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AQUATIC TOXICOLOGY

Antioxidant Status of Hydrophytes with Different Accumulative Ability Illustrated by *Potamogeton alpinus* Balb and *Batrachium trichophyllum* (Chaix) Bosch.

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Abstract—The antioxidant activity of two common species of aquatic plants (*Batrachium trichophyllum* (Chaix) Bosch. and *Potamogeton alpinus* Balb.) with different "accumulation strategies" has been studied. It is shown that the leaves of *Batrachium trichophyllum*, which accumulate metals in large amounts, have a higher intensity of pro- and antioxidant processes than *Potamogeton alpinus*.

Keywords: higher aquatic plants, heavy metals, accumulative capacity, antioxidant stress

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INTRODUCTION

Submerged higher aquatic plants (hydrophytes) considerably differing in the contact surface of leaves with water are characterized by a high absorbing capacity [2, 9]. In the process of life activity, they extract not only biogenic elements from aquatic objects, but also different toxic substances, including heavy metals (HMs).

The excess of HMs in habitats, as a rule, leads to their increased accumulation by plant organisms; however, the magnitude and pattern of accumulation in different species of plants have their own specifics [15]. Depending on the "strategy" of metals accumulation, one can distinguish accumulators, indicators, and excluders [19].

HMs in increased amounts cause accumulation in the cells of plants and other living organisms of reactive oxygen species (ROS), protection from which is provided by different components of the antioxidant system [14].

There are many data on the activity of antioxidant enzymes and the concentration of low molecular weight antioxidants in plants under conditions of oxidative stress induced by the action of HMs [4, 11, 13]. The relationship between antioxidant activity of plants and their "accumulative strategy" has been studied to a smaller degree.

Determining characteristics of oxidative stress and mechanisms of antioxidant system (AOS) action in plants seems especially urgent, since the functioning of this system is one of nonspecific tolerance ways to stress impacts.

The purpose of this work was to study the antioxidant status of submerged higher aquatic plants with different abilities to accumulate HMs.

MATERIAL AND METHODS

Study objects were *Batrachium trichophyllum* (Chaix) Bosch. and *Potamogeton alpinus* Balb., i.e., submerged higher aquatic plants with different accumulative capacities that were selected from the same habitat.

The selection of study objects to achieve the task was performed on the basis of comparing the accumulative ability of hydrophyte species most widespread in water bodies and water courses of Sverdlovsk oblast. It was found that *Batrachium trichophyllum* was distinguished by a high accumulative ability with respect to HMs, while the minimal concentration of HM in leaves was typical for *Potamogeton alpinus* [15]; therefore, these species were chosen as study objects.

Further, a brief characteristic of species is presented [7].

Batrachium trichophyllum (Chaix) Bosch. (Hair-leaved water crowfoot). Dark green, fluccose in the upper part plant, leaves underwater, sessile, vaginate, with a length of 3–5 cm, usually three-fold tripartite, segments with a wide and hairy spurious sheath near leaf base. Flowers 12–15 mm in diameter. Blossoms in summer. Found in rivers and oxbows.

Potamogeton alpinus (Bath.) (Pondweed). Stem branching, leaves stem-clasping, all submerged in water, obtuse. Fruit from outside acute-carinate. In a dry state, all parts of the plant usually assume a reddish

Table 1. Content of HMs in surface waters of the Revda River, leaves of plants, and coefficients of biological accumulation

Index	Cu	Fe	Ni	Zn	Mn
	Surface waters				
HMs, mg/L	0.010	0.170	0.300	0.012	0.022
MAC, mg/L	0.001	0.100	0.010	0.010	0.010
	Leaves of *Potamogeton alpinus*				
HMs, mg per 1 kg of dry mass	11.8	558.9	10.8	110.3	370.5
BCF	1179	3288	36	9234	16842
	Leaves of *Batrachium trichophyllum*				
HMs, mg per 1 kg of dry mass	85.3	14128.2	72.00	416.2	4804.0
BCF	8531	83107	240	34688	218366

tint, according to which it is easily recognizable. Grows in ponds, rivers, and swamps.

Hydrophytes for study were selected in July 2011 in the period of their blooming from the same habitat, the Revda River (Sverdlovsk oblast). Simultaneously, water samples were taken by mixing different specimens up to a depth of 0.5 m.

The Revda River, a left-bank tributary of the Chusovaya River (basin of the Kama River), is formed from the fusion of a great number of small rivers. The river is used both as drinking water and to provide technical water to industrial enterprises [3].

Concentration of metals in leaves of hydrophytes and in unfiltered river water was determined by atomic-absorption spectroscopy after wet washing with 70% nitric acid [5]. The bioconcentarion factor (BCF) was calculated as the ratio of metal content in the plant biomass to the total concentration of this metal in water.

Concentration of soluble protein in leaves of plants was determined using the Shakterle method [23]. The intensity of processes of peroxidation of lipids (POL) and activity of antioxidant enzymes were determined in averaged samples of leaves (weight of weighted portion 0.5 g) that were homogenized in cold in a 0.1 M K/Na-phosphate buffer (pH 7.4) and centrifuged. The resulting supernatant was used to determine the POL, activity of SOD, and guaiacol peroxidase (GP).

The intensity of POL was determined in a butanol extract according to the concentration of products reacting with thiobarbituric acid (TBA-reacting products) [24]. The activity of SOD was determined using a method based on measuring the inhibition of photochemical restoration of nitroblue tetrazolium [21]. The activity of GP was assessed from an increase in the optical density of reactionary medium at a wavelength of 470 nm as a result of guaiacol oxidation [32].

Proline concentration was determined by the common method [20] using an acidninhydrin reagent. The determination of ascorbic acid and glutathione was performed from one averaged weighted portion of leaves by the trinolometric method using parallel titration by 2.6-dichlorophenol-iodophenol and potassium iodate [17]. The content of flavonoids was determined in alcoholic (96% ethanol) extract using a citric-acid boric reagent with spectrophotometer at a wavelength of 420 nm [12].

The significance of differences was assessed according to a nonparametric Mann–Whitney *U*-test at $p < 0.05$. Tables include average arithmetic values from three biological replications and their standard errors.

RESULTS

Results of determining the HM content in surface waters and coefficients of their biological accumulation in leaves of the studied hydrophytes from the Revda River are presented in Table 1.

In the study period, the concentration of the studied metals in waters of the river exceeded the maximum acceptable concentration (MAC) for fishery water bodies. To the maximum degree, it is typical for copper and nickel compounds, the ratio of excess for which comprised 10 and 30 MAC, respectively.

Results of an assessment of HMs accumulation in leaves of the studied hydrophytes reflect considerable differences between the two species: the amount of all studied metals in leaves of *Batrachium trichophyllum* was several times (or by an order of magnitude) higher than in leaves of *Potamogeton alpinus* (Table 1). Excess ratio for zinc was 4, for copper and nickel it was 7, for manganese it was 13, and for iron it was 25.

The intensity of POL processes in leaves of *Batrachium trichophyllum* was an order of magnitude higher than that in *Potamogeton alpinus* (Table 2).

Activity of SOD and GP—key enzymes of the antioxidant system in leaves of *Batrachium trichophyllum* were considerably higher (3.4 and 1.6 times, respectively) (Table 2). Content of soluble protein in leaves of this plant species also exceeded its amount 2.5 times in leaves of *Potamogeton alpinus*.

The content of nonenzymatic antioxidants in leaves of aquatic plants is different (Table 3). *Batrach-*

Table 2. Content of soluble protein, intensity of POL, and antioxidat enzymes activity in leaves of the studied hydrophytes

Plant species	Content of soluble protein, mg per 1 g of dry mass	Intensity of POL, μmol per 1 g of dry mass	Activity of SOD, units per 1 g of dry mass per minute	Activity of GP, μmol per 1 g of dry mass per minute
Potamogeton alpinus	63.3 ± 2.1	90 ± 4.0	383 ± 42	518 ± 13
Batrachium trichophyllum	159.3 ± 6.8	1300 ± 20	1282 ± 34	811 ± 33

Table 3. Content of nonenzymatic antioxidants in leaves of the studied hydrophytes

Plant species	Flavonoids, mg per 1 g of dry mass	Ascorbate	Glutathione	Proline
		μg per 1 g of dry mass		
Potamogeton alpinus	9.8 ± 0.8	179.1 ± 12.8	812.3 ± 12.2	279.0 ± 5.8
Batrachium trichophyllum	70.5 ± 1.0	319.7 ± 22.0	2296.4 ± 55.0	585.5 ± 9.0

ium trichophyllum—a hydrophyte with a high accumulative capacity—differed from *Potamogeton alpinus* in a higher content of all studied low-molecular components of the antioxidant system of plants. For instance, the ascorbate content in leaves of *Batrachium trichophyllum* was 1.8 times higher, that of proline was 2 times higher, glutathione was 2.8 times higher, and flavonoids were 7 times higher than in leaves of *Potamogeton alpinus*.

DISCUSSION

Physiological and biochemical effects of oxidation stress induced by the impact of HMs and the methods of protection from it using antioxidant systems are actively studied. The results of performed theoretical and experimental studies indicate a considerable diversity of antioxidant mechanisms of living systems, which provides their tolerance and reliability of functioning.

The pattern of interaction between components of the system of antioxidant protection remains poorly studied. The functioning of components of the antioxidant system in plants in dynamics and in the loading gradient, as well as the role of different components of antioxidant system against ROS, is also studied insufficiently. Other issues related to the formation of plants tolerance to unfavorable conditions remain unsolved. In connection with this, studies oriented at the expansion and deepening of plants and other living organisms antioxidant systems functioning knowledges and the change in the activity of AOS components under stress conditions also remain urgent. A study of the antioxidant status of living systems directed on the assessment of the possibility of using it for integrated diagnostics of their stability to anthropogenic impact is especially important.

Surface waters of the Revda River, as of most other water bodies of Sverdlovsk oblast, are characterized by increased HMs concentrations. This fact, on the one hand, is a reflection of geochemical specific features of the territory related to the bedding of polymetallic ores and their mining and, on the other hand, it is a consequence of a high degree of urbanization and inflow of waste waters from industrial enterprises [3].

An analysis of coefficients of biological accumulation of HMs in the studied species demonstrated that, in *Potamogeton alpinus*, values of BCF comprised an ascending series: Ni < Ca < Fe < Zn < Mn. The same pattern of metals accumulation was observed in most species of hydrophytes studied previously [15]. However, in *Batrachium trichophyllum*, BCF for iron was higher than for zinc.

The results of studies allow us to suggest that an increase in the intensity of POL in plants with increased accumulative capacity (*B. trichophyllum*) is related to the active accumulation of metals whose excess caused an intensified generation of ROS. This led to the activation of AOS components, i.e., an increase in the intensity of lypoperoxidation could initiate the induction of adaptation mechanisms oriented at the prevention of the subsequent development of oxidation stress, including an increase in the activity of antioxidant enzymes and an increase in the amount of low molecular weight antioxidants.

SOD performs the role of the primary boundary against active ROS. The activation of SOD under unfavorable impacts is a response to an increase in the production of radicals of superoxide under these conditions, which protects cells and tissues from oxidative damages. In the realization of the adaptation potential of plants, a special role is allocated to peroxidase, a polyfunctional enzymatic system capable of responding to a wide spectrum of factors leading to disturbances of metabolic processes [14].

An increase in the activity of antioxidant enzymes under the action of HMs can be determined by the activation of their latent forms and/or the synthesis of their molecules. SOD can also include metals such as iron, copper, manganese, zinc, and the obligatory component of peroxidases: iron [14]. One can suggest that the accumulation of these HMs in cells of plants led to an increase in the activity of SOD and GP. The activation of antioxidant enzymes could also promote the accumulation of low molecular weight antioxidants. In particular, there are published data [1] on the regulating effect of glutathione on the activity of SOD. The restored glutathione induced expression of gene of cytoash SOD in leaves of tobacco, although mechanisms of this effect have not been established [1].

An increase in the rate of antioxidant processes catalyzed by antioxidant enzymes is determined not only by the activation of antioxidant enzymes, but also by their formation in increased amounts upon the development of oxidation stress. The increase in the rate of synthesis of antioxidant enzymes during the accumulation of HMs is evidenced by a higher content of soluble protein in leaves of *B. trichophyllum*.

The higher activity of antioxidant enzymes and the increased content of low molecular weight antioxidants in leaves of *B. trichophyllum* can be regarded as a nonspecific adaptive response providing a high level of tolerance of these plants to the absorbtion and accumulation of a large amount of HMs.

One of the known methods of plants protecting from the harmful effect of HMs is the biosynthesis of low molecular weight proteins and peptides enriched by SH-groups (metallothioneins and phytochelatins). Metal-binding proteins and peptides are synthesized in the norm in inconsiderable amounts. Their content in a cell drastically increases upon an increase in HMs concentration in the nutritive substrate [14].

Batrachium trichophyllum from a habitat with a higher technogenic load (because of which in river waters and leaves of plants an increased amount of HMs was observed) differed in a more considerable synthesis of SH-containing compounds than plants of the same species from a “conventionally clean” habitat [16]. The number of thiolic groups in an environment polluted with HMs significantly increased in soluble and membrane bound proteins. The inactivation of HMs in plants cells at their excessive delivery from the habitat promoted an increase in tolerance for the action of HMs. The increased level of non-enzymatic antioxidants in leaves of *B. trichophyllum* is apparently mediated by the accumulation of ROS. For instance, ascorbic acid acts as a reducing agent, thereby increasing the tolerance of plants for ROS. The formed dihydroascorbate can restore up to ascorbic acid at the expense of the enzymatic and non-enzymatic oxidation of glutathione. Glutathione serves also as the main substrate for the formation of phytochelatins. Thus, an increase in glutathione content and the subsequent formation of phytochelatins in plant species with a high accumulative ability is one mechanism of HMs detoxication.

The increase in soluble proteins content in leaves of plants differing in high levels of HMs accumulation is apparently determined not only by the necessity of synthesis of new molecules of antioxidant enzymes induced by the intensification of prooxidant reactions, but also by the formation of different protective metal-binding proteins. This is proven by the results of studies [10, 16] evidencing an increase in the content of membrane bound proteins in leaves of plants living in aquatic habitat with HMs pollution.

The study of the antioxidant status of hydrophytes in connection with their accumulative potential, especially under conditions of the anthropogenic impact, promotes the detection of relations between physicochemical characteristics of plants and their adaptive abilities.

CONCLUSIONS

Bioconcentration factor of five investigated metals (Cu, Fe, Ni, Zn, and Mn) in leaves of *Batrachium trichophyllum* were several times higher than in leaves of *Potamogeton alpinus*. A comparison of the antioxidant status in these two species demonstrated that the accumulation of metals in leaves of *Batrachium trichophyllum* was accompanied by the intensification of the processes of lipids peroxidation, the activation of superoxidismutase and guaiacol-specific peroxidase, and an increase in the content of low molecular weight antioxidants: flavonoids, ascorbate, glutathione, and proline. The activation of antioxidant enzymes and the accumulation of non-enzymatic antioxidants is one of protection mechanisms against the HMs effect that promotes the survival of the plant organism and its adaptation to an increased content of metals in an aquatic habitat. Antioxidant systems, providing the functioning of non-specific mechanisms of tolerance, play a crucial role in the adaptation potential of plants to metals. Studies of HMs accumulation by plants and means of their adaptation to habitat pollution is the basis for elaborating more effective technologies for treating polluted waters, as well as for perfecting methods of biomonitoring aquatic ecosystems.

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