Journal of Elementology ISSN 1644-2296

Sędzik M., Smolik B., Krupa-Małkiewicz M. 2019.
Effect of nicotinamide in alleviating stress caused by lead in spring barley seedling.
J. Elem., 24(1): 281-291. DOI: 10.5601/jelem.2018.23.2.1582

RECEIVED: 19 March 2018
ACCEPTED: 23 August 2018

ORIGINAL PAPER

EFFECT OF NICOTINAMIDE IN ALLEVIATING STRESS CAUSED BY LEAD IN SPRING BARLEY SEEDLING*

Maja Sędzik[1], Beata Smolik[1], Marcelina Krupa-Małkiewicz[2]

[1] Department of Plant Physiology and Biochemistry
[2] Department of Plant Genetics, Breeding and Biotechnology
West Pomeranian University of Technology in Szczecin, Poland

Abstract

Throughout their lives plants are constantly exposed to abiotic stress. Lead is a highly toxic element and has not yet been shown to have a positive effect on plants. This element causes inhibition of physiological and biochemical processes in plants. Exogenous application of vitamins to plants has been successfully used to minimize the adverse effects of abiotic stresses on plant growth, biochemical and physiological processes. The objective of the present study was to examine whether the adverse effects of 0.5 - 2.0 mM lead nitrate stress on plants could be mitigated by exogenous application of 25 - 100 μM nicotinamide (vitaminum PP). The study material consisted untreated seeds of spring barley (*Hordeum vulgare* var. Eunova). The tolerance to Pb stress was evaluated by measuring morphological traits (root and shoot length, plant fresh weight), biochemical and physiological parameters (malondialdehyde and proline content, catalase activity, total chlorophyll and carotenoids content) of 10-day-old seedlings originated from embryos cultured on the MS which contained lead and exposed to nitrate stress alone or with nicotinamide. All results, when compared to the control, showed that the higher concentration (2.0 mM) of $Pb(NO_3)_2$ had the most remarkable effect on the measured parameters. The reduction of tested parameters in barley seedlings is indicated. The addition of nicotinamide to the MS medium alleviated the adverse effect of $Pb(NO_3)_2$ stress on plant growth and selected biochemical parameters – MDA (malondialdehyd) and proline contents and CAT (catalase activity). Nicotinamide at 50 and 100 μM gives the best effect.

Keywords: abiotic stress, catalase, lead, malondialdehyde, morphological traits, pigments, proline.

Beata Smolik, PhD, Department of Plant Physiology and Biochemistry, Faculty of Environmental Management and Agriculture, West Pomeranian University of Technology in Szczecin, Słowackiego St.17, 71-434 Szczecin, Poland, e-mail: beata.smolik@zut.edu.pl
* The work was financed by a statutory activity subsidy from the Polish Ministry of Science and Higher Education for The Faculty of Environmental Management and Agriculture of West Pomeranian University of Technology (No 518-07-039-3171-03/18).

INTRODUCTION

Throughout their life cycle, plants are subjected to various types of environmental stresses, which include salinity, water deficit, temperature extremes, toxic metal ion concentration and UV radiations (Hayat et al. 2012). High levels of lead in the plant tissues cause the production of reactive oxygen species (ROS), which change the structure and permeability of the membranes (Sharma, Dubey 2005, Najeeb et al. 2014). Formation of aldehydes, especially malondialdehyde (MDA), is an important indicator of the damage in the membranes caused by ROS (Zielinski, Portner 2000). The level of MDA is now being exploited as a biomarker of oxidative stress (Otitoloju, Olagoke 2011, Smolik et al. 2013).

Proline plays three major roles during stress, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule (Hayat et al. 2012). To scavenge high ROS levels, an efficient system of non-enzymatic and enzymatic antioxidants is involved (Gill, Tuteja 2010). Stress factors, such as heavy metals, can also modulate the activities of antioxidative enzymes. Catalases are highly active enzymes that do not require cellular reductants as they primarily catalyse a dismutase reaction (Gill, Tuteja 2010, Mhamdi et al. 2010).

Vitamins could be considered as bio-regulator compounds which in relatively low concentrations exert profound influences on plant growth regulating factors that influence many physiological processes, such as synthesis of enzymes, act as co-enzymes and affect plant growth (Reda et al. 2005, Hassanein et al. 2009). Nicotinamide (vitamin PP) is a water-soluble vitamin from the B group. Nicotinamide is a constituent of the pyridine dinucleotide coenzymes NADH and NADPH, which are involved in many enzymatic oxidation-reduction reactions in living cells (Abdelhamid et al. 2013, Azooz et al. 2013). Exogenous application of vitamins to plants has been successfully used to minimize the adverse effects of abiotic stresses on plant growth, biochemistry and physiology processes (Hassanein et al. 2009, Sadak et al. 2010, Rady et al. 2011, Abdelhamid et al. 2013, Chaparzadeh, Chagharlou 2013). According to Athar et al. (2008), exogenous application of vitamins through the rooting medium can increase the endogenous content of vitamins.

Cereals are grasses that play an important role in the human diet (Paznocht et al. 2018). A widely-consumed cereal is barley (*Hordeum vulgare*) because of its ready availability, reasonable cost, and processing properties for products, such as beer, barley teas, soup, and baked products (Kim et al. 2007). Seed germination is the initial event in the life of a plant. This process is initiated by the regulation of enzymatic reactions which activate catabolic and anabolic process in the storage tissue and the embryonic axis. If a single component of these processes is affected, seed germination could be inhibited. Heavy metals influence the plant physiology and result in several nutritional disturbances (Abdelhamid et al. 2013).

The objective of the present study was to examine whether the adverse effects of 0.5 - 2.0 mM lead nitrate stress on barley plants could be mitigated by exogenous application of 25 - 100 μM nicotinamide.

MATERIAL AND METHODS

Material

The study material consisted untreated seeds of spring barley (*Hordeum vulgare* var. Eunova). The tolerance to Pb stress was evaluated by measuring morphological traits (root and shoot length, plant fresh weight), biochemical and physiological parameters (malondialdehyde and proline content, catalase activity, total chlorophyll and carotenoids content) of 10-day-old seedlings originated from embryos cultured on the MS (Murashige, Skoog 1962) medium containing 0.5 - 2.0 mM Pb salt alone or with 25 - 100 μM nicotinamide (vit PP). The content of the testing media was established on the basis of earlier tests (Sędzik et al. 2015). The control medium was MS. The experiment was carried out in 16 combinations: 1) control, 2) 25 μM Vit. PP, 3) 50 μM Vit. PP, 4) 100 μM Vit. PP, 5) 0,5 mM $Pb(NO_3)_2$, 6) 0,5 mM $Pb(NO_3)_2$ + 25 μM Vit. PP, 7) 0,5 mM $Pb(NO_3)_2$ + 50 μM Vit. PP, 8) 0,5 mM $Pb(NO_3)_2$ + 100 μM Vit. PP, 9) 1 mM $Pb(NO_3)_2$, 10) 1 mM $Pb(NO_3)_2$ + 25 μM Vit. PP, 11) 1 mM $Pb(NO_3)_2$ + 50 μM Vit. PP, 12) 1 mM $Pb(NO_3)_2$ + 100 μM Vit. PP, 13) 2 mM $Pb(NO_3)_2$, 14) 2 mM $Pb(NO_3)_2$ + 25 μM Vit. PP, 15) 2 mM $Pb(NO_3)_2$ + 50 μM Vit. PP, 16) 2 mM $Pb(NO_3)_2$ + 100 μM Vit. PP. Each combination of the experiment was represented by 100 embryos. The embryos were dissected from the seeds soaked in 0.5% sulfuric acid for 20 min, rinsed three times in sterile distilled water. Next, the seeds were treated with 7% sodium hypochloride, rinsed for 15 min in sterile distilled water and soaked in water for 24 h. Then, the embryos were excised with a needle, kept in 10% sodium hypochloride for 10 min., rinsed with sterile distilled water and transferred to a proper medium in test tubes (30 cm^3), 4 embryos in a test tube (9 cm x 3.5 cm). The test tubes were covered with aluminium foil and parafilm and held for 10 days in a growth chamber at 24°C, 16 h photoperiod (40 $\mu mol\ m^{-2}\ s^{-1}$) and 55-60 relative humidity. Morphological and biochemical features of 10-days old barley seedlings were compared with the control. The stages of the experiment are presented in Figure 1.

Determination of proline and malondialdehyde content

The concentration of free proline in barley was measured three times. The proline accumulation was determined according to Bates (1973). The content of malondialdehyde (MDA) in plant tissue was determined by the method described by Sudhakar et al. (2001).

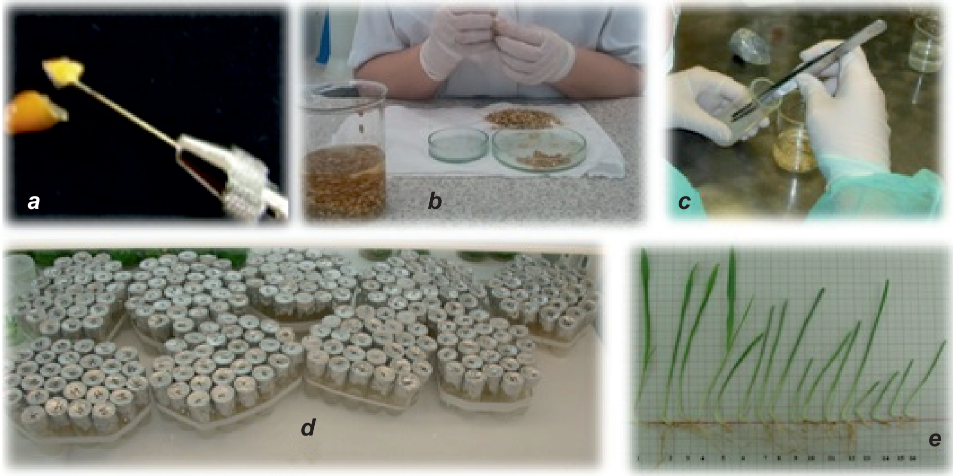

Fig. 1. Preparation steps of spring barley mature embryos under in vitro conditions: *a*, *b* – preparation of embryos using a dissecting needle, *c* – desinfection of embryos, *d* – embryos on a medium in glass tubes in a growth chamber, *e* – ready to measure ten-day-old seedlings

Determination of pigments content

The extraction of leaf pigments was performed with 80% (v/v) acetone. Chlorophyll total (chlorophyll *a*+*b*) and content of carotenoids were determined spectrophotometrically at 663, 645 and 440 nm. The content of chlorophylls was measured according to Arnon et al. (1956) with the modification by Lichtenthaler, Wellburn (1983), whereas the content of carotenoids was determined by the method of Hager, Meyer-Berthenrath (1966).

Determination of CAT activity (EC 1.11.1.6)

The method proposed by LUCK (1963) was used to determine the CAT activity. The decrease in absorbance, caused by decomposition of H_2O_2, was monitored continuously at 240 nm for 90 s. One unit of enzyme is the amount necessary to decompose 1 μM H_2O_2 min^{-1}.

Statistical analysis

The significance of differences was determined by means of variance analysis and the Tukey's test, at the level of significance of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Changes in plant growth and development are often affected by various environmental conditions. A common response of plants to heavy metal stress is growth inhibition (Li et al. 2005, Sędzik et al. 2015). Lead is one of the most dangerous heavy metal pollutants in the environmental that originates from various sources. The main sources of lead are dust and gases from volcanic eruptions, as well as mines, smelters, industry and agriculture. The steadily increasing levels of this metal in the environment inhibit germination of seeds and exert a wide range of adverse effects on the growth and metabolism of plants (Verma, Dubey 2003, Sędzik et al. 2015).

In the present study, the $Pb(NO_3)_2$ treatment inhibited seed germination, root and shoot growth and fresh weight of barley seedlings in comparison to the control (Table 1).

The value of the seed germination index (%) in spring barley seeds under the $Pb(NO_3)_2$ salt stress ranged from 16.77 to 31.40% (Table 1). It was observed, that the lowest germination capacity of barley seeds occurred at the higher Pb salt concentration (2 mM $Pb(NO_3)_2$). Moreover, on the medium with the addition of 0.5 mM $Pb(NO_3)_2$, the addition of PP, regardles of its concentration, did not significantly influence the value of the tested trait. In the case of barley seedlings from MS media supplied with 1.0 and 2.0 mM solution of $Pb(NO_3)_2$, the best efficiency in alleviating the stress effects was

Table 1

Germination index value and summary of morphological characteristics, fresh weight, and the tolerance index of spring barley seedlings growing under stress induced by $Pb(NO_3)_2$ and in the presence of nicotinamide

No.	Treatments	Germination index IG (%)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Tolerance index (%)
1.	Control	100.00	11.71 ± 1.05	9.36 ± 2.93	0,32 ± 0.01	100.00
2.	25 μM PP	50.99	10.86 ± 3.18	9.16 ± 0.81	0.31 ± 0.01	97.86
3.	50 μM PP	58.68	8.81 ± 0.55	7.11 ± 2.31	0.32 ± 002	75.80
4.	100 μM PP	72.48	9.73 ± 0.71	8.76 ± 1.01	0.33 ± 0.01	93.59
5.	0.5 mM $Pb(NO_3)_2$	21.24	7.23 ± 2.21	6.23 ± 1.76	0.22 ± 0.03	66.19
6.	0.5 mM $Pb(NO3)_2$+25 μM PP	21.74	7.41 ± 1.32	7.01 ± 1.45	0.24 ± 0.08	74.73
7.	0.5 mM $Pb(NO_3)_2$+50 μM PP	23.14	8.21 ± 1.93	7.50 ± 1.41	0.27 ± 0.07	80.07
8.	0.5 mM $Pb(NO_3)_2$+100 μM PP	23.97	8.43 ± 2.72	7.06 ± 2.19	0.29 ± 0.05	75.44
9.	1 mM $Pb(NO_3)_2$	31.40	6.33 ± 1.71	3.84 ± 1.85	0.18 ± 0.14	40.57
10.	1 mM $Pb(NO_3)_2$+25 μM PP	17.93	5.76± 0.55	2.16 ± 0,42	0.18 ± 0.10	23.13
11.	1 mM $Pb(NO_3)_2$+50 μM PP	47.11	7.73 ± 3.12	5.70 ± 1.11	0.21 ± 0.12	60.85
12.	1 mM $Pb(NO_3)_2$+100 μM PP	52.64	8.76 ± 0.20	6.36 ± 1.62	0.23 ± 0.10	67.97
13.	2 mM $Pb(NO_3)_2$	16.78	4.06 ± 1.12	2.03 ± 0.46	0.13 ± 0.01	21.71
14.	2 mM $Pb(NO_3)_2$+25 μM PP	18.18	5.13 ± 0.86	3.03 ± 0.26	0.14 ± 0.03	23.49
15.	2 mM $Pb(NO_3)_2$+50 μM PP	19.01	5.46 ± 0.76	2.3 ± 0.78	0.14 ± 0.01	24.55
16.	2 mM $Pb(NO_3)_2$+100 μM PP	22.89	5.71 ± 0.72	2.76 ± 0.37	0.16 ± 0.04	24.55
	LSD (5%)	-	0,46	0,57	0,03	-

demonstrated by the addition of PP vitamin at the highest concentration – 100 μM. The current literature suggests that seed germination is affected by metals in two ways. Firstly, by their general toxicity, and secondly, by their inhibition of water uptake (Kranner, Colville 2011). Seed germination inhibition by heavy metals has been reported by Verma, Dubey (2003) in rice, Li et al. (2005) in *Arabidopsis thaliana* and Sędzik et al. (2015) in various plant species.

Our results indicate a decrease in vigour (length and weight) of barley seedlings when increasing concentrations of $Pb(NO_3)_2$ in MS medium were tested (Table 1). With 2 mM $Pb(NO_3)_2$ in the MS medium, up to a 65% reduction in root length and 78% reduction in shoot length in comparison with the control group was observed in 10-day-old seedlings. Similarly, up to a 59% decline in plant fresh weight compared with the control was noticed in seedlings at 10-days' growth. Moreover, control or stressed seedlings showed a higher plant fresh weight with than without nicotinamide (Table1). This may indicate that that the presence of nicotinamide in the MS medium supplemented with $Pb(NO_3)_2$ raised the Pb tolerance of barley seedlings, as indicated by IT (%). It was observed that the concentration of nicotinamide did not significantly influence the IT value only when 2.0 mM $Pb(NO_3)_2$ were used. According to many authors (Verma, Dubey 2003, Li et al. 2005, Sędzik et al. 2015), roots assimilate Pb better than leaves. However, leaves differ in their abilities to accumulate Pb depending on age. The role of vitamins in modifying the environmental stresses induced changes in the osmoprotectant content and was also investigated by Sadak et al. (2010), Abdelhamid et al. (2013). According to Berglund, Ohlsson (1995), nicotinamide may be of value in biotechnology for the production of valuable substances as well as for plant protection. This vitamin might act as an activator of protein synthesis through modulation of the activity of enzymes involved in the metabolism of proteins or sugars.

Moreover, nictotinamide can significantlt improved physiological and biochemical parameters (Abdelhamid et al. 2013). According to Verma, Dubey (2003), the heavy metals (Cd, Pb, Al, Zn) are known to produce ROS and induce oxidative stress in certain plant species. MDA is one of the end products that are produced as a result of lipid peroxidation damage by free radical. Heavy metal stress significantly increases MDA (from 27.47 to 32.09 nmol g^{-1} fm), and at 2.0 mM $Pb(NO_3)_2$ the effect was more pronounced (Figure 2). Application of nicotinamide (irrespective of the concentrations) attenuated the effect of heavy metal stress, by decreasing the MDA level in comparison to the control (22.90 nmol g^{-1} fm). Proline accumulation is one of the most frequently reported modifications induced by environmental stresses (Kahlaoui et al. 2018). In this study, the content of proline was significantly increased (1.41 μmol g^{-1} fm) when 2.0 mM $Pb(NO_3)_2$ were used in comparison to the control (1.04 μmol g^{-1} fm). A similar response to a Pb treatment was previously noticed in various plants (Zengin, Munzuroglu, 2005, Awaad et al. 2010, Sędzik et al. 2015). Application of nicotinamide into the medium under

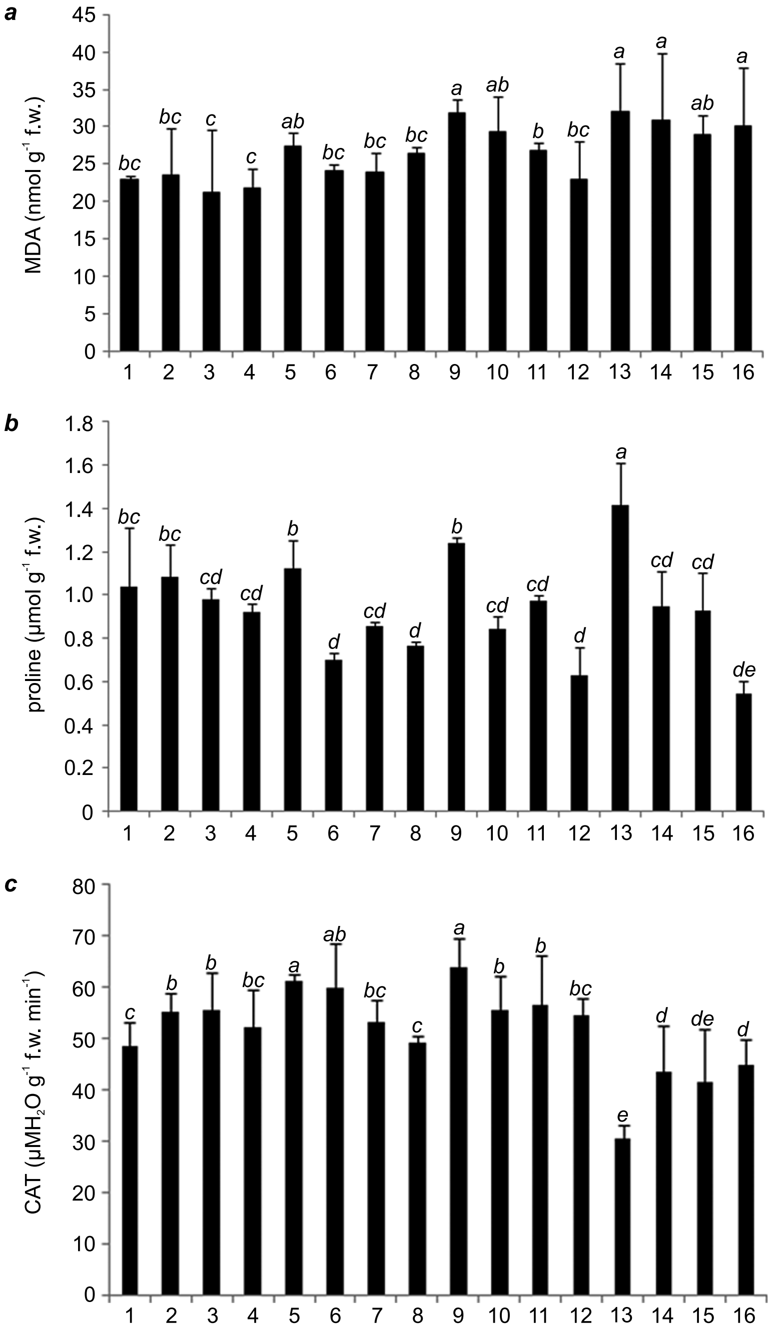

Fig. 2. Summary of biochemical parameters (*a* – MDA, *b* – proline, *c* – CAT activity) in spring barley seedlings growing under stress induced by lead in the presence of nicotinamide; values denoted with the same letters within an experimental variant do not differ statistically ($p < 0,05$); no. 1-16 – see Table 1

heavy metal stress condition significantly decreased the proline level in 10-day-old barley seedlings (0.55 - 0.96 μmol g^{-1} fm) (Figure 2). These results are in agreement with the results observed by Azooz et al. (2013), who suggested that most of the vitamins tend to increase the proline content.

In our study, the activity of catalase (CAT) was observed to have increased during the 10-day-long growth of barley seedlings under heavy metal stress (Figure 2). With increasing levels of $Pb(NO_3)_2$ in the MS medium, the activity of CAT peaked (63.87 μM H_2O_2 g^{-1} fm) in comparison to the control (48.71 μM H_2O_2 g^{-1} fm). However, the activity of CAT significantly decreased (41.42 - 59.98 μM H_2O_2 g^{-1} fm) when nicotinamide was used. Moreover, the higher concentration of nicotinamide (2.0 μM) was more effective than the lower one (0.5 μM). The results are in agreement with the ones obtained by Verma, Dubey (2003), who suggested that the activity of catalase rapidly increased due to the plant's response to a given stressor.

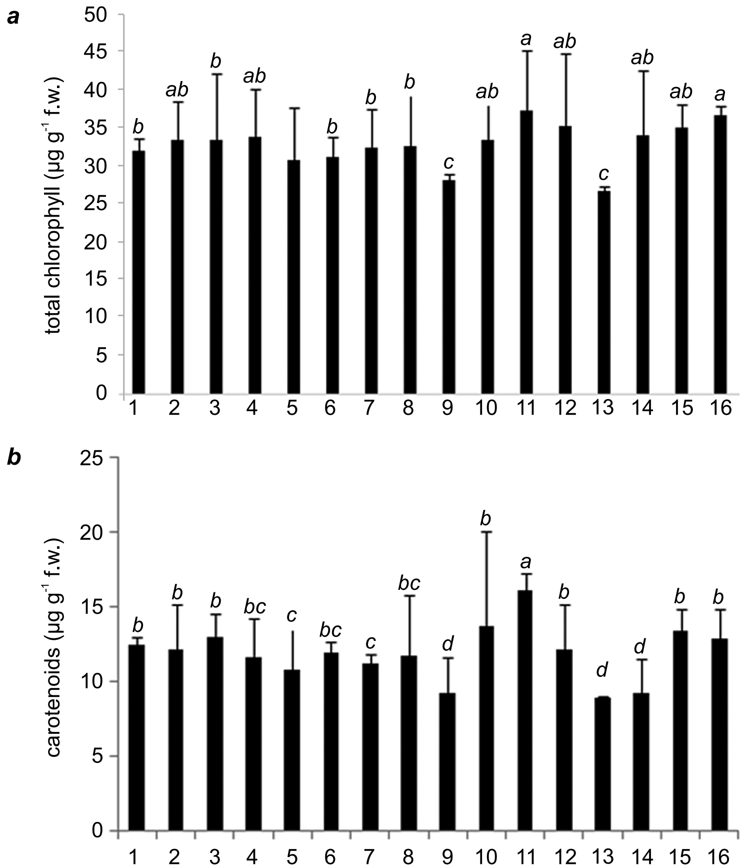

Fig. 3. Physiological parameters (*a* – total chlorophyll, *b* – carotenoids content) barley seedlings growing under stress induced by $Pb(NO_3)_2$ in the presence of nicotinamide.
Values denoted with the same letters within an experimental do not differ statistically ($p < 0,05$); no. 1-16 – see Table 1

According to PURNAMA et al. (2015) and SĘDZIK et al. (2015), a high Pb concentration in the substrate also has a significant influence on the content of photosynthetic (chlorophyll) and non-photosynthetic (carotenoids) pigments. Heavy metals lead to the direct inhibition of the activity of enzymes responsible for the chlorophyll *a* and *b* synthesis and to the malfunctioning of photosynthesis (PURNAMA et al. 2015). In the present study, increasing the $Pb(NO_3)_2$ content in the MS medium caused a decrease in the total chlorophyll and carotenoid concentrations in 10-day-old barley (Figure 3). Seedlings on the MS medium supplemented with 2.0 μg g^{-1} fm showed a 17% and 29% reduction in the total chlorophyll and carotenoids, respectively, compared to the control (32.24 μg g^{-1} fm and 12.52 μg g^{-1} fm, respectively).

However, the addition of nicotinamide to the MS medium supplemented with 1.0 and 2.0 mM $Pb(NO_3)_2$ had a significant effect on chlorophyll and carotenoids. Similar results were obtained by AZOOZ et al. (2013) in *Vicia faba* L., and by SĘDZIK et al. (2015) in experiments on various crop plant.

CONCLUSIONS

In conclusion, the present work has shown that lead exposition to barley seedling induces numerous metabolic disturbances, which result in a dose--dependent inhibition of seeds' germination, root and shoot length, fresh weight, as well as MDA, proline, CAT and total chlorophyll and carotenoids. In this study, the antioxidant properties and role of nicotinamide in lead stress were shown as the compound could inhibit the damaging effects of lead. The application of nicotinamide as an antioxidant increased the apical growth, as well as the development and biochemical parameters of *Hordeum vulgare* var. Eunova in *in vitro* culture. However, it was observed that nicotinamide in concentrations of 50 or 100 μM produced the best effect. The increase of tolerance to heavy metal stress by nicotinamide may be due to the enhanced antioxidant capacity, but may also be through the restoration of the hormone balance, which has to be verified in a future study.

REFERENCES

ABDELHAMID M.T., SADAK M.S.H., SCHMIDHALTER U.R.S., EL-SAADY M. 2013. *Interactive effects of salinity stress and nicotinamide on physiological and biochemical parameters of faba bean plant*. Acta Biol. Colomb., 18(3): 499-510.

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 Protect Sci., 47: 92-108.

ARMON D.J., ALLEN M.B., WHATLEY F. 1956. *Photosynthesis by isolated chloroplast*. Biochim Biophys Acta., 20: 449-461.

ATHAR H.R., KHAN A., ASHRAF M. 2008. *Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat*. Environ Exp Bot., 63: 224-231.

Awaad H.A., Youssef M.A., Moustafa E.S.A. 2010. *Identification of genetic variation among bread wheat genotypes for lead tolerance using morpho – physiological and molecular markers*. J Am Sci., 6(10): 1142-1153.

Azooz M.M., Alzahrani A.M., Youssef M.M. 2013. *The potential role of seed priming with ascorbic acid and nicotinamide and their interactions to enhance salt tolerance in broad bean (Vicia faba* L.*)*. Aust J Crop Sci., 7: 2091-2100.

Bates L.S. 1973. *Rapid determination of free proline for water-stress studies*. Plant Soil, 39: 205-207.

Berglund T., Ohlsson A.B. 1995. *Defensive and secondary metabolism in plant tissue culture, with special reference to nicotinamide, glutathione and oxidative stress*. Plant Cell Tiss Org., 43: 137-145.

Chaparzadeh N., Chagharlou M.G. 2013. *Alleviation of adverse effects of copper on Allium cepa L. by exogenous ascorbic acid application*. J Plant Physiol Breed., 3(2): 1-12.

Gill S.S., Tuteja N. 2010. *Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants*. Plant Physiol Bioch., 48: 909-930.

Hager A., Mayer-Bertenrath T. 1966. *Extraction and quantitative determination of carotenoids and chlorophylls of leaves, algae and isolated chloroplasts with the aid of thin-layer chromatography*. Planta, 69: 198-217. (in German)

Hassanein R.A., Bassuony F.M., Baraka D.M., Khalil R.R. 2009. *Physiological effects of nicotinamide and ascrobic acid on Zea mays plant grown under salinity stress. 1-changes in growth, some relevant metabolic activities and oxidative defense systems*. Res. J. Agric. Biol. Sci., 5(1): 72-81.

Hayat I.S., Hayat Q., Alyemeni M.N., Wani A.S., Pichtel J., Ahmad A. 2012. *Role of proline under changing environments*. Plant Signal Behav., 7(11): 1456-1466. DOI: 10.4161/psb.21949

Kahlaoui B., Hachicha M., Misle E., Fidalgo F., Teixeira J. 2018. *Physiological and biochemical responses to the exogenous application of proline of tomato plants irrigated with saline water*. J Saudi Soc Agric Sci., 17: 17-23.

Khan A., Ahmad M.S., Athar H., Ashraaf M. 2006. *Interactive effect of foliarly applied ascorbic acid and salt stress on wheat (Triticum aestivum* L.*) at the seedling stage*. Pak J Bot., 38(5): 1407-1414.

Kim M.J., Hyun J.N., Kim J.A., Park J.Ch., Kim M.J., Kim J.G., Lee S.J., Chun S.Ch., Chung I.M. 2007. *Relationship between phenolic compounds, anthocyanins kontent and antioxidant activity in colored barley germplasm*. J Agric Food Chem., 55: 4802-4809.

Kranner I., Colville L. 2011. *Metals and seeds: Biochemical and molecular implications and their significance for seed germination*. Environ Exp Bot., 72: 93-105.

Li W., Khan M.A., Yamaguchi S., Kamiya Y. 2005. *Effects of heavy metals on seed germination and early seedling growth of Arabidopsis thaliana*. J Plant Growth Regul., 46: 45-50. DOI: 10.1007/s10725-005-6324-2

Lichtenthaler H.K., Wellburn A.R. 1983. *Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents*. Biochem Soc T., 11: 591-592.

Lück H. 1963. *Catalase*. In: *Methods of enzymatic analysis*. H.U. Bergmeyer (Eds.), Verlag Chemie., 885-888 pp.

Mhamdi A., G., Chaouch S., Vanderauwera S., Van Breusegem F., Noctor G. 2010. *Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models*. J Exp Bot., 61(15): 4197-220. DOI: 10.1093/jxb/erq282

Murashige T., Skoog F. 1962. *A revised medium for rapid growth and bio assays with tobacco tissue cultures*. Physiol Plantarum, 15: 473-497. DOI: 10.1111/j.1399-3054.1962.tb08052.x

Najeeb U., Ahmad W., Zia M., Malik Z., Zhou W. 2014. *Enhancing the lead phytostabilization in wetland plant Juncus effusus* L. *through somaclonal manipulation and EDTA enrichment*. Arab J Chem. DOI: org/10.1016/j.arabjc.2014.01.009

Otitoloju A., Olagoke O. 2011. *Lipid peroxidation and antioxidant defense enzymes in Clarias gariepinus as useful biomarkers for monitoring exposure to polycyclic aromatic hydrocarbons.* Environ Monit Assess., 182: 205-213.

Purnama P.R., Soedarti T., Purnobasuki H. 2015. *The effects of lead [Pb(NO_3)$_2$] on the growth and chlorophyll content of sea grass [Thalassia hemprichii (Ehrenb.)* Aschers.*] ex situ.* Vegetos, 28(1): 9-15.

Rady M.M., Sadak M.S., El-Bassiouny H.M.S., Abdel-Monem A.A. 2011. *Alleviation the adverse effects of salinity stress in sunflower cultivars using nicotinamide and a-Tocopherol.* Aust J Basic Appl Sci., 5(10): 342-355.

Reda F., Abdel-Rahim E.A., El-Baroty G.S., Ayad H.S. 2005. *Response of essential oil, phenolic components and polyphenol oxidative activity of Thyme (Thymus vulgaris* L.*) to some bioregulators and vitamins.* Int J Agric Biol., 7(5): 735-739.

Sadak M.S.H., Abdelhamid M.T., El-Saady M., 2010. *Physiological responses of faba bean plant to ascorbic acid grown under salinity stress.* Egypt J Agron., 32(1): 89-106.

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.* Environ Protect Natur Res., 26(3): 22-27. DOI: 10.1515/OSZN-2015-0009

Sudhakar C., Lakshim A., Giridara-Kumar S. 2001. *Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (Morus alba* L.*) under NaCl salinity.* Plant Sci., 161: 613-619.

Sharma P., Dubey R. 2005. *Lead toxicity in plants.* Braz J Plant Physiol., 17(1): 35-52.

Verma S., Dubey R.S. 2003. *Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants.* Plant Sci., 164: 645-655.

Zengin F.K., Munzuroglu O. 2005. *Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (Phaseolus vulgaris* L.*) seedlings.* Acta Biol Cracov Bot., 47(2): 157-164.

Zieliński S., Pörtner H.O. 2000. *Oxidative stress and antioxidative defense in cephalopods: a function of metabolic rate or age?* Comp Biochem Phys B., 125(2): 147-60.