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Evaluation of Hyper-Tolerance of Aquatic Plants to Metal Nanoparticles

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ABSTRACT

The estimation of the protein content and amino acid composition under the influence of metal nanoparticles (Mn, Cu, Zn, Ag) for seven species of aquatic macrophytes: *Limnobium laevigatum* (Humb. & Bonpl.ExWilld.), *Pistia stratiotes* L., *Salvinia natans* L., *Elodea canadensis* Michx., *Najas guadelupensis* (Spreng.) Magnus, *Vallisneria spiralis* L. and *Riccia fluitans* L. was conducted. The plants were exposed during 7 days on the experimental solutions of metal nanoparticles at the rate of 1 g of plant per 100 ml of the mixture of stock colloidal solutions of metal nanoparticles (Mn – 0.75 mg/l, Cu – 0.37 mg/l, Zn – 0.44 mg/l, Ag^+, Ag_2O – 0.75 mg/l) diluted 200 times. In the five investigated species, reduction of the protein content was observed. However, this indicator remained stable only in *P. stratiotes* (52 µg/ml) and, conversely, increased in *V. spiralis* (46 µg/ml to 51 µg/ml). The content of the studied amino acids in *N. guadelupensis* decreased by 46% (from 112.05 µmol/g to 60.15 µmol/g), in *R. fluitans* – by 44% (from 104.06 µmol/g to 58.25 µmol/g), in *S. natans* – by 23% (from 90.08 µmol/g to 69.59 µmol/g), in *E. canadensis* – by 10% (from 143.92 µmol/g to 129.4 µmol/g), and in *P. stratiotes* as well as in *L. laevigatum* – by 8% (from 210.65 µmol /g to 193.77 µmol/g and with 155.0 µmol/g to 142.60 µmol/g), but in *V. spiralis*, on the contrary, increased by 7% (from 91.31 µmol/g to 97.59 µmol/g). Changes in the composition and content of amino acids for each species of aquatic plant were analyzed. It was suggested that the studied plants, which belong to different families, have different defense mechanisms, according to which the amino acid composition of plants varies.

Keywords: metal nanoparticles, aquatic plants, amino acids, protein, phytoremediation.

INTRODUCTION

Nanotechnology appeared in the late 20th century and is developing rapidly. Its scope is constantly expanding and includes the computer industry, aerospace, electronics, medicine, cosmetology and agriculture [Liu et al., 2020, Diallo et al., 2013]. Currently, heavy metal nanoparticles are used in various fields that can potentially adversely affect the environment and even pose risks to human and animal health. Such risks should be assessed in a timely manner and, if necessary, prevented. Powerful development of nanotechnologies today requires solving the issue of phytoremediation of the environment and processing of nanoproduct waste [Ashraf, 2004].

Due to the rapid involvement of nanotechnology products in various fields of production, their release with industrial emissions into the natural environment, especially aquatic, is inevitable. As the ecological risk of metal nanoparticles to the environment is currently unclear, it is necessary to clarify in advance the possible methods of extracting them from the aquatic environment, in order to preserve the quality of natural waters and biodiversity of aquatic ecosystems [Handy and Shaw, 2007; Owen and Handy, 2007; Odjegba and Fasidi, 2006].

Recently, the interest of scientists around the world has focused on the problem of disposal of nanotechnology products, including metal nanoparticles. Industrial wastewater, which uses metal nanoparticles, requires more careful control and special treatment methods. The search for the species of aquatic organisms capable of removing nanoparticles of metals and at the same time resistant to pollution, for the implementation of remediation of technical reservoirs is an important task today [Parra et al., 2012; Prasad et al., 2001; Taghiganji et al., 2012].

Today, there is a significant increase in interest in phytoremediation of technical and natural reservoirs and the study of patterns of biological transformation of pollutants and, in particular, the ability of vegetation to accumulate or use pollutants during metabolism [Espinoza-Quiñones et al., 2009; Lu et al., 2010; Dhir et al., 2008; Dhir and Srivastava, 2011]. It is known that aquatic plants can intensively absorb various pollutants from water [Murithi et al., 2012; Parra et al., 2012; Odjegba and Fasidi, 2006], due to which they are widely used as phytoremediators [Alvarado et al., 2008; Prasad et al., 2001; Taghiganji et al., 2012].

Phytotechnology is an effective and real alternative for the restoration of polluted waters. The advantages of phytoremediation are a high level of purification, which is not inferior to physical and chemical methods, low cost, safety for the environment, the possibility of further extraction of valuable substances from the green mass of plants and monitoring the purification process [Bennicelli et al., 2004; Smadar et al., 2011].

In the world practice the positive experience of restoration of quality of water of technical and natural reservoirs polluted with ions of metals by methods of phytoremediation has been received [Leblebici and Aksoy, 2011; Rahman and Hasegawa, 2011], but the widespread use of aquatic macrophytes for water treatment is constrained by the lack of appropriate developments and recommendations, which is due to the lack of elaboration of many issues of storage capacity and physiology of plants themselves in terms of pollution. Thus, there is evidence that high concentrations of metals can damage phytoremediator plants. This is manifested in necrotic changes, chlorosis, damage to chloroplasts, cells, stomata, trichomes, decreased biomass, chlorophyll, protein, free amino acids, RNA and DNA, and in some cases plant death, so much attention is today paid to determine the resistance of plants to biochemical indicators to specific metals and threshold concentrations of these metals for remediate plants [Buta et al., 2011; Hoffman et al., 2004; Holtra et al., 2010; Wolff et al., 2012].

Among aquatic plants there are unique species capable of hyperaccumulation of metals [Barce and Poschenrieder, 1990; Dummee et al., 2012]. The ability of such plants to accumulate large amounts of metals is based on the mechanisms of internal hypertolerance to toxic metals [Ingle et al., 2005; Tuomainen et al., 2010; Schneider et al., 2013] associated with specific plant metabolism, primarily detoxification by binding to ligands [Ingle et al., 2005; Alvarez et al., 2009; Tuomainen et al., 2010; Zeng et al., 2011; Schneider et al., 2013], mainly contained in leaf cells. Such ligands are primarily thiol-containing peptides associated with specific plant metabolism, mainly detoxification by binding to ligands. (glutathione, phytochelatins), metallothionein [Roosens et al., 2004; Hassinen et al., 2009] and free amino acids, primarily methionine [Zhao et al., 2011], cysteine and histidine [Ingle et al., 2005].

Important in the mechanisms of plant resistance, including to the action of metals, are the changes in the level of mitochondrial membranes, and especially membrane lipids, which ensure the penetration of certain substances into the cell, especially fatty acids. Acylcarnitines are involved in the transport of fatty acids into mitochondria for β-oxidation [Bohnert et al., 1995], which indirectly characterize the lipid metabolism. Changes in the composition and content of individual acylcarnitines may be a sensitive marker of plant stress tolerance at the level of energy mitochondria [Hare et al., 1999].

The study of the mechanisms of hypertolerance to nanoparticles of metals of aquatic plants at the level of protein, amino acids and acylcarnitines will help create sustainable species that can be used to develop the methods of phytoremediation of natural and technical reservoirs contaminated with metal nanoparticles is currently very promising

SCIENTIFIC HYPOTHESIS

Among aquatic plants, there are the species resistant to metal nanoparticles that can be used in phytoremediation of industrial reservoirs. The resistance of these plants is determined by the presence of specific stress proteins, consisting of certain amino acids, which activate the defense mechanisms of the plant. Biochemical analysis of the amino acid composition of the plant proteins resistant to metal nanoparticles will contribute to understanding the defense mechanisms of these plants.

MATERIALS AND METHODS

Colloidal solutions of nanoparticles of metals were developed by the Department of Construction Materials and Materials Science Technology of National University of Life and Environmental Sciences of Ukraine, and obtained by dispersing granules of manganese, copper, zinc and silver applying impulses of electric current with amplitude of 100–2000 A in water [Handy and Shaw, 2007]. The maximum size of nanoparticles does not exceed 100 nm.

The object of the study was seven species of aquatic plants, including which three species of pleuston (free-floating on the surface of the water) – *Limnobium laevigatum* (Humb. & Bonpl.Ex-Willd.), *Pistia stratiotes* L., *Salvinia natans* (L.) All. and four species of submerged hydrophytes – *Elodea canadensis* Michx., *Najas guadelupensis* (Spreng.) Magnus, *Vallisneria spiralis* L. and *Riccia fluitans* L.

The cultures of aquatic plants were grown in the aquaculture complex of the NSC "Institute of Biology and Medicine" in aquariums of 40–60 liters in the distant water under optimum conditions: illumination of 6000 lux, the period of illumination 12 hours, water temperature 19–25 °C, pH 5–8.

Experimental plants, from the rate of 1 g of plant per 100 ml of the mixture of stock solutions of metal nanoparticles (Mn 0.75 m/l; Cu 0.37 mg/l; Zn 0.44 mg/l; Ag^+, Ag_2O 0.75 mg/l) diluted 200-fold in water, were kept for 7 days under the same illumination and temperature conditions as during control cultivation. At the 7th day, a visual inspection of the plants was performed (Fig. 1, 2) and protein content and amino acids were determined.

To determine the protein and amino acid content, the plant material (0.2 g) was homogenized with 0.5 g of glass powder and 0.5 g of water-free Na_2SO_4. The homogenate was transferred to a glass column with a filter, and then filtered with 3 ml of acetone. The protein content was determined by the biuret-test [Owen and Handy, 2007]. The optical density was measured at 550 nm on a Shimadzu UV-1800 spectrophotometer.

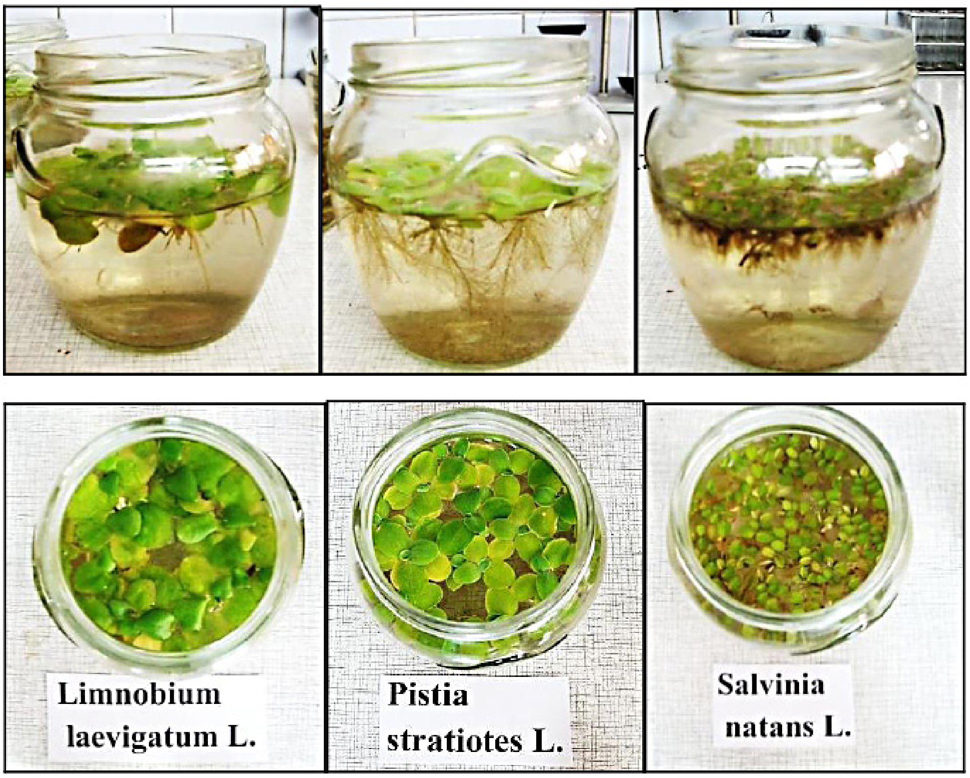

Fig. 1. Appearance of pleuston during exposure in a mixture of colloidal solutions of metals nanoparticles of on the 7th day

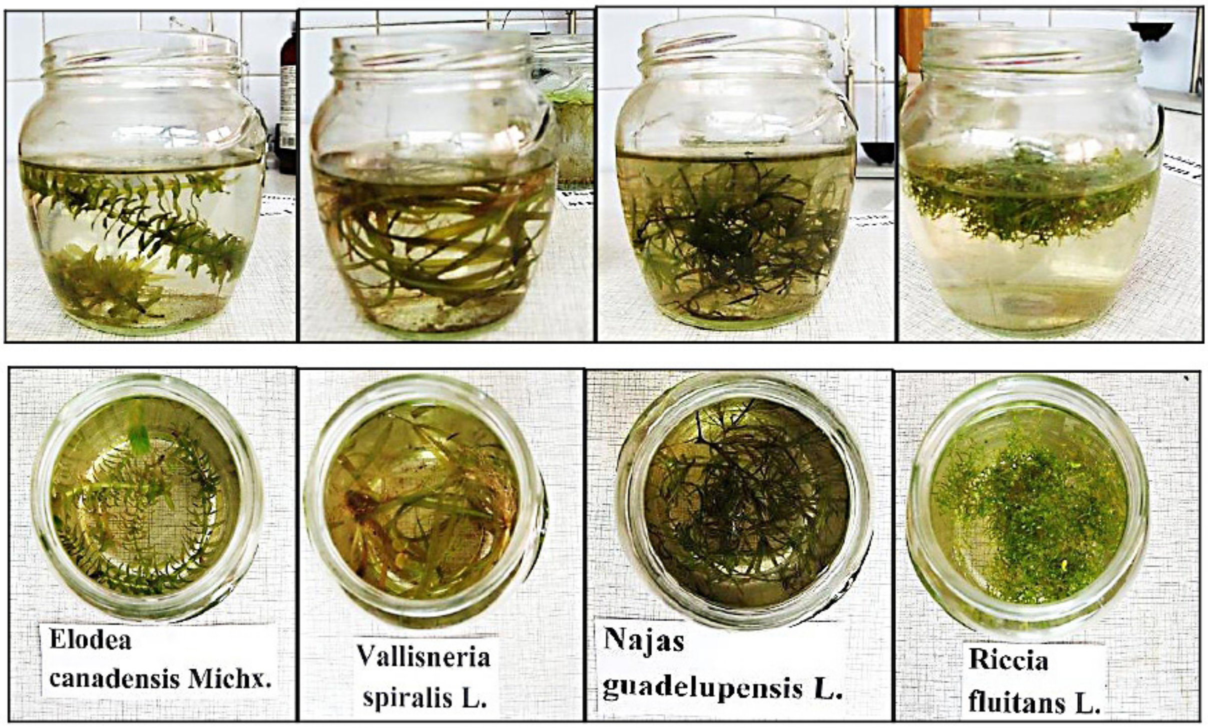

Fig. 2. Appearance of submerged hydrophytes during exposure in a mixture of colloidal solutions metals of nanoparticles of on the 7th day

Determination of the content of amino acids was carried out by the tandem mass spectrometry [Murithi, et al., 2012] using the AB Sciex 2000 mass spectrometer with the Ultimate 3000 autosampler (Dionex).

The statistical processing of the obtained results was conducted using the Microsoft Office Excel software. The results were considered as reliable (according to the t-criterion of Student) at the significance level of $p \leq 0.05$.

STATISTICAL ANALYSIS

The results were statistically evaluated by the Analysis of Variance. All the assays were carried out in triplicates and results are expressed as mean ±SD. All calculations were made with the help of Microsoft Office 2019 software package (Microsoft).

RESULTS AND DISCUSSION

Cultivation of aquatic plants on solutions of metal nanoparticles resulted in decreasing of protein content in five of the seven studied species (Fig. 3).

This indicator was not changed only in *P. stratiotes* and increased in *V. spiralis*. It can be assumed that an increase of the protein content of *V. spiralis* under the treatment by nanoparticles is associated with the synthesis of stress proteins. The decrease of the protein content due to the application of metal nanoparticles is associated with a disturbance of nitrogen metabolism. Since nitrogen is one of the key nutrients in formation of nucleic acids, coenzymes, amino acids, any changes in its content will inhibit growth and reduce plant productivity [Odjegba and Fasidi, 2006].

In the investigated aquatic plants, we identified 17 amino acids (5-Oxo-Pro - 5-hydroxyproline, Ala - alanine, Arg - arginine, Asp - aspartic acid, Cit - citrulline, Glu - glutamic acid, Gly - glycine, His - histidine, Leu - leucine, Met - methionine, Orn - ornithine, Phe - phenylalanine, Pro - Proline, Ser - serine, Trp - tryptophan, Tyr - tyrosine, Val - valine) and their quantity was determined. The obtained results are presented in Figure 4, 5.

The total amount of amino acids and the difference in the number and ratio of individual amino acids, which are part of the protein of the studied hydrophyte species, in the control and experimental options were determined. Alteration in the amino acid composition of protein systems of the studied species after treatment by metal

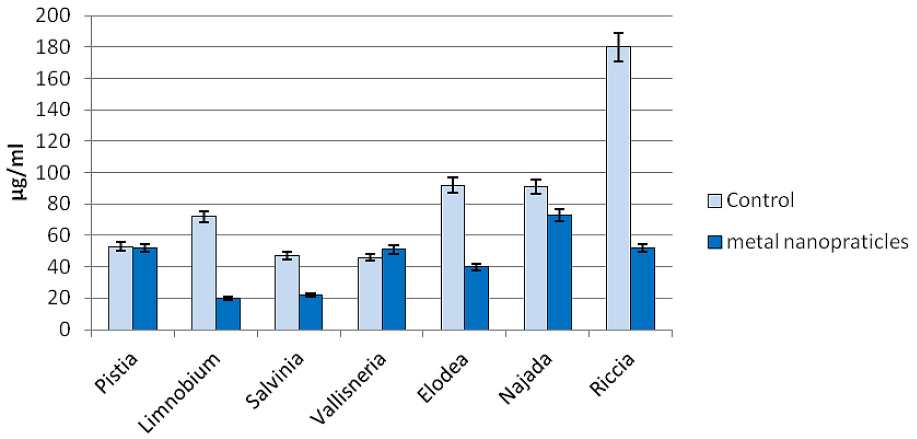

Fig. 3. Protein content in higher aquatic plants by the combined action of nanoparticles of metals on the 7th day

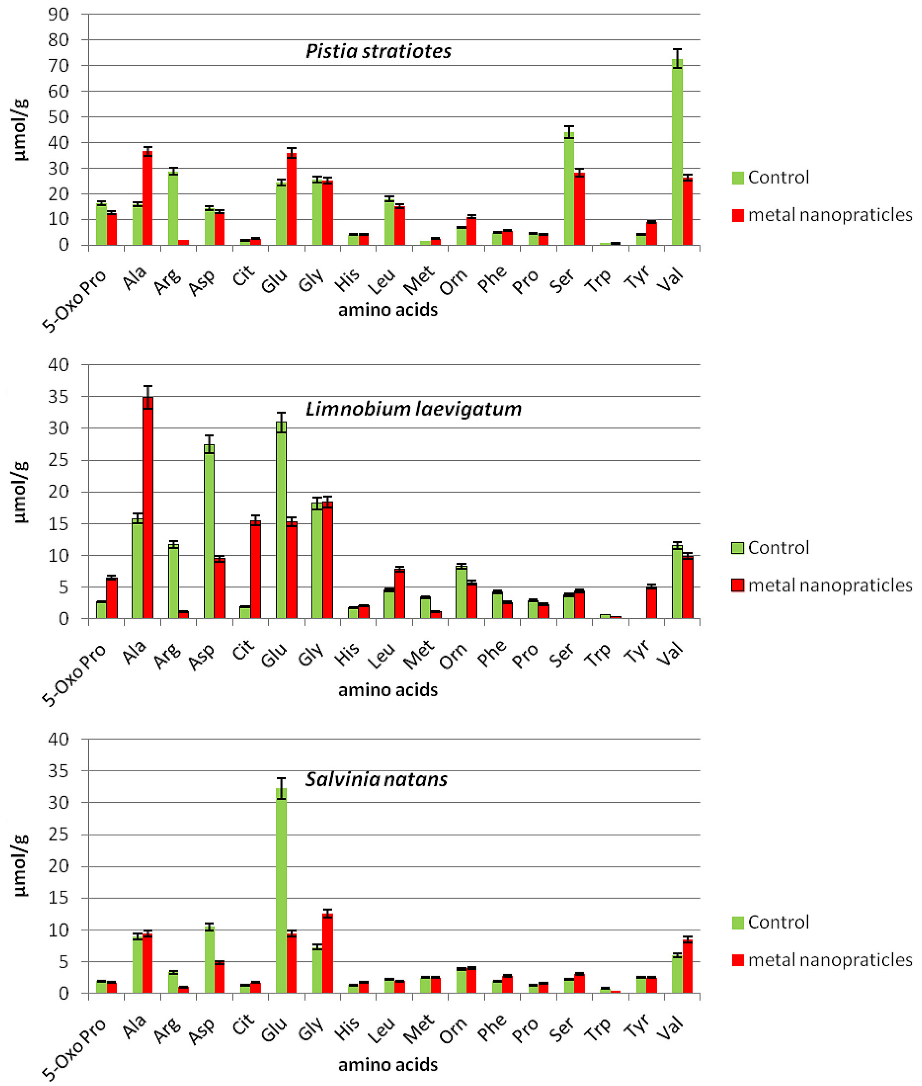

Fig. 4. The content of amino acids in pleuston under treatment by metal nanoparticles on the 7th day of cultivation

nanoparticles was found; moreover, the species with minor changes were the identified. These species, in authors' opinion, correspond to the needs of phytoremediation.

The comparative evaluation showed that the total content of the studied amino acids in the mixture of nanoparticles of metals decreased in six of the seven species of aquatic plants and, on

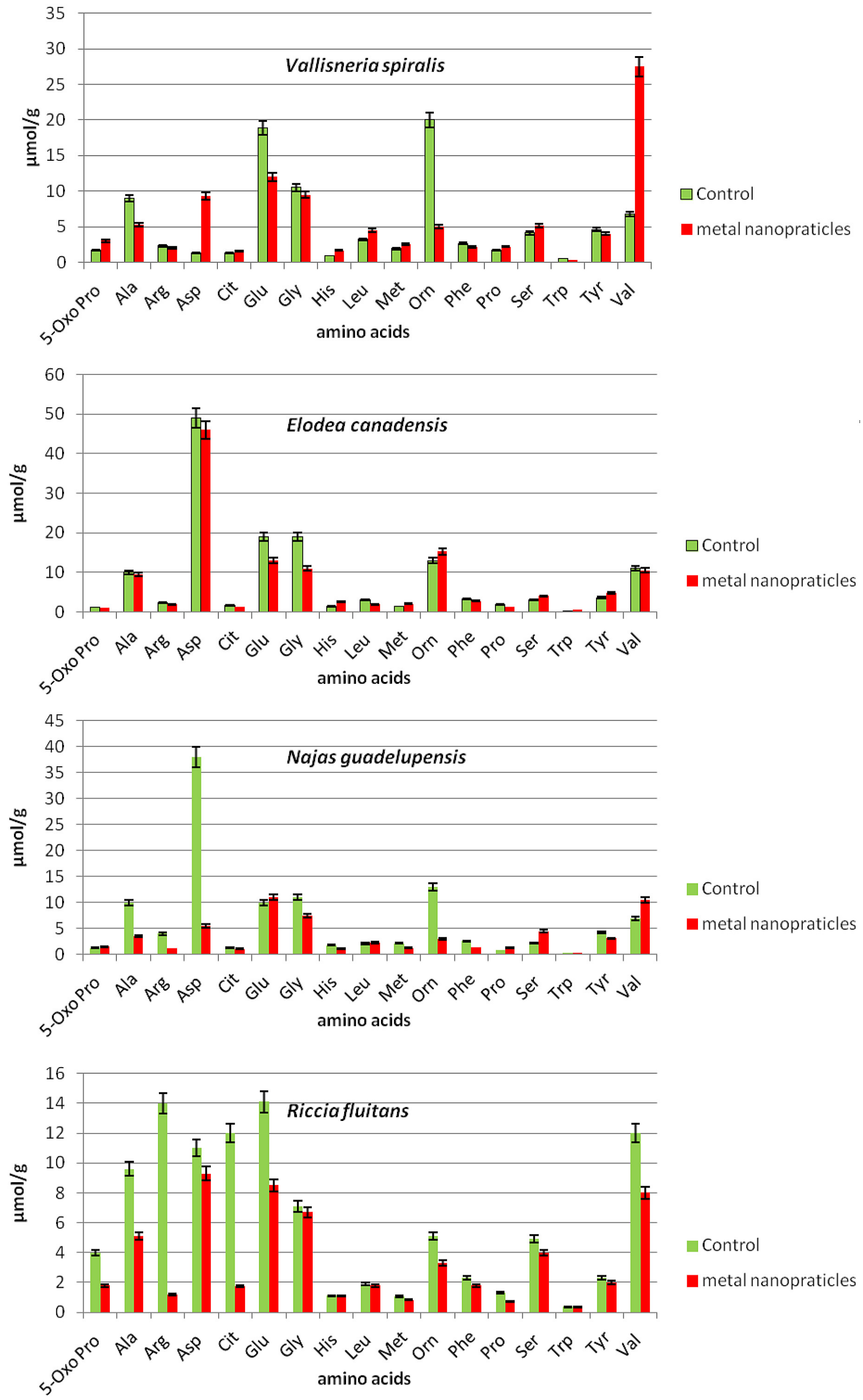

Fig. 5. The content of amino acids in submerged hydrophytes under treatment by metal nanoparticles on the 7th day of cultivation

the contrary, increased in *V. spiralis*. In *N. guadelupensis*, the content of amino acids decreased by 46% (from 112.05 μM/g to 60.15 μM/g), in *R. fluitans* by 44% (from 104.06 μM/g to 58.25 μM/g), in *S. natans* 23% (from 90.08 μM/g to 69.59 μM/g), *E. cannadensis* 10% (from 143.92 μM/g to 129.4 μM/g), and *P. stratiotes* and *L. laevigatum* by 8% (from 210.65 μM/g to 193.77 μM/g and from 155.0 μM/g to 142.60 μM/g, respectively). An increase in the content of the studied amino acids by 7% (from 91.31 μM/g to 97.59 μM/g) was observed in *V. spiralis*.

In each plant species, a decrease in a certain number of individual amino acids, which led to a general decrease of total content, was observed. The quantity and possible dependence between decreasing of particular amino acids and plant species were analyzed. Thus, in *N. guadelupens*, 10 of 17 amino acids decreased, namely, alanine, arginine, aspartic acid, citrulline, glycine, histidine, methionine, ornithine, phenylalanine and tyrosine, with the significant decrease of aspartic acid, ornithine, alanine and arginine content (7-, 4-, and 3-fold respectively).

In *R. fluitans*, the content of almost all identified 15 amino acids decreased, except histidine and tyrosine. The most significant changes were observed in the quantity of arginine (11 times), citrulline (7 times), 5-hydroxyproline (2.2 times), alanine and proline (1.9 times) and glutamic acid (1, 7 times). The content of histidine and tyrosine remained at the control level. An increase of the content of any of the amino acids was not observed.

In *S. natans* the number of 6 amino acids, namely 5-oxoprolin, arginine, aspartic acid, glutamic acid, leucine and tryptophan was reduced. Among them the content of arginine, glutamic acid and aspartic acid decreased by 3-, 3- and 2-times, respectively. Moreover, in this specie the content of 9 out of the 17 amino acids was increased (namely alanine, citrulline, glycine, histidine, ornithine, phenylalanine, proline, serine and valine). Increased content of a significant number of amino acids (more than half of the researched) under the plant cultivation on metal nanoparticles may indicate the launch of protective mechanisms in the plant, which is important for the synthesis of new proteins.

In *E. cannadensis*, where the total amino acid content was not significantly decreased (less than 10 %) compared to the above-mentioned species, the content of 10 amino acids was decreased, but none of them by more than 50%. There was a rising of level of 6 amino acids, namely histidine (1.8 times), methionine and tryptophan (1.6 times), serine and tyrosine (1.3 times), and ornithine (1.2 times). The content of 5-hydroxyproline remained at the control level.

In *P. stratiotes*, the content of 5 amino acids - 5-oxoprolin, arginine, aspartic acid, leucine and serine was decreased however, the quantity of glycine, histidine, proline remained constant on the other hand, the content of alanine, cyttrilin, glutamine acids, methionine, ornithine and phenylalanine was increased.

In *L. laevigatum*, the content of 9 amino acids has been decreased, including arginine (10 times), aspartic acid and methionine (3 times), glutamic acid and tryptophan (2-fold) as well as phenylalanine 1.7 times. The content of 2 amino acids (glycine and tyrosine) remained constant, but the content of other six amino acids - 5-hydroxyproline (2.4 times), alanine (2.1 times), citrulline (even 8 times), leucine (in 1,7 times), and histidine and serine (in 1,2 times) was increased.

Unlike all other species in *V. spiralis*, there was no decrease, but, conversely, an increase of the total content of the studied amino acids by 7% was noted. It was essential to find the amino acids at the expense of which there was an increase in their total content. It was found that in *V. spiralis* the number of 8 amino acids has increased, but the content of only two – aspartic acid (from 1.3 μM/g to 9.3 μM/g, or 7 times) and valine (from 6, 8 μM/g to 27.5 μM/g, or 4 times) has significantly changed, and the content of rest 6 amino acids did not increase significantly. At the same time, in the *V. spiralis* plants, a decreasing of content of other 8 amino acids, among which significant changes were reported by ornithine (4 times), alanine (1.7 times) and glutamic acid (1.6 times), was observed.

In some of the studied plant species, namely: *V. spiralis*, *E. canadensis*, *L. laevigatum*, *S. natans* under the influence of metal nanoparticles there was an increase in the content of the amino acid of histidine. Since it is known that histidine is an amino acid produced by metal hyper-accumulator plants [Parra, et al., 2012], this may indicate a high ability of these plant species to also accumulate metal nanoparticles. The data in the literature indicate that in the plants in which Zn-histidine complex have been detected, the concentration of histidine increased along with the metal concentration in the solution [Alvarado et al., 2008]. Increase of concentration of histidine in plant tissues contributes to the accumulation of a greater amount of metal, which is confirmed by the previous results [Prasad, et al., 2001]. Namely, these plants had advantages over other species in term of accumulation of nanoscale metals. Similarly to Zn-histidine, a complex with aspartic acid [Taghiganji, et al., 2012], which is proper for metal hyper-accumulators, was also found. The properties of aspartic acid as a ligand to Cd, Pb, and Zn have been reported for *in vitro* studies [Bennicelli, et al., 2004]. In the conducted, the increase of the content of aspartic acid was observed only in *V. spiralis*, (7 times), on the contrast, there was a decrease of rate of this amino acid in other plant species (6 times in *N. guadelupensis*, 3 times in *L laevigatum* and 2 in *S. natans*). Amino acids such as glycine and glutamic acid, which are involved in the synthesis of glutathione and phytochelatins, are also important for metal accumulator plants, due to binding metals [Smadar, et al., 2011]. An increase in the glycine content as a response to metal nanoparticles treatment is noted only in the *S. natans*, and glutamic acid – in *P. stratiotes* and *N. guadelupensis*. The simultaneous increase in the content of both glycine and glutamic acid was not observed. It is also known that arginine is involved in the synthesis of polyamines acting as signaling molecules and antioxidants [Smadar, et al., 2011], but in this study, the increase of arginine on the influence of metal nanoparticles has not been found. It was proven that the proline functions as an acceptor of radicals, stabilizer of macromolecules [Leblebici and Aksoy, 2011] and chelating compound [Smadar, et al., 2011]. There is evidence that under stress conditions (water deficit, low and high temperatures, high salinity), many plants accumulate precisely the proline that is synthesized from glutamate or ornithine [Rahman and Hasegawa, 2011]. High accumulation of proline also occurs under the heavy metals stress.

Nanoparticles of metals caused an increase of proline content in such species as *V. spiralis*, *L. laevigatum*, *S. natans* and *N. guadelupensis*, and ornithine - in *E. canadensis*, *S. natans* and *P. stratiotes*.

The content of methionine due to the influence of nanoparticles of metals was reduced in *L. laevigatum*, *N. guadelupensis* and *R. fluitans*, which may indicate that thiol groups of this amino acid are redirected to the biosynthesis of cysteine and glutathione. There is evidence that methionine is converted to S-adenosine methionine, which is a precursor for many biosynthetic pathways, including nicotine amines.

The obtained data showed that the investigated plants have different mechanisms of protection, according to which the amino acid composition of plants has been changed.

The results of the effect of metal nanoparticles on the protein metabolism are consistent with the existing ideas about the synthesis of individual amino acids in the interaction of plants with heavy metal ions. An increase in the content of certain amino acids that take part in the protective reactions to the damaging effects of metal ions and their nanoparticles focuses on supporting of the plant cell homeostasis and inducing of defense mechanisms.

Thus, the conducted studies showed that such free-floating hydrophytes as *P. stratiotes* and *L. laevigatum*, in terms of change in the total content and content of individual amino acids in response to the action of metal nanoparticles, offer advantages over other aquatic macrophytes, especially submerged hydrophytes such as *N. guadelupensis* and *R. fluitans.* Many studies show the high ability of *P. stratiotes* to remove toxic heavy metals, such as As, Cd, Cu, Ni, Zn, Pb, Cr, Mn, Co from contaminated waters, but at high concentrations of pollutants, plant chlorophyll, protein, free amino acids and RNA content are affected [Bohnert et al., 1995; Hare et al., 1999; Espinoza-Quiñones et al., 2009].

S. natans is considered to be an effective bioaccumulator it is already successfully used for the removal of heavy metals from water [Espinoza-Quiñones et al., 2009; Lu et al., 2010]. It was established that this plant species is resistant to aluminum, chromium, boron and lead ions. In authors' studies, among the researched pleuston plants, according to changes in its protein metabolism, *S. natans* revealed to be the most sensitive to the metal nanoparticles stress. However comparing to submerged hydrophytes (*N. guadelupensis* and *R. fluitans*), it showed significant preferences; therefore it, can also be recommended for phytoremediation measures.

CONCLUSIONS

Among the investigated seven species of aquatic plants, the most resistant to the influence of a mixture of metal nanoparticles (Mn, Cu, Zn, Ag), according to the protein content and 17 amino acids, were *P. stratiotes* and *S. natans* (free-floating hydrophytes), and *V. spiralis* (submerged hydrophytes), which can be recommended for phytoremediation of water from metal nanoparticles.

The total content of 17 amino acids was decreased in six of the seven species of aquatic plants (*N. guadelupensis* by 46%, in *R. fluitans* – 44%, *S. natans* – 23%, in *E. cannadensis* – 10%, *P. stratiotes* and *L. laevigatum* – 8%) after cultivation on the mixture of metal nanoparticles (Mn, Cu, Zn, Ag) however this indicator was increased (by 7%) only in *V. spiralis*.

Different mechanisms of protein protection were involved in the studied plant species, but in general, the impact of metal nanoparticles on the protein metabolism of aquatic plants was similar to that of heavy metals ions. The content of amino acids produced by plant hyper-accumulators of metals, namely histidine (*S. natans*, *V. spiralis*, *L. laevigatum*, *E. cannadensis*), aspartic acid (*V. spiralis*), glycine (*S. natans*), glutamic acid (*P. stratiotes*, *N. guadelupensis*), proline (*V. spiralis*, *L. laevigatum*, *N. guadelupensis)* and ornithine (*S. natans*, *P. stratiotes*, *E. cannadensis*) was increased.

The obtained results can be used for developing the chnology for phytoremediation of technical water from metal nanoparticles.

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