Impact of anthropogenic toxic substances on the antioxidant defense of *Ceratophyllum demersum* and *Egeria densa*

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Abstract. Considering the current increase in anthropogenic pollution of the environment, it becomes necessary to monitor the qualitative and quantitative aspects of the impact of pollution on various biological objects. Of great practical interest are the toxic effects of technogenic discharges on various hydrobionts and the basic mechanisms of their development in macrophyte plants. In this regard, this work studied the influence of heavy metal ions, anionic synthetic surfactants and their combinations on the activity of the main antioxidant enzymes in the tissues of *Ceratophyllum demersum* and *Egeria densa*. An experiment was conducted to assess changes in the freshwater macrophytes *Ceratophyllum demersum* and *Egeria densa*. The activity of antioxidant enzymes was taken into account: peroxidase, polyphenol oxidase, ascorbate oxidase and catalase. Their activity was assessed basally and in response to chemical stressors (100 μmol/l lead ions, 1% solution of anionic synthetic surfactants) individually and in combination with different exposures to toxic substances. The identified features of enzymatic activity in the tissues of aquatic macrophytes indicate different levels and power of their antioxidant protection. It has been noted that the activity of antioxidant enzymes when plants are exposed to certain types of pollutants and their combinations is determined by the chemical nature of the pollutant, the mechanism of its action on the plant organism, the duration of exposure and the localization of these enzymes in cell compartments. **Key words:** ecology, environmental friendliness, natural resources, environmentally responsible behavior, biology, lead ions, anionic synthetic surfactants, enzyme composition of tissues, aquatic plants, *Ceratophyllum demersum, Egeria densa.*

1 Introduction

The environment continuously influences the structure and function of living organisms [1]. Under the influence of many environmental factors, changes in the parameters of all body systems occur [2]. They provide adaptation [3] or contribute to the emergence of

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dysfunctions and pre-pathology [4]. For this reason, a detailed clarification of the aspects of environmental influences on the body is very important for assessing the danger of individual environmental factors [5] and clarifying the standards for various parameters of external influences [6]. These studies are of particular practical importance on multicellular organisms [7], as they can reveal their strategies in adapting to various living conditions [8].

Due to the growth of cities in the world and an increase in population density, there is a gradual increase in the amount of transport and the development of the sphere of consumer services. This leads to an increase in the volume of man-made emissions into the biosphere. The situation is complicated by the steady expansion of the chemical composition of pollutants [9,10]. In this regard, the pollution of fresh water bodies with heavy metals and synthetic surfactants is becoming increasingly acute. These compounds pose a serious threat to many forms of life [11,12]. The situation is aggravated by the fact that synthetic surfactants have a pronounced resistance to biodegradation [13,14], and heavy metals, with high toxicity, are very slowly removed from the body [10].

Due to the increase in anthropogenic pollution of the hydrosphere with heavy metals and synthetic surfactants, it becomes necessary to assess the degree of environmental pollution by them and clarify aspects of their biological hazard [15,16,17]. To date, the toxic effects of abiotic stressors on various species of aquatic organisms have been widely studied [18,19,20,21]. However, the mechanisms of influence of chemical factors individually and especially when combined on macrophyte plants still require clarification [22,23]. In particular, aspects of the responses of submerged macrophyte vegetation need to be clarified. It is capable of reflecting the general state of water bodies and changes in their environmental conditions [24,25] and therefore is a significant object of bioindication of natural waters [26,27]. The various reactions of aquatic plants to the appearance of chemical properties with toxic properties in their habitat have been studied to the least extent.

In all organisms, reactive oxygen species are formed during metabolism. These compounds are characterized by high chemical activity. Under conditions of negative influences on the body from the environment, the production of free radicals is especially pronounced. This leads to the development of oxidative stress and the appearance of prepathology and pathology [28]. During evolution, enzymatic mechanisms of protection against the action of reactive oxygen species have been developed, including peroxidase, polyphenol oxidase, ascorbate oxidase and catalase [29]. However, the reaction of these enzymes to negative chemical influences on the body from the environment remains poorly understood. Changes in their activity in the tissues of aquatic plants, which can be considered as biological markers of pollution of fresh water bodies with substances of technogenic origin, have been least studied.

Purpose of the work: to study the effects of the release of heavy metal ions and anionic synthetic surfactants into the environment separately in combination on the activity of the main antioxidant enzymes in the tissues of *Ceratophyllum demersum* and *Egeria densa*.

2 Materials and methods

The following freshwater macrophytes were selected to carry out the study: submerged hornwort (*Ceratophyllum demersum*) and Brazilian elodea (*Egeria densa*).

The experiment was carried out in laboratory conditions at the same sufficient intensity and duration of regular light flux, as well as at a constant temperature (20°C). During the experiment, the plants were divided into groups differing in their growing environment (Table 1).

Table 1. Experimental groups of *Ceratophyllum demersum* and *Egeria densa* in the study performed

Experimental plant groups	Environment
1 group (control *Ceratophyllum demersum*)	Filtered tap water, 20°C
2 group (control *Egeria densa*)	Filtered tap water, 20°C
3 group (*Ceratophyllum demersum*)	100 µmol/l lead ions in filtered tap water, 20°C
4 group (*Egeria densa*)	100 µmol/l lead ions in filtered tap water, 20°C
5 group (*Ceratophyllum demersum*)	1% solution of anionic synthetic surfactants (Dosya laundry detergent, made in Poland), 20°C
6 group (*Egeria densa*)	1% solution of anionic synthetic surfactants (Dosya laundry detergent, made in Poland), 20°C
7 group (*Ceratophyllum demersum*)	100 µmol/l lead ions + 1% solution of anionic synthetic surfactants (Dosya detergent, made in Poland) in filtered tap water, 20°C
8 group (*Egeria densa*)	100 µmol/l lead ions + 1% solution of anionic synthetic surfactants (Dosya detergent, made in Poland) in filtered tap water, 20°C

Taking into account previous work in the field of plant stress response [30,31], experimental control points were determined that corresponded to the phases of its occurrence. Thus, 1, 2, 4 and 12 hours of influence corresponded to the primary inductive stress reaction. In this case, the third day (72 hours of influence) was considered as a manifestation of the second phase of stress.

In plant tissues of *Ceratophylum demersum* and *Egeria densa*, the dynamics of the activity of a number of enzymes of the antioxidant system (peroxidase, polyphenol oxidase, ascorbate oxidase and catalase) was studied basally and in response to the action of very common chemical environmental pollutants (100 µmol/l lead ions and 1% solution of anionic synthetic surface -active substances – laundry detergent "Dosya", produced in Poland) individually and in combination.

The activity of the above enzymes was determined using generally accepted methods: the peroxidase activity method [32]; according to the method for catalase activity [33]; according to the method for polyphenoloxidase activity [32]; according to the method for ascorbate oxidase activity [32]. Statistical processing of the results was performed using the Student's t-test.

3 Results and discussion

In the course of the studies, it was revealed that the plants *Ceratophylum demersum* and *Egeria densa* experience stress in response to the influence of the tested pollutants. The biochemical changes that occurred in the studied plants during the first 12 hours of the experiment reflect the occurrence of the first phase of stress in their tissues - the primary inductive stress reaction [25]. However, the dynamics of enzymatic activities are different in the tissues of the studied plants and ambiguous for various enzymes. For both plant species, the most significant differences from control levels and levels in the first 4 hours of exposure to lead ions and a surfactant were noted.

In the tissues of *Ceratophylum demersum* in the first 4 hours after exposure to lead ions, the activity of catalase and peroxidase decreased by 4 and 2 times, respectively, compared to the control. The activity of ascorbate oxidase and polyphenol oxidase after 4 hours of exposure increased by 2.9 times and 28.5%, respectively (Table 2). Similar changes were noted for the enzymatic activity of Egeria densa tissues, although the differences were less pronounced (Table 3).

Table 2. Activity of antioxidant enzymes in the tissues of the plant *Ceratophylum demersum* located in a medium containing lead ions

Research checkpoints	Catalase, ncat/l	Peroxidase, U/l•s•g plant tissue	Ascorbate oxidase, optical density units	Polyphenol oxidase, relative units/min•g plant tissue
Control	22.3±0.029	12.6±0.20	175.4±0.86	22.8±0.30
1 an hour of experimentation	20.6±0.23	11.4±0.09	177.7±0.89	21.2±0.26
2 hours of experiment	14.8±0.17**	11.2±0.10*	162.6±0.79	24.1±0.28
4 hours of experiment	6.6±0.06**	6.2±0.05**	251.8±1.06**	29.3±0.35**
12 hours of experiment	13.5±0.16**	4.7±0.04**	620.2±3.45**	31.5±0.37**
72 hours of experiment	22.0±0.27	6.5±0.05**	345.4±2.37	42.0±0.44**

Note. Significance of differences between the data in the control and the data during the experiment: * – at the p<0.05 level, ** – at the p<0.01 level.

Analyzing the results obtained, we can say that the appearance of the most significant changes in enzyme activity in the first 4 hours of the toxic effect of lead ions is associated with the development of the primary inductive stress phase in the plant.

In the tissues of *Ceratophylum demersum*, peroxidase activity after 72 hours (the second phase of stress) of exposure to lead ions remained lower (2 times) than the control values. By the same period, catalase activity almost returned to its original value (control). Ascorbate oxidase and polyphenol oxidase activities, starting from 4 hours of exposure, remained higher than the values of similar control indicators. Thus, after 72 hours of exposure to lead ions, ascorbate oxidase and polyphenol oxidase activities in the tissues of *Ceratophylum demersum* were almost 2 times higher than the corresponding control values.

Exposure to lead ions on *Egeria densa* for 72 hours led to an increase (compared to the control) in the activity of all considered enzymes in the tissues of the experimental groups of plants: catalase activity by 1.5 times, peroxidase activity by 39.5%, ascorbate oxidase activity by 1.6 times times, polyphenoloxidase – 2 times (Table 3). At the same time, the activity of ascorbate oxidase was significantly different from the control, starting from 4 hours of exposure, and polyphenol oxidase from 2 hours of exposure.

Table 3. Activity of antioxidant enzymes in the tissues of the plant Egeria densa located in an environment containing lead ions

Research checkpoints	Catalase, ncat/l	Peroxidase, U/l•s•g plant tissue	Ascorbate oxidase, optical density units	Polyphenol oxidase, relative units/min•g plant tissue
Control	32.5±0.34	12.4±0.16	120.3±0.87	7.5±0.09
1 an hour of experimentation	37.2±0.36*	16.3±0.18**	118.4±0.82	8.0±0.11
2 hours of experiment	30.4±0.29	14.6±0.15*	126.4±0.64	13.2±0.15**
4 hours of experiment	28.7±0.25*	12.8±0.12	147.1±0.89**	14.6±0.14**
12 hours of experiment	32.8±0.25	14.2±0.12*	183.7±0.89**	18.2±0.21**
72 hours of experiment	49.5±0.48**	17.3±0.18**	190.0±2.03**	15.7±0.17**

Note. Significance of differences between the data in the control and the data during the experiment: * – at the p<0.05 level, ** – at the p<0.01 level.

Analyzing the enzymatic activity in the tissues of *Ceratophylum demersum* and tissues of *Egeria densa* under the influence of surfactants, faster and more dramatic changes in the activity of the monitored enzymes were revealed than under the influence of lead ions (Tables 4 and 5).

Table 4. Activity of antioxidant enzymes in the tissues of the plant *Ceratophylum demersum* in a solution of an anionic surfactant compound

Research checkpoints	Catalase, ncat/l	Peroxidase, U/l•s•g plant tissue	Ascorbate oxidase, optical density units	Polyphenol oxidase, relative units/min•g plant tissue
Control	22.3±0.29	12.6±0.20	175.4±0.86	22.8±0.30
1 an hour of experimentation	31.9±0.36**	14.4±0.26*	182.3±0.82	26.2±0.37*
2 hours of experiment	30.5±0.40**	14.8±0.20*	190.8±0.78	39.6±0.39**
4 hours of experiment	63.1±0.42**	21.0±0.19**	239.5±1.26**	61.3±0.49**
12 hours of experiment	95.2±0.75**	54.7±0.40**	345.2±1.83**	125.3±0.77**
72 hours of experiment	13.6±0.22**	37.2±0.26**	257.6±1.69**	40.9±0.29**

Note. Significance of differences between the data in the control and the data during the experiment: * – at the p<0.05 level, ** – at the p<0.01 level.

Table 5. Activity of antioxidant enzymes in the tissues of the plant *Egeria densa* in a solution of an anionic surfactant compound

Research checkpoints	Catalase, ncat/l	Peroxidase, U/l•s•g plant tissue	Ascorbate oxidase, optical density units	Polyphenol oxidase, relative units/min•g plant tissue
Control	32.5±0.34	12.4±0.16	120.3±0.87	7.5±0.09
1 an hour of experimentation	39.5±0.53*	16.8±0.17**	143.7±0.78*	9.8±0.10**
2 hours of experiment	45.0±0.48**	18.2±0.25**	158.6±0.91*	10.2±0.07**
4 hours of experiment	59.6±0.71**	31.3±0.38**	137.8±0.86*	13.6±0.15**
12 hours of experiment	63.6±0.58**	48.8±0.32**	126.7±0.91	14.2±0.14**
72 hours of experiment	60.5±0.33**	49.0±0.46**	101.5±0.89*	26.8±0.13**

Note. Significance of differences between the data in the control and the data during the experiment: * – at the p<0.05 level, ** – at the p<0.01 level.

Thus, after 4 hours of exposure to synthetic surfactants on *Ceratophylum demersum*, an increase was noted (compared to the control values of similar enzymes) in the activity of: catalase - 2.8 times, peroxidase - 1.6 times, ascorbate oxidase - 1. 4 times, polyphenoloxidases – 2.7 times. For the enzymes taken into account, the greatest differences from the level of the control group *Ceratophylum demersum* were detected after 12 hours of action of synthetic surfactants. Thus, during these observation periods, catalase activity exceeded control values by 4.2 times, peroxidase activity by 4.3 times, ascorbate oxidase activity by 2 times, and polyphenoloxidase activity by 5.5 times.

In the tissues of *Egeria densa*, when exposed to synthetic surfactants, significant differences were also noted from similar indicators of the control group of plants after 2 hours. Thus, after 2 hours of exposure, catalase activity exceeded control values by 37.2%,

peroxidase activity by 43.3%, ascorbate oxidase activity by 29.5%, and polyphenol oxidase activity by 34.2%. However, if for the activity of catalase, peroxidase and polyphenoloxidase, as the duration of exposure increased (after 72 hours), their activity increased (by 84.4%, 3.8 times, 3.5 times, respectively), then ascorbate oxidase activity by 72 hours decreased compared to the control by 20.6%.

The identified differences in the nature of the response of plants to the action of synthetic surfactants can be explained by the fact that these compounds, once in the aquatic environment, form a film at the interface. This leads to a change in the quality of abiotic factor flows: the amount of light penetrating to plants decreases, gas exchange between the liquid medium and the air deteriorates, leading to a lack of oxygen in the water. Under these conditions, peroxidation processes actively begin in plant cells. In response to increased free radical oxidative processes in plant cells, the working capabilities of the antioxidant defense system, which ensures effective deactivation of reactive oxygen species, increase. This was confirmed by an increase in the activity of the enzymes peroxidase and polyphenoloxidase by 72 hours of exposure to synthetic surfactants.

Enzymatic activity was established in the tissues of *Ceratophylum demersum* and *Egeria densa* under the influence of both toxic substances under consideration (lead ions - 100 μmol/l and 1% solution of a surfactant compound) (Tables 6 and 7).

Table 6. Activity of antioxidant enzymes in the tissues of the plant *Ceratophylum demersum* located in a medium containing lead ions and an anionic surfactant compound

Research checkpoints	Catalase, ncat/l	Peroxidase, U/l•s•g plant tissue	Ascorbate oxidase, optical density units	Polyphenol oxidase, relative units/min•g plant tissue
Control	22.3±0.29	2.6±0.20	175.4±0.86	22.8±0.30
1 an hour of experimentation	25.0±0.27	10.2±0.21*	182.1±0.86	24.0±0.19
2 hours of experiment	28.9±0.23**	9.6±0.19*	179.4±0.95	40.4±0.28**
4 hours of experiment	25.7±0.36*	6.2±0.17**	165.3±0.81	67.5±0.33**
12 hours of experiment	19.0±0.15**	2.3±0.09**	124.3±0.52**	64.8±0.39**
72 hours of experiment	28.7±0.12**	5.5±0.07**	295.4±0.71**	39.4±0.41**

Note. Significance of differences between the data in the control and the data during the experiment: * – at the $p<0.05$ level, ** – at the $p<0.01$ level.

In *Ceratophylum demersum* tissues, catalase activity tended to increase slightly by 72 hours of the experiment. In the tissues of *Egeria densa*, the level of activity of this enzyme by this time of exposure to toxic substances increased 1.6 times.

Peroxidase activity in the tissues of *Ceratophylum demersum* experienced dynamics similar to those when exposed to lead ions only, decreasing by 2.3 times. Dynamics of a similar direction were noted for the activity of *Egeria densa* peroxidase, which after 72 hours of the experiment exceeded the control level by 2.3 times.

Table 7. Activity of antioxidant enzymes in the tissues of the *Egeria densa* plant located in a medium containing lead ions and an anionic surfactant compound

Research checkpoints	Catalase, ncat/l	Peroxidase, U/l•s•g plant tissue	Ascorbate oxidase, optical density units	Polyphenol oxidase, relative units/min•g plant tissue
Control	32.5±0.34	12.4±0.16	120.3±0.87	7.5±0.09
1 an hour of experimentation	34.5±0.32	14.8±0.19*	112.3±0.36	10.2±0.12*
2 hours of experiment	30.8±0.41	18.9±0.17**	128.5±0.75	11.7±0.09**
4 hours of experiment	46.2±0.47**	23.6±0.24**	152.6±0.68**	12.6±0.14**
12 hours of experiment	44.2±0.40**	21.0±0.31**	132.3±0.72*	21.3±0.18**
72 hours of experiment	54.7±0.37**	28.9±0.14**	144.0±0.83**	26.7±0.23**

Note. Significance of differences between the data in the control and the data during the experiment: * – at the $p<0.05$ level, ** – at the $p<0.01$ level.

An assessment of ascorbate oxidase activity in the tissues of *Ceratophylum demersum* under the combined influence of pollutants showed that, as under conditions of exposure to lead ions only, the activity of this enzyme increased by 72 hours of exposure by 19.6% compared to the control. In the tissues of *Egeria densa*, the activity of ascorbate oxidase increased throughout the experiment, reaching a maximum towards the end. These data differed from those observed when *Egeria densa* was exposed to synthetic surfactants, where a decrease in the activity of this enzyme was observed.

Determination of polyphenol oxidase activity in the tissues of *Ceratophylum demersum* and *Egeria densa* showed an increase in the activity of this enzyme, which amounted to 1.7 times for *Ceratophylum demersum* and 3.5 times for *Egeria densa* by 72 hours of exposure to both pollutants. The results obtained can be explained by the genetic characteristics of the organisms affected by the impact taken into account, and their potential to respond to external chemical influences [34,35].

4 Conclusion

The preservation of the viability of the organism in any environmental conditions is largely based on the existing activity of antioxidant enzymes in its cells, including peroxidase, polyphenol oxidase, ascorbate oxidase and catalase. In conditions of increasing anthropogenic pollution of fresh water bodies, it becomes necessary to assess the severity of this process. It is very important to clarify the levels of influence of common technogenic discharges on the antioxidant protection of aquatic organisms. These include heavy metal ions, anionic synthetic surfactants and their combinations. The toxicity of these compounds towards antioxidant enzymes (peroxidase, polyphenol oxidase, ascorbate oxidase and catalase) on *Ceratophyllum demersum* and *Egeria densa* was assessed.

The identified features of enzymatic activity in the tissues of aquatic macrophytes indicate different levels of their biological capabilities. At the same time, the activity of antioxidant enzymes when plants are exposed to individual pollutants and their combinations is determined by the chemical nature of the pollutant itself, the main mechanisms of its action on the plant organism, the duration of exposure and the localization of these enzymes in cell compartments.

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