Impact of anionic synthetic surfactants of lead ions and their combinations on the pigment profile of freshwater plant organisms *Ceratophyllum demersum* and *Egeria densa*

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Abstract. The development of human civilization inevitably leads to the development of production with increasing emissions of many chemicals into the environment. This is especially noticeable in relation to surface waters of fresh water bodies. A very alarming point is that the amount of toxic anthropogenic substances, including heavy metals, is growing every year and is increasingly disturbing the existing ecological balance. Plant organisms of water bodies are among the first to experience the effects of any anthropogenic pollutants and especially heavy metals and surfactants, the amount of which increases in the water of rivers and lakes. Very important in this is the complex of morpho-physiological adaptations associated with restructuring in the structure of chloroplasts. Changes in the content of photosynthetic pigments in plants of two species (*Ceratophyllum demersum* and *Egeria densa*) were assessed when they were exposed to very common technogenic substances (lead ions and a solution of anionic synthetic surfactants). In plants, there is a decrease in the activity of molecular transport, inhibition of H+-ATPase, leading to a decrease in the pH of the cytoplasm and activation of hydrolases, which enhance the breakdown of biopolymers. This leads to changes in the expression of individual genes and increased synthesis of a number of stress proteins. Under these conditions, the assembly of cytoskeletal elements is activated, the structures of membrane proteins and lipids change, the level of ATP decreases, free radical processes are activated and the intensity of photosynthesis is inhibited.

1 Introduction

During the implementation of individual development, the body constantly experiences the dynamics of its many parameters [1]. The life of any organism always occurs under the influence of environmental factors of different nature [2]. These are chemical, physical, mechanical factors [3]. Their influence provides a number of changes in the body, some of

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which have a positive effect, and some of which have a clearly negative effect. These effects have been monitored in humans [4], in productive animals [5] and in non-productive living organisms [6], as well as in different cell populations [7].

In modern conditions, the level of environmental pollution by chemicals of anthropogenic origin has increased significantly [8]. The amount of chemical elements entering the environment as a result of technogenesis, in some cases, significantly exceeds the level of their natural intake. Involving in natural migration cycles, anthropogenic flows lead to the rapid spread of pollutants in the natural components of the urban landscape. The volumes of pollutants containing heavy metals increase annually and disrupt the ecological balance [9]. At the same time, more than 10 heavy metals are now recognized as very dangerous for living organisms, and the first places in terms of toxicity are occupied by mercury, lead and cadmium [10].

Nowadays, interest in the environmental aspects of pollution of water bodies by synthetic surfactants obtained from petroleum hydrocarbons has increased significantly. This is caused by the increasing scale of production and use of synthetic detergents in various industries, as well as their high danger to aquatic ecosystems [11]. Anionic synthetic surfactants are very resistant to biodegradation and pose a great danger to living organisms [11,12].

2 Materials and methods

The work was carried out on freshwater macrophytes: submerged hornwort (*Ceratophyllum demersum L.*) and Brazilian elodea (*Egeria densa*).

The study was carried out in laboratory conditions with the same intensity and regularity of 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. Plant groups *Ceratophyllum demersum* and *Egeria densa* taken into the study

Experimental plant groups	Growing 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*)	Water with 100 µmol/l lead ions, 20°C
4 group (*Egeria densa*)	Water with 100 µmol/l lead ions, 20°C
5 group (*Ceratophyllum demersum*)	1% aqueous solution of anionic synthetic surfactants (Dosya laundry detergent, made in Poland), 20°C
6 group (*Egeriadensa*)	1% solution of anionic synthetic surfactants (Dosya laundry detergent, made in Poland), 20°C
7 group (*Ceratophyllum demersum*)	Water containing 100 µmol/l lead ions and 1% anionic synthetic surfactants (Dosya detergent, made in Poland), 20°C
8 group (*Egeria densa*)	Water containing 100 µmol/l lead ions and 1% anionic synthetic surfactants (Dosya detergent, made in Poland), 20°C

Taking into account the information known from the literature [13,14], experimental control points were determined that correspond to the phases of the stress reaction. The condition of the plants was monitored after 1, 2, 4, 12 and 72 hours of being in the test environment. Since 1, 2, 4 and 12 hours of influence correspond to the primary inductive stress reaction, the third day (72 hours of influence) reflects the picture of the second phase of stress.

The amount of photosynthetic pigments in the composition of plant tissues was assessed (quantitative determination of pigments is based on determining the optical density of pigments at wavelengths that are their absorption maxima) [15]. Acetone was used to extract pigments from plant tissues. To do this, each plant specimen, ground to a pulp, was

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placed in identical flasks containing 10 ml of pre-cooled solvent. The mixture was thoroughly mixed and filtered. The filtrate containing the isolated pigments was diluted to 10 ml. The optical density of the finished solution was analyzed on a standard KFK-3 photometer (Russia) at different wavelengths (440.5; 644; 662 nm). Pigment concentrations were calculated using formulas corresponding to the method. Statistical processing of the study results was carried out by calculating the Student's test.

3 Results and discussion

In the course of our studies, it was revealed that *C. demersum* plants experience stress in response to the action of lead ions at a concentration of 100 µmol/l. The plants showed signs of chlorosis after 12 hours of incubation in the pollutant medium and increased with increasing exposure. The dynamics of the content of photosynthetic pigments is shown in Table 2.

Table 2. Effect of the presence of 100 µmol/l of lead ions in the medium on the content of chlorophyll and carotenoids in the tissues of *Ceratophyllum demersum* under experimental conditions

Experimental conditions	Chlorophyll *a* content, mg/g raw material	Chlorophyll *b* content, mg/g raw material	Carotenoid content, mg/g raw material
Control	1.350±0.026	0.500±0.022	0.281±0.009
1 hour of experiment	1.620±0.043*	0.465±0.019	0.214±0.008*
2 hours of experiment	1.121±0.036*	0.420±0.017	0.365±0.010*
4 hours of experiment	1.121±0.032*	0.395±0.015	0.191±0.007*
12 hours of experiment	0.878±0.015*	0.340±0.012*	1.040±0.014*
72 hours of experiment	2.255±0.047*	0.710±0.019*	0.166±0.006*

* – degree of significance of differences with the control level $p<0.05$.

During the course of the primary stress induction, corresponding to the first 12 hours of incubation in a medium with the addition of 100 µmol/l of lead ions, the content of pigments in the tissues of plants of the experimental group was lower than that of the control group of plants: the content of chlorophyll *a* was 20-35%, chlorophyll *b* was 32% lower, carotenoids – 30%. After 72 hours of incubation in a pollutant environment, the content of photosynthetic pigments in the experimental group of plants exceeded those of the control group (the content of chlorophyll a by 1.7 times, chlorophyll b by 1.4 times), while the content of carotenoids in the tissues of plants of the experimental group was 40% lower, than the control group of plants.

The amount of pigments in a leaf is directly related to the phenomena of stress physiology; the relative concentration of pigments can change under the influence of various abiotic factors [16]. According to the literature, heavy metal ions cause changes in many structural and functional parameters of photosynthesis: inhibition of a number of enzymes [17], various active centers of photosystem II and the enzyme of chlorophyll synthesis - γ-aminolevulenate dehydratase [18], damage to chloroplast granules and stroma [19], disturbances absorption of elements necessary for them, including manganese and iron, damage to the photosynthetic apparatus or destruction of chlorophyll through an increase in chlorophyllase activity [20], as well as slowing down plastocyanin reactions [21]. These effects of heavy metals led to a decrease in the content of photosynthetic pigments during the primary stress induction phase.

Stress factors can affect the level of chlorophyll *a* and *b*. With an increase in the first indicator, the function of the antenna complexes of photosystem II clearly decreases [22, 23]. As a result of the studies, it was revealed that the level of chlorophyll *a* and *b* changed relative to the control group, which indicates the dynamics of the plant pigment complex to the effects of heavy metals.

The pathogenic influence of lead ions in the phase of primary inductive stress led to a decrease in the content of photosynthetic pigments (chlorophyll *a* and *b*) and an increase in the content of carotenoids. Presumably, this is due to the unfavorable effect of blue ions on photosynthesis, causing disruption of the ultrastructure of chloroplasts, inhibiting the synthesis of pigments and enzymes of the Calvin cycle. A reduction in chlorophyll content is possible due to lead inhibition of the enzyme of chlorophyll synthesis - γ-aminolevulenate dehydratase [18], damage to the grana and stroma of *C. demersum* chloroplasts [19], disruption of the absorption of essential elements such as manganese and iron, damage to the photosynthetic apparatus or destruction of chlorophyll through increase in chlorophyllase activity [20]. The increased content of carotenoids is explained by the fact that these pigments are the most common and active metabolite of living organisms, participating in the system of protecting cells from the effects of environmental factors [24].

It is known that during the adaptation phase, membranes are stabilized [25] with subsequent restoration of ion transport, synthesis processes are enhanced, the activity of mitochondria and chloroplasts and the level of energy supply increase. At the same time, the generation of reactive oxygen species decreases [26]. According to the data obtained, the synthesis processes led to an increase in the content of photosynthetic pigments in the tissues of plants of the experimental group.

The dynamics of pigment content in the tissues of *C. demersum* under the influence of a 1% solution of anionic synthetic surfactants is presented in Table 3.

Table 3. Effect of 1% content of anionic synthetic surfactants in the medium on the content of chlorophyll and carotenoids in the tissues of *Ceratophyllum demersum* under experimental conditions

Experimental conditions	Chlorophyll *a* content, mg/g raw material	Chlorophyll *b* content, mg/g raw material	Carotenoid content, mg/g raw material
Control	1.350±0.034	0.500±0.025	0.281±0.007
1 hour of experiment	0.872±0.028*	0.492±0.031	0.081±0.005*
2 hours of experiment	2.746±0.075*	0.897±0.035*	0.337±0.012
4 hours of experiment	2.158±0.093*	1.109±0.056*	0.146±0.007*
12 hours of experiment	3.225±0.115*	0.559±0.039	0.679±0.010*
72 hours of experiment	1.670±0.062*	0.586±0.026	0.290±0.011

* – degree of significance of differences with the control level $p<0.05$.

During the first 12 hours of incubation in a pollutant medium, corresponding to the phase of primary stress induction, the content of chlorophyll a was 1.5-2.2 times higher in the plant tissues of the experimental group, chlorophyll b was 1.7-2 times higher, and carotenoids were higher – 2.4 times, which indicated the strength of the stress factor and damage to the antenna complexes of photosystem II in the tissues of *C. demersum* [22].

The change in the amount of pigments in the tissues of *C. Demersum* under the influence of a combination of pollutants is reflected in Table 4.

Table 4. Effect of 100 µmol/l lead ions and 1% solution of anionic synthetic surfactants on the content of chlorophyll and carotenoids in the tissues of *Ceratophyllum demersum* during the experiment

Experimental conditions	Chlorophyll *a* content, mg/g raw material	Chlorophyll *b* content, mg/g raw material	Carotenoid content, mg/g raw material
Control	1.350±0.036	0.500±0.034	0.281±0.008
1 hour of experiment	0.872±0.022*	0.235±0.012*	0.270±0.010
2 hours of experiment	2.528±0.096*	0.304±0.011*	0.270±0.011
4 hours of experiment	2.022±0.097*	0.152±0.015*	0.272±0.008
12 hours of experiment	3.112±0.120*	0.221±0.012*	0.147±0.009*
72 hours of experiment	1.682±0.032*	0.274±0.016*	1.298±0.016*

* – degree of significance of differences with the control level $p<0.05$.

The phase of primary stress induction as a result of the influence of 100 µmol/l of lead ions and a 1% solution of anionic synthetic surfactants proceeded similarly to the phase of primary stress induction as a result of the influence of only lead ions. The content of chlorophyll a was 1.5-2 times lower than that of the control group, the content of chlorophyll b was 2-3.2 times lower.

The adaptation phase to the action of a combination of pollutants turned out to be similar to that to the action of a 1% solution of anionic synthetic surfactants. The content of chlorophyll a and b in the tissues of *C. demersum* of the experimental group was 2 times lower than that of the control group, while the content of carotenoids was 4.7 times higher.

It was possible to find out that the content of chlorophylls and carotenoids in the studied plants was different. The absolute amount of pigments is a hereditarily determined value, which is associated with the structural and anatomical features of the leaves of a particular species. However, the trend in the dynamics of pigment content in the tissues of *Egeria densa* was at some points similar to that in the tissues of *Ceratophyllum demersum*.

When *Egeria densa* entered a medium containing lead ions at a concentration of 100 µmol/l, the plants also experienced slight chlorosis, but after 4 hours of incubation in the pollutant medium. The dynamics of the content of photosynthetic pigments of *Egeria densa* during the observation is reflected in Table 5.

Table 5. Effect of 100 µmol/l lead ions on the content of chlorophyll and carotenoids in *Egeria densa* tissues under experimental conditions

Experimental conditions	Chlorophyll *a* content, mg/g raw material	Chlorophyll *b* content, mg/g raw material	Carotenoid content, mg/g raw material
Control	0.150±0.019	0.075±0.014	0.035±0.010
1 hour of experiment	0.128±0.017*	0.073±0.012	0.030±0.011

2 hours of experiment	0.178±0.021*	0.068±0.010	0.038±0.009
4 hours of experiment	0.162±0.018*	0.057±0.009*	0.027±0.007
12 hours of experiment	0.181±0.020*	0.050±0.010*	0.054±0.013*
72 hours of experiment	0.159±0.016	0.078±0.013	0.021±0.006*

* – degree of significance of differences with the control level $p<0.05$.

During the primary stress induction, corresponding to the first 12 hours of incubation in a medium supplemented with 100 μmol/l lead ions, the content of pigments in the tissues of *Egeria densa* of the experimental group was higher than that in the tissues of the control group of plants. However, by the same time, the proportion of carotenoids increased and the ratio of chlorophylls to carotenoids increased to approximately the level of 4 (in the control, this indicator corresponded to 6). The increased content of carotenoids, which was noted during the 12-hour exposure period, is explained by the fact that these pigments are the most active metabolites involved in the system of protecting cells from the influence of environmental factors, including the appearance of heavy metals in the environment [24].

As a result of incubation for 72 hours in a pollutant medium, the content of photosynthetic pigments in the experimental group of plants was inferior to that in the control group. The content of carotenoids in plant tissues of this experimental group of *Egeria densa* was 40% lower than this indicator in the control group of plants.

The dynamics of pigment content in *Egeria densa* tissues under the influence of a 1% solution of anionic synthetic surfactants is presented in Table 6.

Table 6. Effect of a 1% solution of anionic synthetic surfactants on the content of chlorophyll and carotenoids in *Egeria densa* tissues under experimental conditions

Experimental conditions	Chlorophyll *a* content, mg/g raw material	Chlorophyll *b* content, mg/g raw material	Carotenoid content, mg/g raw material
Control	0.150±0.023	0.075±0.012	0.035±0.012
1 hour of experiment	0.136±0.014*	0.062±0.016*	0.020±0.005*
2 hours of experiment	0.178±0.026*	0.081±0.012*	0.041±0.006
4 hours of experiment	0.162±0.018	0.078±0.019	0.025±0.009
12 hours of experiment	0.208±0.015*	0.071±0.013	0.042±0.005
72 hours of experiment	0.159±0.028	0.070±0.011	0.041±0.003

* – degree of significance of differences with the control level $p<0.05$.

After an hour of action of the surfactant, the content of chlorophyll a in the tissues of *Egeria densa* increased by 65%. During the first 12 hours of being in the pollutant environment, which corresponded to the phase of primary stress induction in the plant tissues of the experimental group, the amount of chlorophyll gradually decreased and by 12 hours exceeded the value of the same indicator in the control group by only 28%, which indicated the strength of the stress factor and the development of damage antenna complexes of photosystem II in tissues of *Egeria densa* [22].

Changes in the amount of pigments in the tissues of *Egeria densa* under the influence of a combination of pollutants are reflected in Table 7.

Table 7. Effect of 100 μmol/l lead ions and 1% solution of anionic synthetic surfactants on the content of chlorophyll and carotenoids in tissues of *Egeria densa* under experimental conditions

Experimental conditions	Chlorophyll *a* content, mg/g raw material	Chlorophyll *b* content, mg/g raw material	Carotenoid content, mg/g raw material
Control	0.150±0.018	0.075±0.012	0.035±0.012
1 hour of experiment	0.148±0.028	0.050±0.011*	0.030±0.007
2 hours of experiment	0.244±0.025*	0.062±0.009	0.028±0.006
4 hours of experiment	0.199±0.034*	0.034±0.011*	0.027±0.006
12 hours of experiment	0.258±0.037*	0.044±0.009*	0.022±0.005*
72 hours of experiment	0.189±0.029*	0.041±0.010*	0.064±0.009*

* – degree of significance of differences with the control level $p<0.05$.

Assessing the content of photosynthetic pigments in the tissues of *Egeria densa* under the influence of combined factors (100 μmol/l lead ions and 1% solution of anionic synthetic surfactants), it was found that the response of plants to the action of pollutants was similar to that when exposed only to lead ions. During the beginning of the primary stress induction phase (hour of exposure), the content of both chlorophyll a and chlorophyll b (by 34%) was lower than the values of this indicator in the control group of *Egeria densa*.

Changes in the content of photosynthetic pigments in plants of two species (*Ceratophyllum demersum* and *Egeria densa*) and under the influence of various pollutants (100 μmol/l lead ions and 1% solution of anionic synthetic surfactants) characterize the patterns of response of aquatic plants to anthropogenic impacts. The duration of the influence of the factors corresponded to the transition of plants from a normal state to a state of stress and the subsequent possible adaptation of plants to the action of these pollutants. Such changes in the quantitative content of photosynthetic pigments are characteristic of the phases of emergence, development and some adaptation to stress influences.

Under the conditions of the first phase, the permeability of membranes increases as a result of changes in the molecular composition of their components, and the membranes gradually depolarize, which causes inhibition of H+-ATPase. A decrease in the activity of the latter leads to a decrease in the pH of the cytoplasm and activation of hydrolases, which enhance the processes of biopolymer decomposition. Under these conditions, the rate of hormonal metabolism decreases, acetylcholine, catecholamine, histamine, and serotonin are synthesized, which interact with phytohormones. At the same time, at this stage, the expression of repressed genes and the synthesis of a number of stress proteins occur. Also, under these conditions, the assembly of cytoskeletal elements is activated, which leads to an increase in the viscosity of the cytoplasm [27]. The intensity of photosynthesis is inhibited due to changes in the structure of proteins and lipids of thylakoid membranes [28]. Respiration in plant cells is initially activated, but then inhibited as photosynthesis weakens and the level of ATP decreases. The situation is aggravated by the activation of free radical processes in living cells [29,30].

4 Conclusion

Various aspects of ecology are now the focus of research as a result of pollution of freshwater bodies by synthetic surfactants. This is due to the increasing scale of production of detergents and the large volumes of their use in industry and the extremely wide range of their negative effects on aquatic ecosystems and the human body. Plant organisms of water bodies are among the first to experience the effects of anthropogenic pollution with heavy metals and surface-active compounds. Unfortunately, many aquatic plants are not highly resistant to pollutants. Ecological plasticity is largely associated with changes in the structure of chloroplasts. The work revealed changes in the content of photosynthetic pigments in plants of two species (*Ceratophyllum demersum* and *Egeria densa*) and under the influence of lead ions and a solution of anionic synthetic surfactants. They were considered as a response of aquatic plants to anthropogenic impacts. As the duration of the influence of these toxic factors in plants increased, a transition occurred from a normal state to a state of stress and subsequent adaptation of plants to the negative influence. It is clear that as a result of the action of pollutants (lead ions, anionic synthetic surfactants and their combinations) negative changes occur in the tissues of aquatic plants. In this regard, *Ceratophyllum demersum* and *Egeria densa* can rightly be considered reliable objects for monitoring environmental distress associated with assessing changes in the level of photosynthetic pigments in them.

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