell wall only). Moreover, a certain amount of Pb<sup>2+</sup> was observed in the parenchymal cells of the stele. However, there was no intensive staining of vascular bundles, indicating the accumulation of Pb<sup>2+</sup> rather than long-distance transport through the plant. In general,

slice staining due to  $Pb^{2+}$  accumulation was less intense compared to control plants. In plants whose seeds had been treated with 10 nM TDZ, the colouring of slices was much more intense compared to the control plants except for vascular root bundles. Abundance of  $Pb^{2+}$  was found in the cells of the root cortex, especially in the exoderm and rhizodermis, and the pericycle. In the root cortex, both the strong colouring of cell walls and the entire cytoplasm was detected. Finally, the pre-treatment of maize seeds with 1  $\mu$ M Epin-extra caused strong accumulation of  $Pb^{2+}$  in cells of the pericycle and 1-2 layers of the ectoderm cells (Figure 1). Cells of untreated plants. Another feature of Epin-extra pre-treatment is that  $Pb^{2+}$  was bound to concretions visible on the surface (cell walls) of the cortex, especially in the endoderm and rhizoderm cells.

### Effects of Pb<sup>2+</sup> and PGRs on catalase (CAT) activity

As compared to control plants,  $Pb^{2+}$  induced CAT activity in maize plants by a factor of 1.6–1.75 under all studied  $Pb^{2+}$  concentrations (Figure 2).

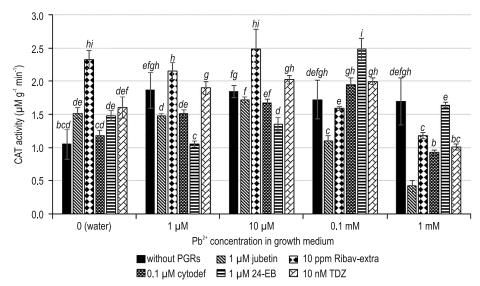


Fig. 2. The effects of lead ions (Pb<sup>2+</sup>) on catalase (CAT) activity in maize leaves in relation to synthetic and natural plant growth regulators (PGRs). Different letters indicate differences between means assessed by the Duncan's multiple range test at  $P \leq 0.05$ 

The activity of CAT tended to decrease with an increasing  $Pb^{2+}$  concentration in the growth medium (differences between  $Pb^{2+}$  concentrations were insignificant). Almost all applied PGRs, except Cytodef, tended to induce CAT activity in maize leaves growing without  $Pb^{2+}$  treatment. Under  $Pb^{2+}$  exposure, the effect of the pre-treatment of seeds with each PGR on CAT activity in maize leaves depended on the  $Pb^{2+}$  concentration in growth medium. Weak  $Pb^{2+}$  solutions (1 or 10  $\mu$ M) showed a positive effect on CAT activity in maize whose seeds were pre-treated with Ribav-extra (35% compared to plants untreated by PGRs). Against 0.1 mM Pb<sup>2+</sup>, seed pre-treatment with Epin-extra was the most efficient (44%) for CAT activity in maize seedling leaves. Finally, against the highest Pb<sup>2+</sup> concentration (1 mM), only the pre-treatment of seeds with Epin-extra was effective for CAT activity in maize seedling leaves, as it remained at the level of water control.

## Effects of Pb<sup>2+</sup> and PGRs on the growth of axial organs

The effect of seed pre-treatment with PGRs on the growth of maize seedlings exposed to  $Pb^{2+}$  were evaluated by roots and shoots growth rate of pre-treated and non-pre-treated plants (Figure 3). In most studied cases,

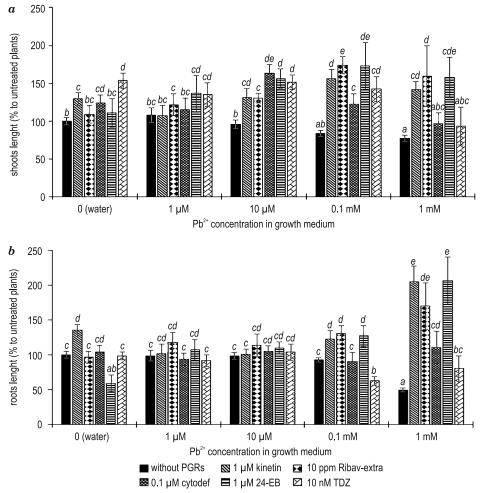


Fig. 3. The effects of lead ions (Pb<sup>2+</sup>) on the growth of maize seedlings in relation to plant growth regulators (PGRs), a – shoot length, b – root length. Different letters indicate differences between means assessed by the Duncan's multiple range test at  $P \leq 0.05$ 

the pre-treatment of seed with PGRs stimulated the elongation of axial organs of maize plant. The pre-sowing treatment with PGRs Epin-extra and TDZ mostly stimulated the growth of seedling shoots exposed to the weakest Pb<sup>2+</sup> concentration (1  $\mu$ M, 37% and 35%, respectively). The application of all studied PGRs on maize seeds was the most impressive for stimulating the growth of shoots at 10  $\mu$ M Pb<sup>2+</sup> concentration, and pre-treatment with Cytodef (63%) was the most efficient for shoots growth of maize plants. At moderate (0.1 mM) and high (1 mM) Pb<sup>2+</sup> concentrations, the most effective PGRs for shoots growth were Ribav-extra and Epin-extra (57% and 74%, respectively), while the most effective PGRs for root growth were Kinetin, Ribav-extra, and Epin-extra (1.2 to 2.1 times).

#### **Effectiveness of PGRs**

To determine the most efficient PGRs, the efficiency index (IE) formula was applied as proposed by GRUZNOVA et al. (2017). The results of our calculations are shown in Figure 4. The efficiency of all studied PGRs against 1  $\mu$ M Pb<sup>2+</sup> in growth medium was very low. At 10  $\mu$ M Pb<sup>2+</sup> Cytodef, Ribav-extra and TDZ showed low efficiency, while Ribav-extra and Epin-extra showed moderate efficiency against 0.1 mM Pb<sup>2+</sup> concentration in the growth medium. Finally, at a high (1 mM) Pb<sup>2+</sup>concentration, Kinetin, Ribav-extra, and Epin-extra demonstrated very high efficiency (Figure 4).

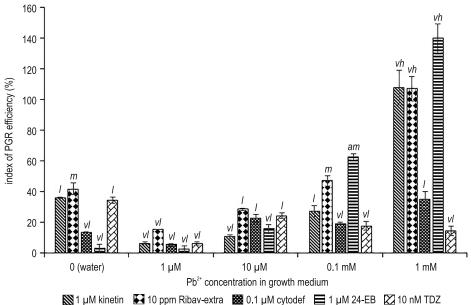


Fig. 4. The efficiency of seed pre-treatment with plant growth regulators (PGRs) against lead ions (Pb<sup>2+</sup>) negative effects on the maize seedlings. Different letters indicate efficiency of the PGRs: vl – very low, l – low, m – moderate, am – above moderate, h – high, vh – very high

## DISCUSSION

Heavy metals are usually very toxic elements for plants (DUCHOVSKIS et al. 2006, ANJUM et al. 2015). Several studies have investigated the metal--specific mechanisms of heavy metal deficiency or excess affecting plant growth characteristics. Delaying or stopping the elongation of axial organs in plants under the treatment of essential or non-essential heavy metals has been discussed in many reviews and research articles (ATTA et al. 2014, AMBIKA et al. 2016).  $Pb^{2+}$  is a non-essential heavy metal (GUPTA et al. 2013, KHAN et al. 2020) that is unnecessary or even toxic for plants (KUMAR et al. 2017). In our research,  $Pb^{2+}$  inhibited the growth of roots and shoots of maize seedlings with an increasing  $Pb^{2+}$  concentration in the growth medium (Figure 3). However, this effect was more pronounced in the roots at lower  $Pb^{2+}$  concentrations. It is known that in a Pb-contaminated environment, the growth of plants may be reduced due to  $Pb^{2+}$  inhibitory effect on the mitotic index (MALAR et al. 2014). Exposure to Pb<sup>2+</sup> reduces the activity of meristematic cells and enzymes in cotyledons and the endosperm, thus reducing the length, fresh and dry weights of wheat (Triticum aestivum L.) seedlings (DATTA et al. 2009). A low Pb<sup>2+</sup> concentration can stimulate the metabolic processes and enzymes involved in these processes, as shown in *Pisum sativum* seedlings (PINHO, LADEIRO 2012). In some cases, Pb<sup>2+</sup> can accelerate germination, but at the same time may have an adverse effect on roots and/or aerial parts length, like in Hordeum vulgare, Elsholtzia argyi, Spartina alterniflora, Pinus halepensis, Oryza sativa, Zea mays, due to its interaction with protease and amylase enzymes (SENGAR et al. 2008, NAS, ALI 2018). In general, high levels of Pb<sup>2+</sup> in the soil result in abnormal morphology of many plant species at the whole plant or cell level.

Thus, the main plant response to Pb<sup>2+</sup> toxicity is growth inhibition and induction of oxidative stress in plants (PINHO, LADEIRO 2012, MALAR et al. 2014), mainly due to the inhibition of the electron transport system (AMBIKA et al. 2016). Plant resistance to  $Pb^{2+}$  depends on the success of the plant antioxidant system. Antioxidant enzymes and some plant metabolites play a vital role in encouraging plants to adapt to stressful conditions (ZHANG et al. 2007). In our research, any concentration of  $Pb^{2+}$  increased the activity of catalase – CAT (it was particularly pronounced against the 1 or 10  $\mu$ M Pb<sup>2+</sup>), indicating the successful resistance of the maize antioxidant system to oxidative stress caused by  $Pb^{2+}$  (Figure 2). The presence of plant growth regulators (PGRs) in the growth medium has a strong effect on CAT activity in maize leaves growing without Pb<sup>2+</sup> treatment. It is known that CAT activity is generally a relevant indicator of plant resistance to heavy metals. However, excessive heavy metal-induced reactive oxygen species (ROS) production can inactivate CAT activity at high metal concentrations, as shown for Lemna gibba (PARLAK, YILMAZ 2013) and *Glycine max* (MISHRA et al. 2013). Apparently, in some cases, the decrease in CAT activity can be compensated by an increased activity of other antioxidant enzymes that decompose  $H_2O_2$  (FENG-TAO et al. 2013, MALAR et al. 2014).

Based on previous research, exogenous PGRs enhance the growth and production of plants exposed to various heavy metals (ALI et al. 2018, NABAEI, AMOOAGHAIE 2019). In general, PGRs increase the activity of stress-reducing enzymes (SOD and CAT) and decrease the malondialdehyde concentration (DALYAN et al 2018). Also, PGRs improve photosynthesis by increasing Rubisco and carbonic anhydrase activity. They have the ability to reduce oxidative stress by reducing the production of free radicals and maintaining free radical scavenging enzymes (AHMAD et al. 2018). PGRs enhance the concentrations of anthocyanins, proline and total phenols (KHAVARI-NEJAD et al. 2016). In our study, some PGRs increased CAT activity, indicating the stimulation of the plant's antioxidant system activity (Figure 2). PGR Ribay-extra had the greatest positive effect on CAT activity against low Pb<sup>2+</sup> concentrations (1 or 10  $\mu$ M), while Epin-extra was the most effective against increased (0.1 mM) or sublethal (1 mM) Pb<sup>2+</sup> concentrations. The literature describes various mechanisms to explain the protective effect of PGRs, such as their effect on the regulatory mechanisms of cells at the genetic and metabolic levels (GHORBANI et al. 1999), changes in endogenous levels of natural plant hormones, which allows to shift the plants growth and development (CHEN et al. 2017), phytohormone or PGRs signalling pathways are triggered by a metal that initiates the expression of genes encoding protective proteins that regulate heavy metal uptake, translocation, or binding in cell compartments (SINGH et al. 2016, BÜCKER-NETO et al. 2017). In our study, it was visualized that Cytodef and Epin-extra slowed the radial transport of Pb<sup>2+</sup> in maize root tissues; also, these PGRs inhibited the xylem translocation of metal, while thidiazuron (TDZ) increased the accumulation of the metal in the root tissues. Moreover, in plants pre-treated with any PGR, especially TDZ and Epin-extra, Pb<sup>2+</sup> was bound to concretions that are visible on the surface (cell walls) of the cortex cells (especially of the endoderm and mesoderm). All these changes led to modifications in plant growth parameters. Thus, in some studied cases, PGRs stimulated the elongation of the axial organs of pre-treated plants. PGR Epin-extra mostly stimulated the growth of seedlings exposed to the wide range of  $Pb^{2+}$  concentrations (from 1  $\mu M$ to 0.1 mM). Against 10  $\mu$ M of Pb<sup>2+</sup> the pre-treatment of seeds with Cytodef was the most effective for the growth of maize shoots. With increasing Pb<sup>2+</sup>concentrations in growth medium up to 0.1 mM or 1 mM, the most efficient PGRs were Epin-extra and Ribav-extra for shoots or Epin-extra and Kinetin for root growth. Thus, the different PGRs used for seed pre-treatment have ambiguous effects on plants, resulting in different responses of physiological and biochemical processes in maize seedlings exposed to different Pb<sup>2+</sup>concentrations.

The results of the research show that the use of PGRs for the pre-sowing treatment of maize seeds in order to protect plants from heavy metal absorp-

tion and toxic effects is an inexpensive and a very promising procedure. It is possible that some PGRs studied will find application in solving phytoremediation problems.

## CONCLUSION

The pre-treatment of maize seeds with solutions containing different plant growth regulators modified the allocation of lead ions (Pb<sup>2+</sup>) in root tissues. Plant growth regulator Thidiazuron (TDZ) enhanced while Epin-extra impaired the absorption of Pb<sup>2+</sup> in root tissues. The effect of the pre-treatment of maize seeds with each plant growth regulator on seedling growth depended on the concentration of Pb<sup>2+</sup> in the growth medium. At low Pb<sup>2+</sup> concentrations in the growth medium, the pre-treatment of maize seeds with plant growth regulator Ribav-extra was the most effective for young maize plants. Against moderate (0.1 mM) or highest (1 mM) Pb<sup>2+</sup> concentrations, the pre-treatment of seeds with Epin-extra was the most effective. The efficiency of the pre-treatment of seeds with plant growth regulators against Pb<sup>2+</sup> concentrations decreased as follows: Epin-extra  $\approx$  Ribav-extra > Kinetin > Cytodef > TDZ. This study provides a theoretical basis for the practical use of PGRs for maize cultivation in lead-contaminated soils.

#### REFERENCES

- AHMAD B., JALEEL H., SADIQ Y., KHAN M.M., SHABBIR A. 2018. Response of exogenous salicylic acid on cadmium induced photosynthetic damage, antioxidant metabolism and essential oil production in peppermint. Plant Growth Regul., 86(2): 273-286. DOI: 10.1007/s10725--018-0427-z
- ALI S., RIZWAN M., ZAID A., ARIF M.S., YASMEEN T., HUSSAIN A., SHAHID M.R., BUKHARI S.A.H., HUSSAIN S., ABBASI G. 2018. H5-Aminolevulinic Acid-Induced heavy metal stress tolerance and underlying mechanisms in plants. J. Plant Growth Regul., 37: 1423-1436. DOI: 10.1007/s00344-018-9875-y
- ALI H., KHAN E. 2019. Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs – Concepts and implications for wildlife and human health. Human Ecol Risk Assess., 25: 1353-1376. DOI: 10.1080/ /10807039.2018.1469398
- AMBIKA A., MOHNISH P., KUMAR N. 2016. *Effect of heavy metals on plants: An overview*. Int J Appl Innov Engin Manage, 5(3): 56-66.
- ANJUM N.A., SINGH H.P., KHAN M.I., MASOOD A., PER T., NEGI A., BATISH D., KHAN N.A., DUARTE A.C., PEREIRA E. 2015. Too much is bad – an appraisal of phytotoxicity of elevated plant-beneficial heavy metal ions. Environ. Sci. Pollut. Res., 22: 3361-3382. DOI: 10.1007/s11356-014-3849-9
- ATTA M.I., GULSHAN A.B., AHMAD N., SAEED S. 2014. Toxicological study of heavy metals on early growth responses of sunflower (Helianthus annuus L.). ARPN J. Agric. Biol. Sci. 9(2): 46-50. http://www.arpnjournals.com/jabs/research\_papers/rp\_2014/jabs\_0214\_632.pdf
- BÜCKER-NETO L., PAIVA A.L.S., MACHADO R.D., ARENHART R.A., MARGIS-PINHEIRO M. 2017. Interactions between plant hormones and heavy metals responses. Genet. Mol. Biol., 40(1 Suppl 1): 373-386. DOI: 10.1590/1678-4685-GMB-2016-0087

- CHEN Q., WU K., TANG Z., GUO Q.X., GUO X., WANG H. 2017. Exogenous ethylene enhanced the cadmium resistance and changed the alkaloid biosynthesis in Catharanthus roseus seedlings. Acta Physiol. Plant., 39: 267. DOI: 10.1007/s11738-017-2567-6
- DALYAN E., YÜZBAŞIOĞLU E., AKPINAR I. 2018. Effect of 24-Epibrassinolide on antioxidative defence system against lead-induced oxidative stress in the roots of Brassica juncea L. seedlings. Russ. J. Plant Physiol., 65(4): 570-578. DOI: 10.1134/S1021443718040118
- DATTA J.K., GHANTY S., BANERJEE A., MOUDAL N.K. 2009. Impact of lead on germination physiology of certain wheat cultivar (Triticum aestivum L.). J. Ecophysiol. Occup. Hlth., 9: 145-151.
- DENTON B. 2007. Advances in phytoremediation of heavy metals using plant growth promoting bacteria and fungi. MMG 445. Basic Biotechnol., 3: 1-5.
- DUCHOVSKIS P., BRAZAITYTE A., JUKNYS R., JANUŠKAITIENE I., SLIESARAVIČIUS A., RAMAŠKEVIČIENE A., BURBULIS N., ŠIKŠNIANIENE J.B., BARANAUSKIS K., DUCHOVSKIENE L., STANYS V., BOBINAS Č. 2006. Changes of physiological and genetic indices of Lycopersicon esculentum Mill. by cadmium under different acidity and nutrition. Pol. J. Environ. Stud., 15(2): 235-242.
- FENG-TAO L.I., JIAN-MIN Q.I., GAO-YANG Z., LI-HUI L., PING-PING F., FEN T.A., JIAN-TANG X.U. 2013. Effect of cadmium stress on the growth antioxidative enzymes and lipid peroxidation in two kenaf (Hibiscus cannabinus L.) plant seedlings. J. Int. Agric., 12: 610-620.
- GHORBANI M., KAREH S.H., SEREHR M.F. 1999. Effect of cadmium and gibberellins on growth and photosynthesis of Glycine max. Photosynthetica, 37: 627-631.
- GRUZNOVA K.A., BASHMAKOV D.I., BRAZAITYTE A., DUCHOVSKIS P., LUKATKIN A.S. 2017. Efficiency index as the integral indicator of Triticum aestivum response to growth regulators. Žemdirbystė=Agriculture, 104(4): 299-304. DOI: 10.13080/z-a.2017.104.038
- GRUZNOVA K.A., BASHMAKOV D.I., MILIAUSKIENĖ J., VAŠTAKAITĖ V., DUCHOVSKIS P., LUKATKIN A.S. 2018. The effect of a growth regulator Ribav-Extra on winter wheat seedlings exposed to heavy metals. Žemdirbystė=Agriculture, 105(3): 227-234. DOI: 10.13080/z-a.2018.105.029
- GUPTA D.K., CORPAS F.J., HUANG H.G. 2013. Lead tolerance in plants: strategies for phytoremediation. Environ. Sci. Pollut. Res., 20(4): 2150-2161. DOI: 10.1007/s11356-013-1485-4
- HADI F., BANO A., FULLER M.P. 2010. The improved phytoextraction of lead (Pb) and the growth of maize (Zea mays L.): the role of plant growth regulators (GA<sub>3</sub> and IAA) and EDTA alone and in combinations. Chemosphere, 80(4): 457-462. DOI: 10.1016/j.chemosphere.2010.04.020
- KAUR G., SINGH H.P., BATISH D.R., KOHLI R.K. 2012. Growth, photosynthetic activity and oxidative stress in wheat (Triticum aestivum) after exposure of lead to soil. J. Environ. Biol., 33(2): 265-269.
- KHAN M., SAMRANA S., ZHANG Y., MALIK Z., KHAN M.D., ZHU S. 2020. Reduced glutathione protects subcellular compartments from Pb-induced ROS injury in leaves and roots of upland cotton (Gossypium hirsutum L.). Front Plant Sci., 11:412. DOI: 10.3389/fpls.2020.00412
- KHAVARI-NEJAD R.A., NAJAFI E., RANJBARI M. 2016. The interactive effects of cadmium and GA3 on tomato (Lycopersicon esculentum Mill. cv. CH) plants photosynthesis, anthocyanin, proline and total phenolic contents. Rom. J. Biol. - Plant Biol., 61(1-2): 43-52.
- KUMAR B., SMITA K., FLORES L.C. 2017. Plant mediated detoxification of mercury and lead. Arabian J. Chem., 10: 2335-2342. DOI: 10.1093/pcp/pcu117
- LOPEZ M.L., PERALTA J.R., PARSONS J.G., GARDEA J.L., DUARTE-GARDEA M. 2009. Effect of indole-3-acetic acid, kinetin, and ethylenediaminetetraacetic acid on plant growth and uptake and translocation of lead, micronutrients, and macronutrients in alfalfa plants. Int. J. Phytoremediat., 11: 131-149.
- LUKATKIN A.S. 2002. Contribution of oxidative stress to the development of cold-induced damage to leaves of chilling-sensitive plants: 2. The activity of antioxidant enzymes during plant chilling. Russ. J. Plant. Physiol., 49: 782–788.
- LUKATKIN A.S., BASHMAKOV D.I., KIPAIKINA N.V. 2003. Protective role of thidiazuron treatment on cucumber seedlings exposed to heavy metals and chilling. Russ. J. Plant Physiol., 50(3): 305-307.

- LUKATKIN A.S., GRACHEVA N.V., GRISHENKOVA N.N., DUKHOVSKIS P.V., BRAZAITITE A.A. 2007. Cytokinin-like growth regulators mitigate toxic action of zinc and nickel ions on maize seedlings. Russ. J. Plant Physiol., 54(3): 381-387.
- MALAR S., VIKRAM S. SH., FAVAS P.J.C., PERUMAL V. 2014. Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths (Eichhornia crassipes (Mart.)). Bot. Stud., 55: 1-11. DOI: 10.1186/s40529-014-0054-6
- MAŁKOWSKI E., SITKO K., SZOPIŃSKI M., GIEROŃ Ż., POGRZEBA M., KALAJI H.M., ZIELEŹNIK-RUSINOWSKA P. 2020. Hormesis in plants: the role of oxidative stress, auxins and photosynthesis in corn treated with Cd or Pb. Int. J. Mol. Sci., 21: 2099. DOI: 10.3390/ijms21062099
- MISHRA P.K., KUMAR U., MISHRA M., PRAKASH V. 2013. Antioxidative defense responses to leadinduced oxidative stress in Glycine max L. cv. Merrill grown in different pH gradient. J. Stress Physiol. Biochem., 9: 131-147.
- NABAEI M., AMOOAGHAIE R. 2019. Interactive effect of melatonin and sodium nitroprusside on seed germination and seedling growth of Catharanthus roseus under cadmium stress. Russ. J. Plant Physiol., 66: 128-139. DOI: 10.1134/S1021443719010126
- NAS F.S., ALI M. 2018. The effect of lead on plants in terms of growing and biochemical parameters: a review. MOJ Ecol. Environ. Sci., 3(4): 265-268. DOI: 10.15406/mojes.2018.03.00098
- PARLAK K.C., YILMAZ D.D. 2013. Ecophysiological tolerance of Lemna gibba L. exposed to cadmium. Ecotoxicol. Environ. Saf., 91: 79-85. DOI: 10.1016/j.ecoenv.2013.01.009
- PINHO S., LADEIRO B. 2012. Phytotoxicity by lead as heavy metal focus on oxidative stress. J. Bot., Article ID 369572. DOI: 10.1155/2012/369572
- SAZANOVA K.A., BASHMAKOV D.I., BRAZAITYTÉ A., BOBINAS Č., DUCHOVSKIS P., LUKATKIN A.S. 2012. The effect of heavy metals and thidiazuron on winter wheat (Triticum aestivum L.) seedlings. Žemdirbystė=Agriculture, 99(3): 273-278.
- SEREGIN I.V., IVANOV V.B. 1997. Histochemical investigation of cadmium and lead distribution in plants. Russ. J. Plant Physiol., 44: 915-921.
- SENGAR R.S., GAUTAM M., SENGAR R.S., SENGAR R.S., GARG S.K., SENGAR K., CHAUDHARY R. 2008. Lead stress effects on physiobiochemical activities of higher plants. Rev. Environ. Contam. Toxicol., 196: 73-93. DOI: 10.1007/978-0-387-78444-1\_3
- SINGH S., PARIHAR P., SINGH R., SINGH V.P., PRASAD S.M. 2016. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front. Plant Sci., 6: 1143-1179. DOI: 10.3389/fpls.2015.01143
- SYTAR O., KUMARI P., YADAV S., BRESTIC M., RASTOGI A. 2019. Phytohormone priming: regulator for heavy metal stress in plants. J. Plant Growth Regul., 38: 739-752. DOI: https://doi. org/10.1007/s00344-018-9886-8
- TASSI E., POUGET J., PETRUZZELLI G., BARBAFIERI M. 2008. The effects of exogenous plant growth regulators in the phytoextraction of heavy metals. Chemosphere, 71: 66-73. DOI: 10.1016/j. chemosphere.2007.10.027
- ZHANG F.Q., WANG Y.S., LOU Z.P., DONG J.D. 2007. Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (Kandelia candel and Bruguiera gymnorrhiza). Chemosphere, 67: 44-50. DOI: 10.1016/j. chemosphere.2006.10.007