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# The Effect of Use of the Biologically Active Substances in Alleviating the Stress Caused by Lead in Barley Seedling on the Basis of Biochemical and Physiological Parameters

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**ABSTRACT**

Plants are constantly exposed to a variety of stressors during their lives. One of such stressors is contamination of the environment with heavy metals. Lead is one of highly toxic metals and it significantly inhibits normal plant growth. The study aimed at assessing the degree of relieving the stress caused by 1 mM $Pb(NO_3)_2$ via different biologically active substances (AsA, GSH, PP, α-Toc, SA) on the basis of the measurement of morphological (root length, coleoptile length, fresh weight), biochemical (Pro, MDA, CAT, POX) and physiological (Chl a+b, Car) traits in 10-day leaves of spring barley of the cultivar Eunova under laboratory conditions. Pb-stress reduced the fresh weight, root length and coleoptiles of the barley tested. Lead increased lipid peroxidation and Pro content, enhanced CAT and POX activity, and significantly suppressed the photosynthetic pigments content. Among the substances used in the experiment, PP, α-Toc and GSH generally relieved the toxic effect of lead to the barley seedlings to the greatest degree.

**Keywords:** barley, biologically active substances, catalase, lead, malondialdehyde, peroxidase, pigments, proline.

## INTRODUCTION

An increase in the addition of heavy metals in the environment causes losses to agricultural crops. Lead is a toxic element, easily assimilated by plants and accumulated in various parts thereof. Plant responses to environmental stresses are complex, limiting plant growth and yield by affecting morphological, physiological and biochemical parameters (Sharma and Dubey, 2005; Sędzik et al., 2015). One of the biochemical changes occurring when plants are subjected to heavy metals, including Pb, is the production of reactive oxygen species (ROS) (Shahid et al., 2014). Enhanced production of ROS in plants during stress can enhance oxidative processes (such as: membrane lipid peroxidation, protein oxidation), enzyme inhibition and DNA and RNA damage (Asada, 2006). To control ROS levels and protect cells from damage, plants produce numerous efficient defense mechanisms, known as the antioxidant defense system. This system consists of low-molecular weight antioxidant compounds (ascorbic acid (AsA), reduced glutathione (GSH), carotenoids, tocopherols) and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX) and enzymes of the ascorbateglutathione cycle (AsA-GSH) (Mishra et al., 2009).

Effective solutions are necessary to increase the tolerance of plants to environmental stresses, including heavy metals. This can be achieved through the use of biologically active substances. Under stress conditions in plants, endogenous levels of these substances are low, which can be counteracted by their exogenous application, which is an environmentally friendly approach. Such exogenous application of plant

non-enzymatic antioxidants, plant growth regulators, plant extracts with secondary metabolites can mitigate the adverse effects of heavy metals on growth, yield as well as biochemical and physiological processes in plants (Chen et al., 2007; Jazi et al., 2011; Al-Hakimi and Hamada, 2011; Son et al., 2014; Jazi and Oregani, 2014).

Vitamin C [ascorbic acid (AsA)] is an antioxidant molecule and a crucial substrate for the detoxification of ROS, molecule essential for the regulation of key physio-biochemical processes in plants (Ishikawa et al., 2006; Moghadam, 2016). Studies by several authors have shown that AsA is effective in improving the plant stress tolerance. It can improve the tolerance to abiotic stresses by enhancing plant growth; it is also involved in the regulation of photosynthesis (protection of photosynthetic pigments), transpiration, protection of lipids, proteins, and enzymes (Al-Hakimi and Hamada, 2011; Venkatesh and Park, 2014).

Glutathione (GSH) is a tripeptide composed of glutamate, cysteine, and glycine. As a non-enzymatic antioxidant, GSH protects plants from oxidative damage caused by stress factors. It is present in all plant cells. It is involved in detoxification of ROS, as well as heavy metals. Glutathione is a substrate for the synthesis of phytochelatins, which are involved in the detoxification of heavy metal ions (Foyer and Noctor 2005, Sharma and Dietz 2006). Nicotinamide (vitamin PP) is a water-soluble vitamin. It is part of the NADH and NADPH coenzymes, which are involved in many enzymatic oxidations, i.e. reduction reactions in cells. It participates in the repair of damage caused by ROS (Abdelhamid et al., 2013). α-Tocopherol (α-Toc, vitamin E) is low molecular a lipid-soluble antioxidant. It is synthesized in chloroplasts by all plants. α-Toc is a key molecule for the detoxification of ROS and protects against oxidation damage. Studies by numerous authors have shown that tocopherol proves to be effective in improving the plant tolerance to various stress environments (Kumar et al., 2012; Sadak and Dawood, 2014).

Salicylic acid (SA) belongs to a group of phenolic compounds. It is a phytohormone with a signaling function in plants (Miura and Tada, 2014). SA is involved in the regulation of important physiological and biochemical processes such as seed germination, growth, development, ion uptake and transport, membrane permeability, photosynthesis, and amino acid metabolism. This acid induces the production of certain stress proteins, thereby participating in plant defense against abiotic and biotic stresses (Khan et al, 2012; Naser et al, 2014; Miura and Tada, 2014).

Barley is one of the basic cereals cultivated in Europe. The high barley yielding potential causes the species to increase its popularity in Europe, and its cultivation area is constantly extended. Barley is grown mainly for animal feed and malt for brewing (Fischbeck, 2003). The study of Sędzik et al. (2015) determined that barley is sensitive to the effect of lead. The experiment investigated whether the addition of exogenous application of biologically active substances (BAS) such as ascorbic acid, glutathione, nicotinamide, α-tocopherol and salicylic acid alleviates the harmful effect of 1 mM lead nitrate stress on barley seedlings.

## MATERIAL AND METHODS

The research was carried out under laboratory conditions at the Department of Microbiology and Environmental Biochemistry of the West Pomeranian University of Technology in Szczecin (lat. 53°26′17″ N, long. 14°32′32″ E). The plant material consisted of naked seeds of spring barley (*Hordeum vulgare* L.) of the 'Eunova' cultivar. They were purchased from a specialist shop as certified seeds in class (C/1).

The sensitivity to the presence of 1 mM $Pb(NO_3)_2$ and the extent to which its toxicity was mitigated by the biologically active substances: ascorbic acid (1 mM AsA), glutathione (100 μM GSH), nicotinamide (50 μM PP), a-tocopherol (1 mM α-Toc), salicylic acid (1 mM SA) were assessed for barley seeds and seedlings. Seed disinfection was carried out according to the method described by Krupa-Malkiewicz et al. (2018). Firstly, 1 mM $Pb(NO_3)_2$ and BAS were dissolved in water and poured into vessels and then disinfected barley seeds were introduced into the appropriate combinations of the experiment. For treatments: 30 $cm^3$ of 1 mM $Pb(NO_3)_2$, 30 $cm^3$ of (BAS) like: AsA, GSH, PP, α-Toc, SA, respectively and their combinations (AsA+Pb, GSH+Pb, PP+Pb, α-Toc+Pb, SA+Pb) were used. The experiment was set up in six replications (six Petri dishes Ø10 cm with 10 seeds for each treatment).

The seed dishes were incubated for 72 hours in the dark at 21°C, then the pre-germinated seeds were transferred to the phytotron where the light intensity was set to 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ and kept for a further 10 days at 25°C. During the experiment, the photoperiod was set to 16:8 hours. After 10

days, morphological parameters (root length, coleoptile and fresh weight of the seedling), biochemical parameters (Proline - Pro, malondialdehyde – MDA, *catalase – CAT and peroxidase – POX activities*) and physiological parameters (concentrations of total chlorophyll – Chl a+b and carotenoids – Car) were measured.

### Determination of the MDA content

The content of malondialdehyde (MDA), as a product of lipid peroxidation, was determined with thiobarbituric acid (TBA) according to the method of Sudhakar et al. (2001). The absorbance of the supernatant was measured at 532 nm and 600 nm using a spectrophotometer UV-1800 produced by Shimadzu. The MDA content was expressed in μmol·g$^{-1}$ of fresh plant weight (FW).

### Determination of the Pro content

Proline content was determined by ninhydrin reaction according to the method of Bates et al. (1973). The mixture obtained by the reaction was then extracted with toluene. In the collected toluene layer, the absorbance of the dyed chromophore against toluene was determined on a spectrophotometer at 520 nm. The Pro content was expressed in μmol·g$^{-1}$ of fresh plant weight (FW).

### Determination of the catalase and peroxidase activities

The catalase (CAT) activity of [EC 1.11.1.6] was determined spectrophotometrically according to the method of Lück (1963). The CAT activity was expressed as μmol $H_2O_2$·g$^{-1}$·min$^{-1}$ fresh plant weight (FW). In contrast, total peroxidase activity (POX) [EC 1.11.1.7] was measured spectrophotometrically according to Chance and Maehly (1955). The POX activity was presented as μmol purpurogallin·g$^{-1}$ fresh plant weight (FW).

### Determination of pigment content

The Chl a and Chl b contents were determined in fresh weight according to the method presented by Arnon et al. (1956) and modified by Lichtenthaler and Wellburn (1983). The Car content was assessed according to the method of Hager and Meyer-Berthenrath (1966). The results obtained for pigment content were expressed in μg·g$^{-1}$ plant fresh plant weight (FW).

### Statistical analysis

Statistical analyses were performed using Statistica 13 (TIBCO Software Inc.). The results obtained were analyzed using descriptive statistics (mean, standard deviation) and two-ways ANOVA. The means were compared using Tukey's HSD test with a significance level at $p < 0.05$. The significance was set at $p < 0.05$. Means were used to carry out cluster analysis using Ward's agglomerative method (using Euclidean distances). Created groups were used to perform MDS (MultiDimensional Scaling), stress and bootstrap in R (R Core Team R), package SMACOF (de Leeuw and Mair 2009). In addition, the differences between effects of BAS *per se* have been shown in MDS using Eta^2 and 'raw' effect of interactions BAS×Pb. The data for Eta^2 has been taken from ANOVA, whereas 'raw' effect of interactions BAS×Pb has been calculated using formula presented in Table 1.

## RESULTS AND DISCUSSION

Abiotic stresses (heavy metals, salinity, ozone, UV-B radiation, extreme temperatures, or drought) are among the most challenging threats to agricultural system and economics of yield of crop plants. Numerous studies demonstrate, that physiological and biochemical processes are disturbed under the conditions of abiotic stress, which limits the growth and yielding in plants (Sharma and Dubey, 2005; Khan, 2015). In recent years, with the development of modern cultivation methods, increased interest of different types of chemical compounds is observed, which could fulfill different anti-stress functions in a plant, relieving unfavorable abiotic effects in crops.

### Effects of BAS on seedling growth parameters under lead stress

An analysis of morphological traits of the barley seedlings allowed observing that the lead salt influenced on average on the reduction of root's and coleoptile's length and fresh weight of the seedlings with respect to the control, by 81.8%, 36.6% and 41.2%, respectively.

The use of exogenous BAS like: GSH, and SA reduced by 0.9 to 10.75% both roots and coleoptiles length in comparison to the control, although not all means were significantly different (Table

**Table 1.** The influence of BAS on *morphological* parameters, MDA, Pro, Chl a+b, Car contents, CAT and POX activities in barley seedlings exposed to 1 mM $Pb(NO_3)_2$ treatment under laboratory conditions

| Parameter | CL [cm] | | RL [cm] | | FW [g] | | MDA [µmol·g$^{-1}$FW] | | Pro [µmol·g$^{-1}$FW] | | Chl a+b [µg·g$^{-1}$FW] | | Car [µg·g$^{-1}$FW] | | CAT [µmol $H_2O_2$·g$^{-1}$FW·min$^{-1}$] | | POX [µmol purpurogallin ·g$^{-1}$FW] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| [1]Control | 10.37 ±1.63 | a | 9.58± 1.69 | a | 0.27± 0.02 | a | 22.42 ±0.85 | c | 0.21± 0.01 | c | 286.28± 5.3 | c | 98.81 ±1.73 | b | 19.8 ±2.94 | d | 7.09± 2.36 | b |
| [1]Pb | 6.57± 0.99 | b | 1.74± 0.43 | b | 0.16± 0.03 | b | 62.04 ±4.32 | a | 0.54± 0 | a | 190.4 ±2.76 | d | 67.9 ±0.98 | d | 67.66 ±1.63 | a | 56.48 ±9.12 | a |
| ASC | 11.42 ±2.26 | a | 10.27 ±1.72 | a | 0.29± 0.04 | a | 22.58 ±1.86 | c | 0.23± 0.01 | c | 325.2 ±3.39 | a | 105.29 ±1.38 | a | 26.7 ±1.58 | c | 14.88 ±2.54 | b |
| ASC+PB | 8.33± 1.09 | c | 2.38± 0.28 | b | 0.17± 0.03 | b | 49.3± 1.14 | b | 0.47± 0.01 | b | 306.03 ±3.7 | b | 90.73 ±2.06 | c | 42.14 ±0.41 | b | 21.46 ±6.54 | b |
| Mean±SD | 9.17± 1.49 | | 5.99± 1.03 | | 0.22± 0.03 | | 39.09 ±2.04 | | 0.36± 0.01 | | 276.98 ±3.79 | | 90.68 ±1.54 | | 39.07 ±1.64 | | 24.97 ±5.14 | |
| [2]Eta^2 | 0.013 | | 0.000 | | 0.000 | | 0.721 | | 0.838 | | 0.973 | | 0.909 | | 0.966 | | 0.833 | |
| [3](control-ASC) - (Pb - ASC+Pb) | 0.701 | | -0.055 | | -0.002 | | -12.903 | | -0.082 | | 76.711 | | 16.342 | | -32.432 | | -42.814 | |
| Control | 10.37 ±1.63 | a | 9.58± 1.69 | a | 0.27± 0.02 | a | 22.42 ±0.85 | c | 0.21± 0.01 | d | 286.28 ±5.3 | b | 98.81 ±1.73 | b | 19.8 ±2.94 | d | 7.09 ±2.36 | c |
| Pb | 6.57± 0.99 | c | 1.74± 0.43 | b | 0.16± 0.03 | c | 62.04 ±4.32 | a | 0.54± 0 | a | 190.4 ±2.76 | d | 67.9 ±0.98 | d | 67.66 ±1.63 | a | 56.48 ±9.12 | a |
| GSH | 9.63± 0.98 | a | 9.5± 1.45 | a | 0.27± 0.02 | a | 25.43 ±0.25 | c | 0.25± 0 | c | 322.99 ±8.06 | a | 113.74 ±2.85 | a | 27.94 ±3.73 | c | 16.93 ±2.82 | b |
| GSH+Pb | 7.88± 1.86 | b | 2.22± 0.49 | b | 0.19± 0.04 | b | 43.44 ±0.52 | b | 0.48± 0.02 | b | 253 ±13.16 | c | 83.26 ±0.56 | c | 43.83 ±1.18 | b | 22.98 ±1.71 | b |
| Mean±SD | 8.61± 1.36 | | 5.76± 1.02 | | 0.22± 0.03 | | 38.33 ±1.48 | | 0.37± 0.01 | | 263.17 ±7.32 | | 90.93 ±1.53 | | 39.81 ±2.37 | | 25.87 ±4 | |
| Eta^2 | 0.120 | | 0.015 | | 0.069 | | 0.899 | | 0.867 | | 0.478 | | 0.005 | | 0.935 | | 0.876 | |
| (control-GSH) - (Pb - GSH+Pb) | 2.044 | | 0.553 | | 0.029 | | -21.613 | | -0.097 | | 25.882 | | 0.425 | | -31.976 | | -43.350 | |
| Control | 10.37 ±1.63 | a | 9.58± 1.69 | b | 0.27± 0.02 | b | 22.42 ±0.85 | c | 0.21± 0.01 | c | 286.28 ±5.3 | b | 98.81 ±1.73 | b | 19.8 ±2.94 | d | 7.09 ±2.36 | c |
| Pb | 6.57± 0.99 | c | 1.74± 0.43 | c | 0.16± 0.03 | d | 62.04 ±4.32 | a | 0.54± 0 | a | 190.4 ±2.76 | c | 67.9 ±0.98 | c | 67.66 ±1.63 | a | 56.48 ±9.12 | a |
| PP | 10.69 ±1.86 | a | 10.75 ±1.31 | a | 0.31± 0.07 | a | 22.96 ±2.23 | c | 0.22± 0.01 | c | 360.28 ±2.62 | a | 113.65 ±5.13 | a | 31.19 ±3.18 | c | 9.31 ±1.62 | c |
| PP+Pb | 8.59± 2.02 | b | 2.4± 0.39 | c | 0.21± 0.02 | c | 42.37 ±0.65 | b | 0.42± 0.01 | b | 291.1± 12.75 | b | 91.36 ±3.56 | b | 44.02 ±1.85 | b | 22.42 ±2.41 | b |
| Mean±SD | 9.06± 1.62 | | 6.12± 0.96 | | 0.24± 0.03 | | 37.45 ±2.01 | | 0.35± 0.01 | | 282.02 ±5.86 | | 92.93 ±2.85 | | 40.67 ±2.4 | | 23.82 ±3.88 | |
| Eta^2 | 0.064 | | 0.014 | | 0.002 | | 0.861 | | 0.930 | | 0.566 | | 0.393 | | 0.949 | | 0.835 | |
| (control-PP) - (Pb - PP+Pb) | 1.700 | | -0.514 | | 0.007 | | -20.215 | | -0.123 | | 26.699 | | 8.613 | | -35.037 | | -36.285 | |
| control | 10.37 ±1.63 | b | 9.58± 1.69 | b | 0.27± 0.02 | b | 22.42 ±0.85 | c | 0.21± 0.01 | c | 286.28 ±5.3 | a | 98.81 ±1.73 | ab | 19.8 ±2.94 | d | 7.09 ±2.36 | b |
| Pb | 6.57± 0.99 | d | 1.74± 0.43 | c | 0.16± 0.03 | d | 62.04 ±4.32 | a | 0.54± 0 | a | 190.4 ±2.76 | c | 67.9 ±0.98 | c | 67.66 ±1.63 | a | 56.48 ±9.12 | a |
| α-Toc | 11.47 ±1.05 | a | 10.65 ±1.81 | a | 0.31± 0.05 | a | 24.62 ±0.57 | c | 0.22± 0.02 | c | 288.12± 2.92 | a | 107.1 ±13.36 | a | 32.95 ±2.7 | c | 12.55 ±0.53 | b |
| α-Toc+Pb | 8.36± 1.03 | c | 2.08± 0.36 | c | 0.2± 0.03 | c | 42.53 ±0.81 | b | 0.44± 0.01 | b | 250.1 ±17.1 | b | 81.41 ±5.64 | bc | 43.5 ±1 | b | 15.89 ±2.94 | b |
| Mean±SD | 9.19± 1.18 | | 6.01± 1.07 | | 0.24± 0.03 | | 37.9 ±1.64 | | 0.35± 0.01 | | 253.73 ±7.02 | | 88.8 ±5.43 | | 40.98 ±2.07 | | 23± 3.74 | |
| Eta^2 | 0.021 | | 0.022 | | 0.001 | | 0.897 | | 0.867 | | 0.789 | | 0.046 | | 0.964 | | 0.891 | |
| (control-α-Toc) - (Pb-α-Toc +Pb) | 0.697 | | -0.741 | | 0.005 | | -21.720 | | -0.116 | | 57.861 | | 5.242 | | -37.316 | | -46.053 | |
| Control | 10.37 ±1.63 | a | 9.58± 1.69 | a | 0.27± 0.02 | a | 22.42 ±0.85 | c | 0.21± 0.01 | c | 286.28 ±5.3 | b | 98.81 ±1.73 | ab | 19.8 ±2.94 | d | 7.09 ±2.36 | c |
| Pb | 6.57± 0.99 | b | 1.74 ±0.43 | b | 0.16± 0.03 | c | 62.04 ±4.32 | a | 0.54± 0 | a | 190.4 ±2.76 | d | 67.9 ±0.98 | c | 67.66 ±1.63 | a | 56.48 ±9.12 | a |
| SA | 9.25± 1.34 | a | 9.36± 1.01 | a | 0.25± 0.02 | b | 25.11 ±1.62 | c | 0.27± 0.01 | b | 355.43 ±2.65 | a | 105.33 ±5.85 | a | 33.02 ±1.88 | c | 9.92 ±1.64 | bc |
| SA+PB | 7.16± 1.6 | b | 1.79± 0.29 | b | 0.17± 0.03 | c | 34.19 ±1.28 | b | 0.51± 0.02 | a | 266.01 ±7 | c | 93.63 ±5.72 | b | 40.05 ±1.41 | b | 19.94 ±1.9 | b |
| Mean±SD | 8.34± 1.39 | | 8.34± 1.39 | | 0.22± 0.02 | | 35.94 ±2.02 | | 0.38± 0.01 | | 274.53 ±4.43 | | 91.42 ±3.57 | | 40.13 ±1.96 | | 23.36 ±3.75 | |
| Eta^2 | 0.087 | | 0.004 | | 0.122 | | 0.937 | | 0.795 | | 0.146 | | 0.661 | | 0.974 | | 0.859 | |
| (C ontrol-SA) - (Pb - SA+Pb) | 1.701 | | 0.257 | | 0.034 | | -30.538 | | -0.085 | | 6.456 | | 19.208 | | -40.833 | | -39.372 | |

**Note:** [1] For the clarity of the interpretation of the results, in the Table 1 it has been repeated means for the dependent variables (traits investigated) obtained in the tests for the control and Pb; [2]Eta^2 - values from teh ANOVA analysis. They describe the percentage of the variance explained by the BAS×Pb interaction for each described dependent variable. They were used in the study for the MDS analysis. [3]the ‚raw' effect of variance was calculated for each BAS according to the formula (control - AsA) - (Pb - AsA+Pb), and the differences were used in the MDS analysis; a-d - homogeneous groups.

1). The use of AsC, PP and α-Toc had the opposite effect. The use of biologically active substances stimulated the seedling fresh weight from 6.2 to 14.5% and stimulated by 3.1 to 12.3% both roots and coleoptiles length with regards to the control (Table 1). On the other hand, the use of combination BAS with lead salt caused a reduction in the toxic effect of lead ions on the formation of morphological parameters of the barley seedlings. In this case, the highest "relieving" role on the inhibition of the root and coleoptile length and fresh weight of the seedlings was determined after the use of nicotinamide (Table 1). Such effect of the used nicotinamide may stem from the fact that it fulfills numerous functions within plants. Nicotinamide influences the induction and regulation of the metabolic response in a plant organism exposed to a stress factor and is a component and manifestation of the defense metabolism in plants (Berglund and Ohlsson, 1995). In addition, the compound has been shown to have a positive effect on many physiological processes, such as the biosynthesis of enzymes, nucleic acids and proteins as a growth-regulating factor, in addition to acting as a coenzyme (Hathout, 1995). The results obtained in the present study are in line with the results reported by other authors (Dawood et al., 2019; Mohamed et al., 2020; Sadak et al., 2010). They demonstrated that the addition of exogenous BAS caused a growth of the roots, coleoptiles and plant fresh weight stressed by different abiotic factors. Reports on negative effects resulting from the use of SA on the growth of plants can be found in the scientific literature. The study conducted by Klocek and Mioduszewska (2001) determined the influence of SA on the length of the shoots, as well as the number of leaves, roots, and tubers formed in a potato. According to Basra et al. (2007) the inhibition of plant growth under the influence of SA is observed in its higher concentrations. These authors suggest that this may stem from the restriction in the absorption of nutrients due to the disturbances of membranes integrity. On the other hand, the study of Jazi et al. (2011) on the alleviating of the negative effect of $Pb(NO_3)_2$ showed that the most efficient SA dose turned out to be 10 μM. A similar limitation in growth efficiency was found in goji in response to 1 mM $Pb(NO_3)_2$ (Krupa-Małkiewicz et al., 2018). These authors reported that in the presence of 1 mM ascorbic acid in MS medium with 1 mM $Pb(NO_3)_2$ the shoot and root lengths of goji were enhanced by 31% and 74.5%, respectively, compared to lead-treated explants. In the study of Al-Hakimi and Hamada (2011), the negative effects of Cu toxicity to the growth of roots and shoots were partially alleviated by treatment of plants with ascorbic acid solutions, thiamine (vitamin $B_1$) and salicylic acid.

### Effects of BAS on the MDA content in leaves of barley under lead stress

Lead stress significantly increased the MDA content (by 176.7%) in coleoptiles with regards to the control (Table 1). Application of lead with combination of BAS effectively alleviated lipid peroxidation as reduced MDA content in the barley leaves. All means (Pb *versus* BAS+Pb) were significantly different (Table 1). The greatest decrease of MDA (by 44.9%) content in barley seedlings was observed after the use of SA, the lowest (20.5%) – after the use of AsA. Similar results were obtained by Khattab (2007) on canola, and Cao et al. (2013) on rice seedlings. In contrast, in a study by Krupa-Małkiewicz et al. (2018), it was observed that the presence of 1 mM AsA with 1 mM $Pb(NO_3)_2$ in MS medium had an inhibitory effect on the MDA content by 4% in goji seedlings compared to Pb treatment. However, 1 mM ASA induced MDA accumulation in goji seedlings by 24% when compared to the control. Lokhande et al. (2011) and Cao et al. (2013) suggested that the higher MDA concentration in plant tissues may be responsible for the reduction in membrane lipid peroxidation, which is related to high membrane oxidative damage and therefore higher $H_2O_2$ production. In contrast, Metwally et al. (2003) reported an increase in MDA of about 50% after the exposure to Cd in roots of SA-free controls. The effect of SA on lipid peroxidation was not due to a reduction in Cd accumulation in roots and shoots.

### Effects of BAS on Pro in leaves of barley seedlings under lead stress

Application of lead salt significantly increased the proline content by an average of 153% compared to the control (Table 1). Although for all applied BAS the proline content was higher if compared to the control ($H_2O$) on average by 7.9% (AsA), 18.0% (GSH), 3.0% (PP), 5.3% (α-Toc) and 27.7% for SA, only for GSH and SA these differences were described as significant. The application of BAS in the Pb stressed plants significantly reduced the proline content in coleoptiles of

the tested seedlings if compared to the Pb treated seedlings on average by 12.7% (AsA), 10.9% (GSH), 21.7% (PP), 18.4% (α-Toc) and only 5% for SA. The most beneficial effect of minimizing lead-induced stress was found when AsA, PP and α-Toc were applied, respectively. Pavliková et al. (2008) showed that the formation of large amounts of proline at high heavy metal concentrations leads to an increase in the glutamate kinase activity. This gives rise to an increase in the concentration of glutamic acid required for the synthesis of glutathione and phytochelatins in plant cells. Thus, the ability of plants to synthesize large amounts of proline after exposure to Pb stress suggests the ability to tolerate this element (Ozturk and Demir 2002). Pro is an indicator of oxidative stress. It accumulates in plant tissues when exposed to many environmental factors (Zhu et al., 2008) as well as has chelating and antioxidant properties, protecting enzymes from the denaturing effects of ROS. By capturing singlet oxygen, it protects CAT, POX, and polyphenol oxidase, among others (Verbruggen and Hermans 2008).

## Effects of BAS on the photosynthetic pigments content in leaves of barley under lead stress

Application of Pb salt in growth media significantly suppressed the photosynthetic pigments content (total chlorophyll and carotenoids) of tested barley seedlings (Table 1). The Pb-induced stress decreased the content of both total chlorophyll and carotenoid, by 33.5 and 31.3% in comparison to the control. However, it was observed that BAS application had a positive effect on the tested traits in comparison to the control. The greatest increase of the total chlorophyll and carotenoids content was determined after the use of SA, by 24.1% and 6.6% and PP by 25.8 and 15.0%, respectively.

The use of all exogenous biologically active substances in combination with Pb significantly reduced the toxicity of lead ions in comparison to the level of photosynthetic pigment content in the seedlings treated only with 1 mM $Pb(NO_3)_2$ (Table 1). According to Chen et al. (2007), Pb inhibits the synthesis and even increases the degradation of chlorophyll, resulting in impaired uptake of essential elements such as Mg and Fe by plants. In their study, they used exogenous SA pretreatment on young rice seedlings. The obtained result showed that SA could enhance the chlorophyll content under Pb stress significantly higher compared to the control. Similar results were obtained by Matewaly et al. (2003) on the influence of the stress caused by the effect of Cd on barley seedlings. In turn, Krupa-Małkiewicz et al. (2018) studied the exposure the goji seedlings to 1 mM $Pb(NO_3)_2$ and observed marked reduction on the contents of chlorophylls a and b and carotenoid, by 21%, 51% and 54%, respectively, with respect to the control. Addition of AsA to MS medium significantly mitigated the negative effect of Pb-stress factor. Khattab (2007) to alleviate saline stress in canola used GSH and Poly (A) (poliadenylic acid) as biologically active substances. According to many authors (Zechmann et al., 2008; Noctor et al., 2012) glutathione shows effects in various cellular functions in biosynthetic pathways, sulfur transport, gene expression, eliminates the reactive oxygen radicals (ROS), and resistance to biotic and abiotic stresses. Glutathione also has a protective function for the plant in forming conjugates with xenobiotics, and acts as a precursor for the synthesis of phytochelatins, which are involved in the detoxification of heavy metals (Cobbett and Goldsbrough, 2002). The canola seeds treated with GSH and Poly (A) significantly increased the contents of chlorophylls a and b. The ameliorating effects of glutathione may be due to their protective role in salinity tolerance by maintaining the redox status. In turn, Abdelhamid et al. (2013) showed that foliar treatment of nicotinamide on faba bean plant in two concentrations (200 and 400 mg) alleviated the effect on the contents of chlorophyll a, chlorophyll b and total pigments. In order to alleviate the effects of temperature stress in corn, Ahmad et al. (2014) used exogenous AsA, SA and $H_2O_2$. The chlorophyll b contents were increased with exogenous application of AsA, SA and $H_2O_2$ in maize. This increase in the chlorophyll b content might be due to enhancement in antioxidant production at low temperature which may have protected chlorophyll from degradation.

## Effects of BAS on enzyme activities in leaves of barley seedlings under lead stress

The use of lead salt had a significant influence on increase of antioxidant enzyme activities: CAT and POX by mean from 241.7% to 696.6% with respect to the control (Table 1). The increase of the activities of the above-mentioned enzymes in coleoptiles was also observed after the addition

of BAS. The increase for CAT was in the range from 34.9% to 66.8% and for POX from 16.4% to 110%, respectively. The use of BAS influenced the reduction of the activities of the tested enzymes in the Pb-stressed plants, in comparison to the test combination with lead only (Table 1). In the CAT case, the significantly decreased activity of the enzyme was observed for all combinations at a similar level. The use of combination of α-Toc+Pb was most favorable for POX, decreasing the activity of the enzyme by 71.86%. According to Krieger-Liszkay and Trebst (2006), Maeda and DellaPenna (2007) α-tocopherol is an antioxidant that has been demonstrated to deactivate reactive oxygen species from photosynthesis. It also scavenges peroxyl radicals in thylakoid membranes, thereby preventing lipid peroxidation. In turn, Chen et al. (2007) indicated that SA pretreatment in the absence of Pb was the most favorable for decreased CAT activity in rice seedlings. Rucińska-Sobkowiak (2010) indicated that the reports on the changes of antioxidant enzymes under the effect of environmental stresses differ. As reported by Dey et al. (2007), the reason for such different reactions of enzymes under similar stress conditions, may consist in not entirely identical experimental conditions. CAT, POX, SOD are significant antioxidant enzymes, which function in the cells; they are important in order to prevent the excess reactive oxygen species accumulation (Passardi et al., 2005). Hydrogen peroxide is removed by CAT and POX, among others (Cui and Zhao, 2011). The increase of activity of these enzymes indicates oxidative stress in the cells. A significant decrease of the activity of SOD, CAT and POX in plants, as provided by Dey et al. (2007) indicates the weakening of the scavenging systems for the reactive oxygen species, which are found during the effect of a stressor. These authors believe that the decrease of enzyme activity may be caused by enzyme inhibition. These proteins are sensitive to numerous factors. According to Rucińska-Sobkowiak (2010) as well as Cui and Zhao **(2011)** such different reactions in the activity of enzymes (increase, decrease or lack of changes) depend on the plant species, the treatments used, its concentration and the exposure time. The range of plant response to stress differs within one species; a variable tolerance to the same factor is exhibited (Malik et al., 2010).

Ward's agglomerative method was used to group treatments according to the morphological, biochemical and physiological parameters in order to reveal the effective influence of Pb and BAS on seedlings barley growth (Fig. 1). Treatments were clustered in six discrete groups (a–f). Separate groups were formed by the independent variables (control and Pb, respectively) and SA+Pb (Fig. 1). The other two groups included, respectively, BAS – added to investigate the effect *per se* on the

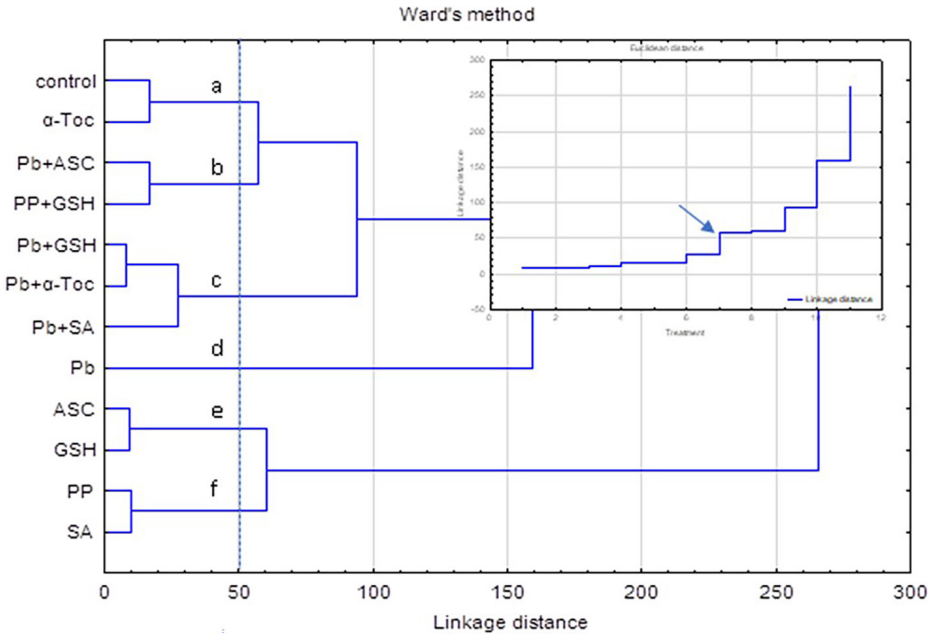


**Fig. 1.** Dendrogram of cluster analyses of Ward's method of dependent variables determined for seedlings of barley cv. Eunova after using twelve treatments. The vertical lines indicate the cuts-off used to form the five groups (a–f)

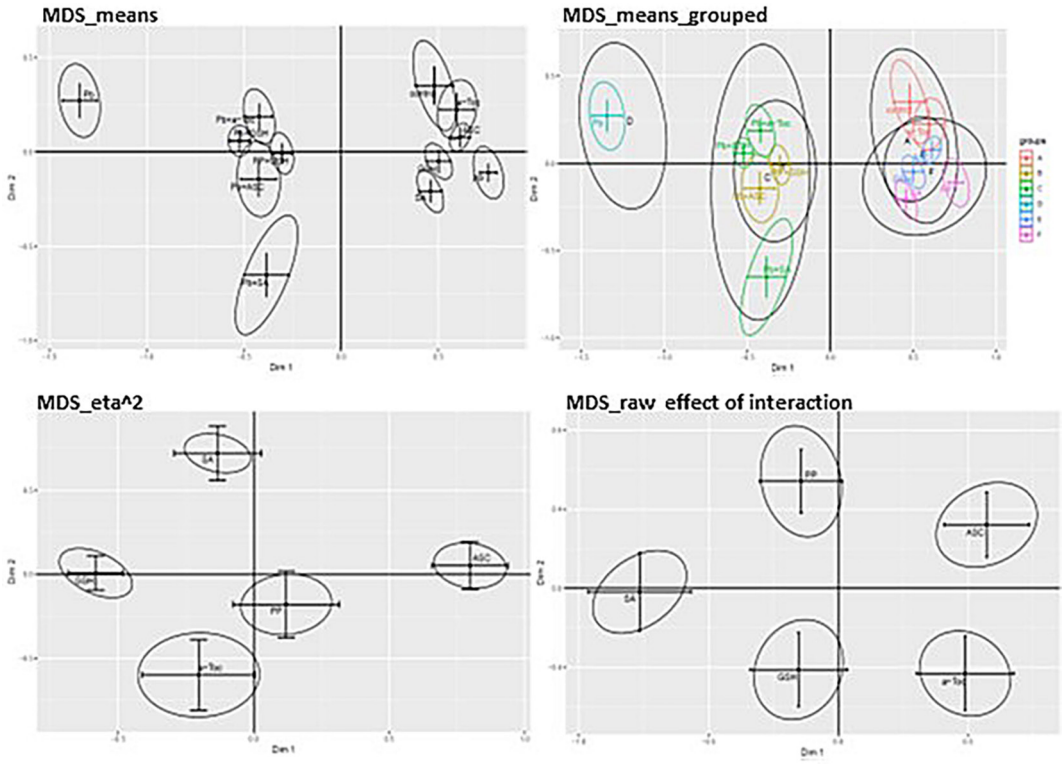


**Fig. 2.** Two-dimensional plots of MDS performed for treatment datasets using Euclidean distance matrix

studied seedling traits and BAS+Pb to investigate their ability to minimize the stress caused by the presence of lead salts in the solution. It has been shown that BAS inhibits the toxic effect of lead to varying degrees if selected morphological, physiological and biochemical parameters are analyzed (groups b and c). It should be added that Ward's agglomerative method was used by Manschadi et al. (2008) to group wheat genotypes differing in tolerances to drought in terms of growth angle and seminal root number. The effect of Ward's grouping has been used in MDS (Fig. 2). Clearly different coordinates were observed in both graphs for the five different groups. Distinct groups were formed by BAS and BAS+Pb. Others were control, PB and SA+Pb. The splits observed in the two-dimensional spaces of MDS clearly reflect the effect of BAS in alleviating the abiotic stress induced by lead salts.

To better visualize the differences in the interaction of various BASs with Pb used in the experiment, MDS was performed for the Eta^2 values generated in the ANOVA analyses for the interactions described for each BAS and the investigated dependent variables. The distribution of points (individual BAS) in different places of the two-dimensional MDS space suggests differences in the influence of individual BASs on minimizing the negative impact on the toxic effects of Pb. Similarly, for individual BASs, the 2D MDS space is presented after the analysis of the value mapping 'raw' inteaction BAS×Pb variance effect (Fig. 2).

## CONCLUSIONS

Lead salts negatively affect the determined morphological, biochemical and physiological parameters in 10-day-old spring barley seedlings. Of the BAS tested, the best effects in mitigating the negative effects of Pb stress were shown by: PP, α-Toc and GSH. In contrast, the lowest were determined for SA. Changes in the values of Pb-induced abiotic stress indicators, such as MDA, Pro, Chl a+b, Car, CAT and POX, reflect the stress status of the plant. They allow seeing and expressing the beneficial effect of BAS in minimizing it.

## REFERENCES

1. Abdelhamid M.T., Sadak M.S.H., Schmidhalter U.R.S., El-Saady A.K.M. 2013. Interactive effects of salinity stress and nicotinamide on physiological and biochemical parameters of Faba bean plant. Acta Biológica Colombiana, 18(3), 499–510.

2. Ahmad I., Maqsood S., Basra A., Wahid A. 2014. Exogenous application of ascorbic acid, salicylic acid and hydrogen peroxide improves the productivity of hybrid maize under at low temperature stress. International Journal of Agriculture & Biology, 16, 825–830.
3. Al-Hakimi A.B.M., Hamada A.M. 2011. Ascorbic acid, thiamine or salicylic acid induced changes in some physiological parameters in wheat grown under copper stress. Plant Protection Science, 47, 92–108.
4. Arnon D.J., Allen M.B., Halley F. 1956. Photosynthesis by isolated chloroplasts. Biochimica et Biophysica Acta, 20, 449–461.
5. Asada K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiology, 141, 391–396.
6. Barbero P., Beltrami M., Baudo R., Rossi D. 2001. Assessment of Lake Orta sediments phytotoxicity after limiting treatment. Journal of Limnology, 60(2), 269–276.
7. Basra S.M.A., Farooq M., Rehman H., Saleem B.A. 2007. Improving the germination and early seedling growth in melon (Cucumis melo L.) by pre-sowing salicylate treatments. International Journal of Agriculture and Biology, 9(4), 550–554.
8. Bates L.S. 1973. Rapid determination of free proline for water-stress studies Plant and Soil, 39, 205–207.
9. Berglund T., Ohlsson A.B. 1995. Defensive and secondary metabolism in plant tissue cultures, with special reference to nicotinamide, glutathione and oxidative stress. Plant Cell, Tissue and Organ Culture, 43, 137–145.
10. Cao F., Wang N., Zhang M., Dai H., Dawood M., Zhang G., Wu F. 2013. Comparative study of alleviating effects of GSH, Se and Zn under combined contamination of cadmium and chromium in rice (Oryza sativa). BioMetals 26(2), 297–308.
11. Chance B., Maehly A.C. 1955. Assay of catalase and peroxidases. Methods in enzymology. Vol 2 Eds CP Calonic, NO Kaplan New.
12. Chen J., Zhu C., Li L., Sun Z., Pan X. 2007. Effects of exogenous salicylic acid on growth and $H_2O_2$-metabolizing enzymes in rice seedlings under lead stress. Journal of Environmental Sciences, 19, 44–49.
13. Cobbett C., Goldsbrough P. 2002. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annual Review of Plant Biology, 53, 159–182.
14. Cui Y., Zhao N. 2011. Oxidative stress and change in plant metabolism of maize (Zea mays L.) growing in contaminated soil with elemental sulfur and toxic effect of zinc. Plant Soil and Environment, 57(1), 34–39.
15. Dawood M.G., Abdel-Baky Y.R., El-Awadi M.E.S., Bakhoum G.S. 2019. Enhancement quality and quantity of faba bean plants grown under sandy soil conditions by nicotinamide and/or humic acid application. Bulletin of the National Research Centre, 43, 28.
16. de Leeuw J., Mair P. 2009. Multidimensional scaling using majorization: SMACOF in R. Journal of Statistical Software, 31(3), 1–30.
17. Dey S.K., Dey J., Patra S., Pothal D. 2007. Changes in the antioxidative enzyme activities and lipid peroxidation in wheat seedlings exposed to cadmium and lead stress. Brazilian Journal of Plant Physiology, 19(1), 53–60.
18. Ebrahim M.K. 2005. Amelioration of sucrose-metabolism and yield changes, in storage roots of NaCl-stressed sugarbeet, by ascorbic acid. Agrochimica, 49(3–4), 93–103.
19. Fischbeck G. 2003. Diversification through breeding. Diversity in barley (Hordeum vulgare). Edit. von Bothmer R, van Hintum T, Knüpffer H, Sato K., Elsevier Science, Amsterdam.
20. Foyer C.H., Noctor G. 2005. Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant, Cell Environment, 28, 1056–1071.
21. Hager A., Mayer-Berthenrath T. 1966. Die Isolierung und quantitative Bestimung der Carotenoide und Chlorophyll von Blatern, Algen und isolierten Chloroplasten mit Hilfe Dunnschicht-chromatographischer Methoden. Planta, 69, 198–217.
22. Hathout T.A. 1995. Diverse effects of uniconazole and nicotinamide on germination, growth, endogenous hormones and some enzymic activities of peas. Egyptian Journal of Physiological Sciences, 19, 77–95.
23. Ishikawa T., Dowdle J., Smirnoff N. 2006. Progress in manipulating ascorbic acid biosynthesis and accumulation in plants. Physiologia Plantarum, 126, 343–355.
24. Jazi B.S., Yazdi L.X., Ranjbar M. 2011. Effect of salicylic acid on some plant growth parameters under lead stress in Brassica napus var. Okapi. Iranian Journal of Plant Physiology, 1(3), 177–185.
25. Jazi S.B., Oregani K.E. 2014. Impact of salicylic acid on the growth and photosynthetic pigment of canola (Brassica napus L.) under lead stress. International Journal of Biosciences, 4(10), 290–297.
26. Khan M.I.R., Iqbal N., Masood A., Khan N.A. 2012. Variation in salt tolerance of wheat cultivars: role of glycinebetaine and ethylene. Pedosphere, 22, 746–754.
27. Khan M., Fatma M., Per T., Anjum N., Khan N. 2015. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Frontiers in Plant Science, 6(462), 1–16.
28. Khattab H. 2007. Role of glutathione and polyadenylic acid on the oxidative defense systems of two different cultivars of Canola seedlings grown under saline condition. Australian Journal of Basic and Applied Sciences, 1(3), 323–334.
29. Klocek J., Mioduszewska H. 2001. Wpływ kwasu salicylowego i salicylohydroksamowego na wzrost

i rozwój ziemniaka w kulturach in vitro. Biotechnologia, 2(53), 148–151. [in Polish]

30. Krieger-Liszkay A., Trebst A. 2006. Tocopherol is the scavenger of singlet oxygen produced by the triplet states of chlorophyll in the PSII reaction centre. Journal of Experimental Botany, 57(8), 1677–1684.
31. Krupa-Małkiewicz M., Kruczek A., Pelc J., Smolik B., Ochmian I. 2018. Alleviating effects of ascorbic acid on lead toxicity in goji (Lycium barbarum L.) in vitro. Folia Pomer. Univer Stetin., Agric., Aliment., Pisc., Zootech., 340(45)1, 55–64.
32. Kumar S., Singh R., Nayyar H. 2012. α-Tocopherol application modulates the response of wheat (Triticum aestivum L.) seedlings to elevated temperatures by mitigation of stress injury and enhancement of antioxidants. Journal of Plant Growth Regulation, 32(2), 307–314.
33. Lichtenthaler H., Wellburn A. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions, 11, 591–592.
34. Lokhande V.H., Nikam T.D., Patade V.Y., Ahire M.L., Suprasanna P. 2011. Effects of optimal and supra-optimal salinity stress on antioxidative defence, osmolytes and in vitro growth responses in Sesuvium portulacastrum L. Plant Cell, Tissue and Organ Culture, 104, 41–49.
35. Lück H. 1963. Catalase, in: Methods of enzymatic analysis. Eds HU Bergmeyer Verlag Chemie, New York.
36. Maeda H., DellaPenna D. 2007. Tocopherol functions in photosynthetic organisms. Current Opinion in Plant Biology, 10, 260–265.
37. Malik A.A., Li W., Lou L.N., Weng J.F. 2010. Biochemical/physiological characterization and evaluation of in vitro salt tolerance in cucumber. African Journal of Biotechnology, 9(22), 3284–3292.
38. Manschadi A.M., Hammer G.L., Christopher J.T., deVoil P. 2008. Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (Triticum aestivum L.). Plant and Soil, 303(1–2), 115–129.
39. Metwally A., Finkemeier I., Georgi M., Dietz K.J. 2003. Salicylic acid alleviates the cadmium toxicity in Barley seedlings. Plant Physiology, 132, 272–281.
40. Mishra M., Mishra P.K., Kumar U., Prakash V. 2009. NaCl Phytotoxicity Induces Oxidative Stress and Response of Antioxidant Systems in Cicer arietinum L. cv. Abrodhi. Botany Research International, 2(2), 74–82.
41. Miura K., Tada Y. 2014. Regulation of water, salinity, and cold stress responses by salicylic acid. Frontiers in Plant Science, 23(5), 4.
42. Moghadam H.R.T. 2016. Application of super absorbent polymer and ascorbic acid to mitigate deleterious effects of cadmium in wheat. Pesquisa Agropecuária Tropical, 46(1), 9–18.
43. Mohamed, M.H., Badr, E.A., Sadak, M.S., Khedr H.H. 2020. Effect of garlic extract, ascorbic acid and nicotinamide on growth, some biochemical aspects, yield and its components of three faba bean (Vicia faba L.) cultivars under sandy soil conditions. Bulletin of the National Research Centre, 44, 1–8.
44. Naser Alavi S.M., Arvin M.J., Kalantari K.M. 2014. Salicylic acid and nitric oxide alleviate osmotic stress in wheat (Triticum aestivum L.) seedlings. Journal of Plant Interactions, 9, 683–688.
45. Noctor G., Mhamdi A., Chaouche S., Han Y., Neukermans J., Marquez-Garcia B. 2012. Glutathione in plants: an integrated overview. Plant, Cell & Environment, 35(2), 454–484.
46. Öztürk L., Demir Y. 2002. In Vivo and in Vitro Protective Role of Proline. Plant Growth Regulation, 38(3), 259–264.
47. Passardi F., Cosio C., Penel C., Dunand C. 2005. Peroxidases have more functions than a Swiss army knife. Plant Cell Reports, 24(5), 255–265.
48. Pavlíková D., Pavlík M., Staszková L., Motyka V., Száková J., Tlustoš P., Balík J. 2008. Glutamate kinase as a potential biomarker of heavy metal stress in plants. Ecotoxicology and Environmental Safety, 70, 223–230.
49. R Core Team R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/ (2017).
50. Rucińska-Sobkowiak R. 2010. Stres oksydacyjny wywołany działaniem metali ciężkich na rośliny. Postępy Biochemii, 56(2), 191–200. [in Polish]
51. Sadak M.S., Rady M.M., Badr N.M., Gaballah M.S. 2010. Increasing sunflower salt tolerance using nicotinamide and α-tocopherol. International Journal of Academic Research, 2(4), 263–270.
52. Sadak M.S., Dawood M.G. 2014. Role of ascorbic acid and α tocopherol in alleviating salinity stress on flax plant (Linum usitatissimum L.). Journal of Stress Physiology & Biochemistry, 10(1), 93–111.
53. Sędzik M., Smolik B., Krupa-Małkiewicz M. 2015. Effect of lead on germination and some morphological and physiological parameters of 10-day-old seedlings of various plant species. Environmental Protection and Natural Resources, 26, 3(65), 22–27.
54. Shahid M., Pourrut B., Dumat C., Nadeem M., Aslam M., Pinelli E. 2014. Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. Reviews of Environmental Contamination and Toxicology, 232, 1–44.
55. Sharma P., Dubey R.S. 2005. Lead toxicity in plants. Brazilian Journal of Plant Physiology, 17, 35–52.
56. Sharma S.S., Dietz K.J. 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress.

Journal of Experimental Botany, 57, 711–726.

57. Smolik M., Ochmian I., Bobrowska-Chwat A., Chwat G., Arus L., Banaszczak P., Bocianowski J., Milczarski P., Ostrowska K. 2022. Fingerprinting, structure, and genetic relationships among selected accessions of blue honeysuckle (Lonicera caerulea L.) from European collections. Biotechnol. Rep., 34, e00721.
58. Son J.A., Narayanankutty D.P., Roh K.S. 2014. Influence of exogenous application of glutathione on rubisco and rubisco activase in heavy metal-stressed tobacco plant grown in vitro. Saudi Journal of Biological Sciences, 21(1), 89–97.
59. Sudhakar C., Lakshim A., Giridarakumar S. 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (Morus alba L.) under NaCl salinity. Plant Science, 161, 613–619.
60. Venkatesh J., Park S. 2014. Role of L-ascorbate in alleviating abiotic stresses in crop plants. Botanical Studies, 55, 38.
61. Verbruggen N., Hermans C. 2008. Proline accumulation in plants: a review. Amino Acids, 35(4), 753–759.
62. Zechmann B., Mauch F., Sticher L., Müller M. 2008. Subcellular immunocytochemical analysis detects the highest concentrations of glutathione in mitochondria and not in plastids. Journal of Experimental Botany, 59(14), 4017–4027.
63. Zhu B., Xiong A., Peng R., Xu J., Zhou J., Xu J., Jin X., Zhang Y., Houl X., Yao X. 2008. Heat stress protection in Aspen sp1 transgenic Arabidopsis Thaliana. Biochemistry and Molecular Biology Reports, 41(5), 382–387.