ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Antioxidant Properties of Stevioside Under the Influence of Heavy Metals.

Alexander Leonidovich Mikhailov*, Julia Yuryevna Nevmerzhitskaya, and Olga Arnoldovna Timofeeva.

Kazan Federal University, Kremlevskaya St. 18, Kazan, Republic of Tatarstan.

ABSTRACT

This paper deals with the study of the antioxidant activity of diterpene glycoside of stevioside in the absence of stress factors and under the influence of heavy metals. Results showed different response of stevioside to – it reduced catalase activity by 34% as compared to control, but virtually had no effect on the enzymatic activity of an ascorbate peroxidase. Heavy metals at suboptimal concentrations (10 µM) also did not significantly change the activity of the studied enzymes. Growing of plants at a sublethal concentration of pollutants (1 mM) was accompanied by a sharp increase in the activity of ascorbate peroxidase, and, otherwise, decrease in catalase activity. Plants pretreatment with diterpene glycosides ensured reduction of the negative effects of heavy metals on the activity of these enzymes, i.e., ascorbate peroxidase activity was lower and catalase activity was higher. The influence of stevioside also led to 4-fold increase in the content of non-enzymatic antioxidant – proline that may indicate an increasing stress resistance of plants to negative environmental factors.
Keywords: *Triticum aestivumL.*, steviozide, heavy metals, catalase, ascorbate peroxidase, proline.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Urbanization and industrialization develop rapidly, which in turn causes an intensive development of agriculture, a colossal mining, which leads to both the depletion of natural resources and the negative effects on the biosphere. These effects include the spread and accumulation of heavy metals (HM). The HM ions can quite easily get into plants and tend to accumulate in them, thereby having a negative influence on many physiological processes that somehow leads to retardation or stops their growth and development, and in certain cases causes their death [1 2]. As a rule, the negative HM impact is associated with oxidative stress, caused by the generation of reactive oxygen species (ROS).

However, plants have antioxidant defense system consisting of enzymatic and non-enzymatic compounds, which ensure plants adaptation and survival in the pollution of the environment with HM [1, 2, 3]. The most important role among antioxidant enzymes in ROS detoxification belongs to enzymes of the ascorbate-glutathione cycle, a catalase, a superoxide dismutase, the peroxidases group, etc. [4, 5]. ROS detoxification also involves non-enzymatic antioxidants such as ascorbic acid, proline, tocopherol, polyamines, reduced glutathione, etc. Their activity mechanism lies in the fact that they react with ROS and through their oxidation interrupt dangerous for the cell chain reactions [6].

It is known that the preparations with growth-regulatory activity can increase the resistance of plants to hypo- and hyperthermia, drought, salinity, and toxic effects (HM) [7, 8]. To date, the substances released from plants seem to be very promising because they are environmentally safe and react at very low concentrations. Most interesting among the tetracyclic compounds are kaurene derivatives, which, among all, include a tetracyclic diterpenoid steviol being the glycoside aglycone derived from the extract of herb *Stevia Rebaudiana Bertoni* [9]. One of the glycosides of the plant is stevioside [10].

Objective of this study was to determine the antioxidant properties of stevioside under the oxidative stress caused by heavy metals.

MATERIAL AND RESEARCH METHODS

Object of the study were the roots of winter wheat (*Triticum aestivum* L.) of Kazanskaia 560 variety. Stevioside was obtained from vegetable raw materials in A.E. Arbuzov IOPC in the laboratory of phosphate analogs of natural compounds of the correspondent member of RAS V.F. Mironov. Wheat plants were grown in vitro in cells with illumination of 100 W/m^2, in 12-hour photoperiod and at a temperature of 23^{o}C. During 5 days, the control plants were growing with addition of water, and the experimental - with stevioside solution at a concentration of 10^{-8}M. Then the 5-day plants were placed in $CdSO_4$, $CuSO_4$ and $ZnSO_4$solutions at concentrations of 10 µM and 1 mM where they were growing for 4 days more.

We determined catalase activity by using a spectrophotometric method proposed by Aeby [11]. The method is based on determining the decomposition rate of hydrogen peroxide by the studied catalase sample with further formation of water and oxygen. Ascorbate peroxidase activity was determined by the method of [12]. The method is based on the ability of ascorbate peroxidase to increase the decomposition rate of H_2O_2 through the action of ascorbic acid (AA). Free proline content was determined by ninhydrin test.

RESULTS AND DISCUSSION

According to research conducted by our group, stevioside shows both the growth-stimulating and anti-stress activity, since it improves the frost resistance, as well as reduces the toxic effect of HM on lectin activity and growth of winter wheat plants [13].

Catalase is a heme-containing enzyme composed of four subunits. It catalyzes the decomposition of hydrogen peroxide to water and molecular oxygen. Catalase has low affinity for H_2O_2, but less specificity toward organic peroxides (R-O-O-R). It has a very high reaction rate (6×10^6 molecules of H_2O_2 in H_2O and O_2 min^{-1}) and is unique among the antioxidant enzymes, because it requires no reducing equivalents [5]. Catalase decomposes H_2O_2 with minimum energy consumption [14].

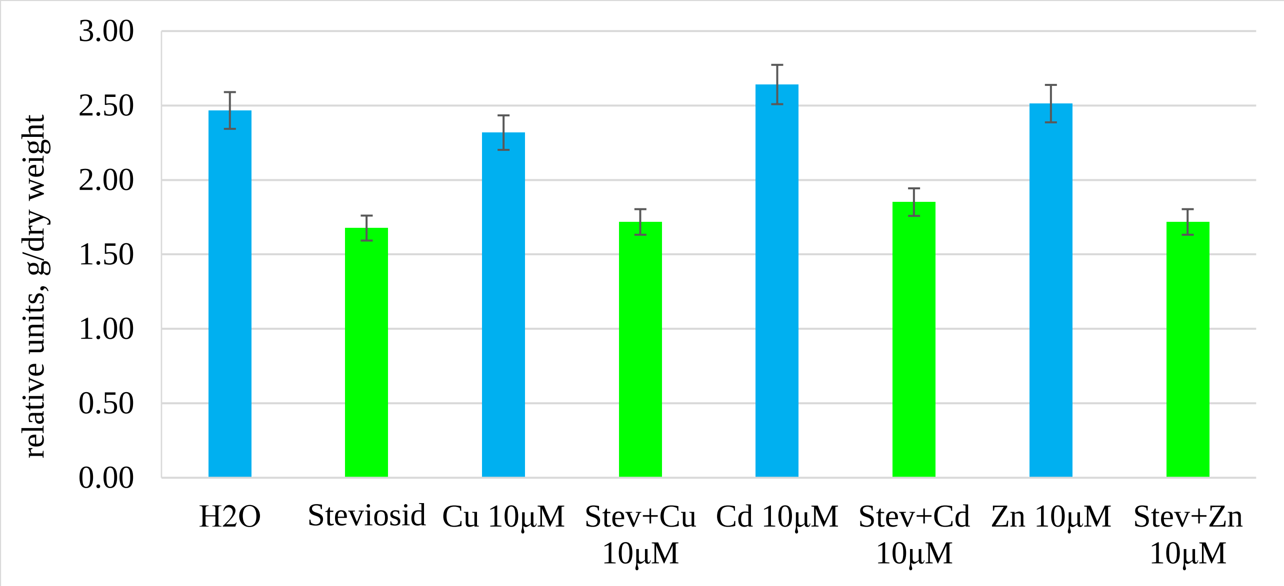

Fig. 1 - Effect of stevioside (10^{-8} M) on catalase activity in winter wheat seedling roots during operation $CdSO_4$, $ZnSO_4$ and $CuSO_4$ concentration (10 µM).

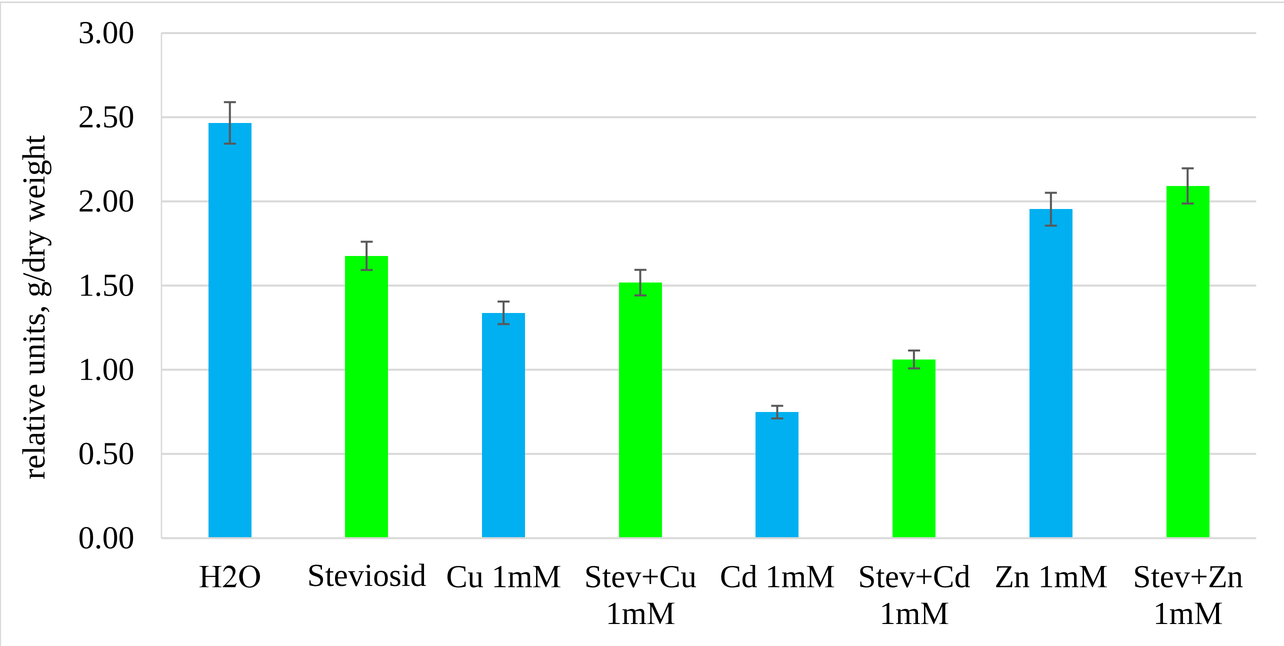

Fig. 2 - Effect of stevioside (10^{-8} M) on catalase activity in winter wheat seedling roots during operation $CdSO_4$, $ZnSO_4$ and $CuSO_4$ concentration (1 mM).

As can be seen from Fig. 1, 2, stevioside (10^{-8}M) reduced the catalase activity as compared to the control, and the difference was about 34%. This reduction in enzyme activity under the action of stevioside is possibly due to the fact that it reduces the flow of micronutrients to plants, including iron ions, which are necessary for the catalase since being a heme-containing protein.

Suboptimal concentration of metals ($CuSO_4$, $CdSO_4$ and $ZnSO_4$) (10 µM) did not affect the activity of the studied enzyme (Fig. 1), which is consistent with the literature data [15]. Pretreatment with diterpene glycoside has led to lower catalase activity than in the control group, which remained at the same level throughout the experiment, with the difference equal to 30%, as well as in the experiment with one stevioside.

Action of HM at a sublethal concentration (1 mM) inhibited the catalytic activity of catalase, but the obtained data were different under the influence of pollutants (Fig. 2). There is evidence that at moderately toxic concentration of HM (0.5 mM) the activity of catalase increased, whereas at highly toxic level of HM (1 mM) there was a substantial inhibition of the enzyme activity [16].

Under the action of $CuSO_4$ and $CdSO_4$, catalase activity decreased by 51% and 66%, respectively, as compared to the control (Fig. 2). $ZnSO_4$ at a concentration of 1 mM also reduced the enzyme activity of the test enzyme, and the difference with the control was 23%.

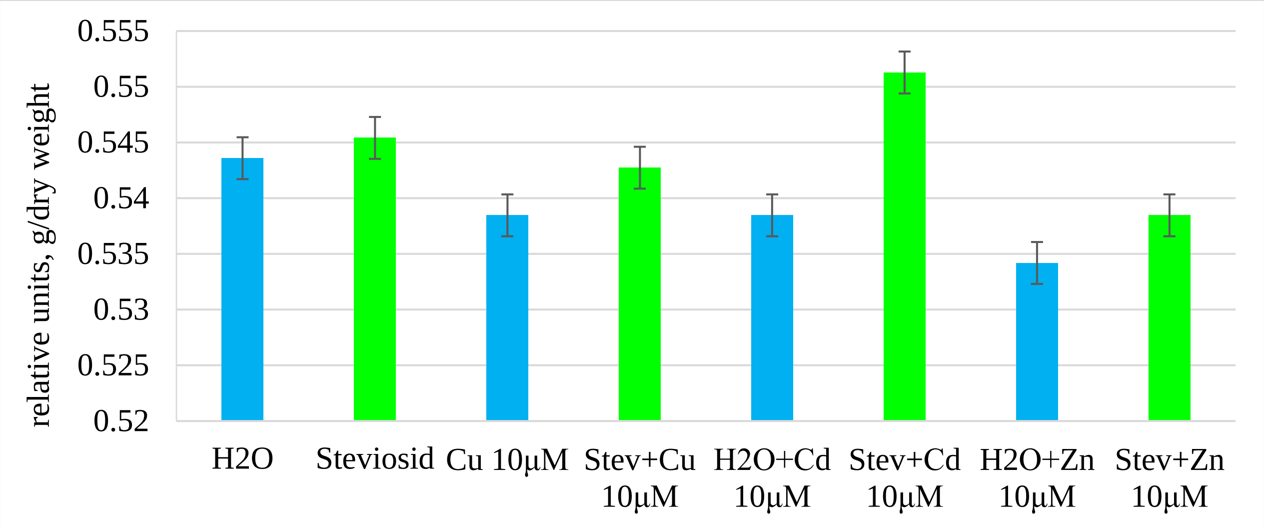

Fig. 3 - Effect of stevioside (10^{-8} M) and heavy metals ($CuSO_4$, $CdSO_4$, $ZnSO_4$) at a concentration (10 μM), ascorbate peroxidase activity on winter wheat roots.

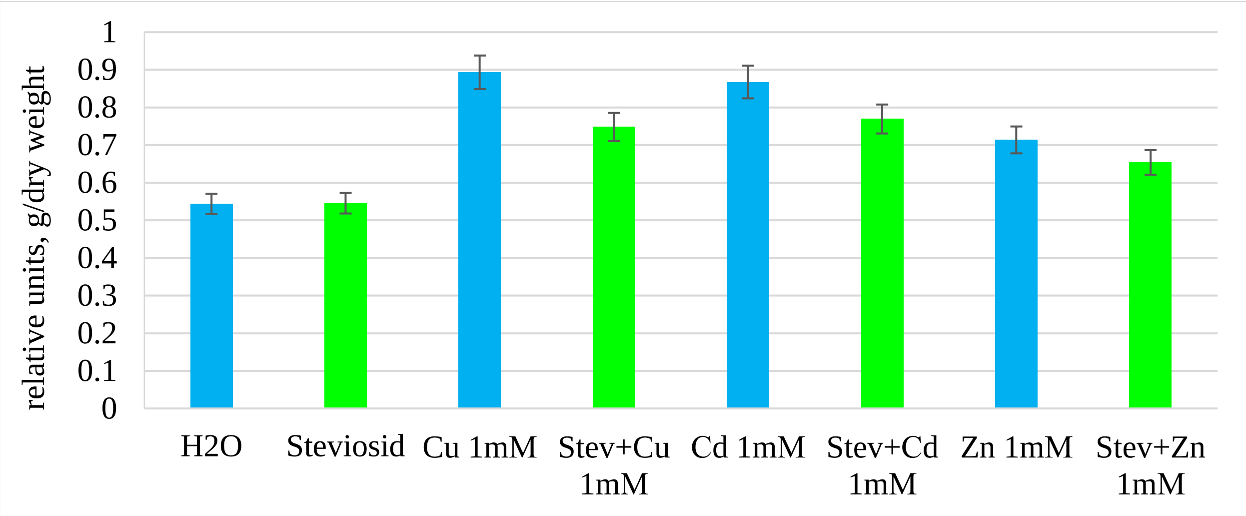

Fig. 4 - Effect of stevioside (10^{-8} M) and heavy metals ($CuSO_4$, $CdSO_4$, $ZnSO_4$) concentration (1 mM), ascorbate peroxidase activity on winter wheat roots.

On the background of stevioside, under the action of $CuSO_4$, $CdSO_4$ and $ZnSO_4$ 1 at a concentration of mM, the enzyme activity increased slightly as compared with the action of heavy metals only. The greatest increase in activity was observed in the experiment with cadmium, where the difference was 13%, and in the case of copper and zinc, the increase was 7%. Perhaps, this effect of stevioside on the enzyme activity is associated with a decrease in HM uptake by plants under its influence.

Ascorbate peroxidase (APO) is an integral part of the ascorbate-glutathione cycle. While catalase utilizes mainly H_2O_2 in peroxisomes, the ascorbate peroxidase performs the same function in the cytosol and chloroplasts. Ascorbic acid [17] is used as the ascorbate peroxidase reducing agent.

APO has better affinity for H_2O_2 than catalase, which leads to a more efficient utilization of H_2O_2 during stress [18].

Stevioside (10^{-8} M) had little effect on the activity of the test enzyme (Fig. 3). The activity of ascorbate peroxidase also remained the same under the influence of all investigated HMs at a low concentration, and did not virtually differ from control plants (Fig. 3). Otherwise, high HM concentrations caused an increase in APO activity as compared to control, but to various extent (Fig. 4).

Changes in this indicator were similar to those under the action of copper and cadmium. $CuSO_4$ and $CdSO_4$ at concentration of 1 mM caused an increase in the enzyme activity by 64% and 60%, respectively, as compared to the control. Zinc, in a sublethal concentration (1 mM), in turn, caused an increase in the activity of the test enzyme by 31%.

With the application of stevioside, the APO activity under the influence of low HM concentrations did not change. At the same time, stevioside partially removed the effect of copper, cadmium, and zinc at a high concentration (1 mM) on the enzyme activity (Fig. 4). Experiments with copper and cadmium with the application of stevioside show increase in this indicator by 38% and 41%, respectively (as compared to 64% and 60% in the experiments without stevioside). Zinc under the influence of stevioside increased the enzyme activity by 20%, while the experiment without stevioside pretreatment showed increase in activity equal to 31%.

Proline - an amino acid that refers to one of the twenty proteinogenic amino acids. Under stress, its share in the total pool of free amino acids of plant cells may be about 5% [19].

There have been a lot of data received about the accumulation of proline in different varieties in response to the toxic ions Cd^{2+}, which correlates with a decrease in the level of cadmium-induced ROS production [20]. It has been shown that proline treatment is also effective for the reduction of the level of cadmium damaging effect on cowpea plants, due to the maintenance of high glutathione levels and its metabolic enzyme activity [20].

Adding HM into the plant growth environment resulted in the increased proline levels, which indicates a quick response of plants to the pollutant-induced stress. There is a clear dependence of the proline biosynthesis on the metal and its concentration. Copper and zinc at suboptimal concentrations had virtually no effect on the level of proline, since these two metals are a part of the trace elements and their low concentration causes no stress in plants. While their sublethal concentration resulted in significant increase in proline content: copper - by 17 times, and zinc - by 5 times. Cadmium, in turn, caused elevated levels of the studied amino acid already at low concentration by almost 4 times, and at high - by 14 times.

Table. 3. The content of proline (µg/g dry weight) in the roots of winter wheat grown in water and stevioside at $CuSO_4$ action, $CdSO_4$ and $ZnSO_4$.

variant	H_2O	Steviosid
Control	4.0±1.1	16.05±1.2
Cu (10 µM)	5.3±2.1	15.9±2.5
Cu (1 mM)	68.4±2.3	80.5±3.7
Cd (10 µM)	15.2±1.3	44.5±1.1
Cd (1 mM)	56.07±2.1	63.9±2.3
Zn (10 µM)	4.04±1.7	15.4±1.7
Zn (1 mM)	20.5±2.4	35.08±3.1

Stevioside stimulated the accumulation of proline in roots of wheat seedlings by 4 times in comparison with the plants grown with the addition of water. Probably, the proline level in this case may indicate an increasing adaptive capacity of the plants treated with stevioside.

Seedlings treated with stevioside and HM has shown increase in proline concentration higher than in the experiment with only HM treatment. We should note that the maximum content of proline was detected in the experiments with sublethal doses (1 mM) of HM (Cd, Zn, Cu). Probably, stevioside induces the formation of low molecular weight antioxidant, aimed at protecting the cell and its compartments from HM exposure.

SUMMARY

The data obtained indicate that stevioside shows its protective properties under the influence of pollutants, reducing thereby the level of oxidative stress in plants. Based on the available results of the study of the antioxidant properties of stevioside, we can assume that the diterpene glycoside can be used as anti-stress agent.

CONCLUSION

Changes in the activity of the studied enzymes, which are components of the antioxidant system of plants, indicates reduction in the level of stress caused by heavy metals under the action of stevioside. Perhaps, the obtained effect of the diterpene glycoside is associated with an increase in the content of a stress protein proline, which can act as a low molecular weight antioxidant.

ACKNOWLEDGEMENTS

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

REFERENCES

[1] Titov A.F. Plant resistance to heavy metals / A.F. Titov, V.V. Talanova, N.M. Kaznina, G.F. Laidinen. - Petrozavodsk: Karelian Research Centre of Russian Academy of Sciences, – 2007. – 172 p.

[2] Gallego, S.M. Unravelling cadmium toxicity and tolerance in plants: Insight into regulatory mechanisms / S.M. Gallego, L.B. Pena, R.A. Barcia, C.E. Azpilicueta, M.F. Iannone, E.P. Rosales, M.S. Zawoznik, M.D. Groppa, M.P. Benavides // Environ. Exp. Bot. – 2012. – V. 83. – P. 33-46.

[3] Blaby-Haas, C.E. The ins and outs of algal metal transport / C.E. Blaby-Haas, S.S. Merchant // Biochim. Biophys. Acta. – 2012. – V.1823. – Pp. 1531-1552.

[4] Menshikova E.B. Antioxidant and radical inhibitors of oxidative processes / E.B. Menshikova, N.K. Zenkov // Successes of modern biology. – 1993. – V.113. – Pp. 442-455.

[5] Mitteler, R. Oxidative stress, antioxidants and stress tolerance / R. Mitteler // Trends in Plant Science. – 2002. – V.7. – Pp. 405-410.

[6] Siess, H. Antioxidant Functions of Vitamins – Vitamin E and Vitamin C, в-Carotene, and Other Carotenoids and Intercellular Communication via Gap Junctions / H. Siess, W. Stahl // Int. J. Vitamin Nutr. Res. – 1997. – V.67. – Pp. 364-367.

[7] Chekurov V.M. Effect of natural bio-stimulants / V.M. Chekurov, S.I. Sergeev. – M., 2001. – 292 p.

[8] Timofeeva O.A. Kartolin influence on oryzalin-induced changes in the lectin activity at low-temperature hardening of plants / O.A. Timofeev, L.D. Garaeva, Iu.Iu. Chulkova, L.P. Khokhlova // Plant Physiology. – 2008. – V.55. – Pp. 333-337.

[9] Kataev V.E. Ent-kaurene diterpenoids and glycosides: isolation, properties, chemical transformation / V.E. Kataev, R.N. Khaibullin, R.R. Sharipova, I.Iu. Strobykina // Review Journal of Chemistry. – 2011. – V. 1, – No. 1. – Pp. 1-69.

[10] Mishra, H. Antidiabetic activity of medium-polar extract from the leaves of *Stevia rebaudiana* Bert. (Bertoni) on alloxan-induced diabetic rats / H. Mishra, M. Soni, N. Silawat, D. Mehta, B.K. Mehta, D.C. Jain // Journal of Pharmacy and Bioallied Sciences. – 2011. – V. 3. – Pp. 242-248.

[11] Aeby, H. Catalase in vitro // Methods Enzymol. - 1984. - V. 105. - Pp. 121-126.

[12] Nakano, Y. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts / Y. Nakano, K. Asada // Plant Cell Physiol. – 1981. – V. 22. – Pp. 867-880.

[13] Nevmerzhitskaia Iu.Iu. Stevioside increases the winter wheat resistance to low temperatures and heavy metals / Iu.Iu. Nevmerzhitskaia, O.A. Timofeeva, A.L. Mikhailov, A.S. Strobykina, I.Iu. Strobykina, V.F. Mironov // Reports of the Academy of Sciences. – 2013. – V. 452. – Pp. 346-349.

[14] Mhamdi, A. Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models / A. Mhamdi, G. Queval, S. Chaouch, S. Vanderauwera, F. Van Breusegem, G. Noctor // Journal of Experimental Botany. – 2010. – V.61. – No.15. – Pp. 4197-4220.

[15] Maleva M.G. The impact of heavy metals on water weed photosynthetic apparatus and antioxidant status / M.G. Maleva, G.F. Nekrasova, G.G. Borisova, N.V. Chukina, O.S. Ushakova // Plant Physiology. - 2012. - V.59. - No.2. - Pp. 216-224.

[16] Verma, S. Lead Toxicity Induces Lipid Peroxidation and Alters the Activities of Antioxidant Enzymes in Growing Rice Plants / S. Verma, R.S. Dubey // Plant Science. – 2003. – V. 164. – Pp. 645-655.

[17] Shalata, A. Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation / A. Shalata, P.M. Neumann, // Journal of Experimental Botany. – 2001. – V. 52. – P. 2207-2211.

[18] Sharma, P. Ascorbate peroxidase from rice seedlings: properties of enzyme isoforms, effects of stresses and protective roles of osmolytes / P. Sharma, R.S. Dubey // Plant Science. – 2007. – V. 167. – Pp. 541-550.

[19] Matysik, J. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants / J. Matysik, Alia, B. Bhalu, P. Mohanty // Current Science. – 2002. – V. 82, – Pp. 525-532.

[20] Hossain, M.A. Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation / M.A. Hossain, P. Piyatida, J.A. Teixeira da Silva, M. Fujita // J. Bot. – 2012. – V. 2012. – 37 p.