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Usage of Enzymes of Algae-macrophytes Antioxidant System for Monitoring Water Pollution By Oil Products

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Abstract

Macrophyte algae are perspective indicator species for assessing the environmental impact of pollutants. One of the ways to estimate the negative impact is to change the activity of enzymes of the antioxidant system. The possibility of using hydrogen peroxide, catalase and superoxide dismutase (SOD) is analyzed in the research for assessing the anthropogenic load on algae macrophytes of different systematic groups: *Palmaria palmata* (L.) O. Kuntze; *Fucus vesiculosus* L., *Ulvaria obscura* (Kützing) Gayral ex Bliding. The natural level of enzymes (in July) and its change under the influence of oil products (diesel fuel) on algae growing in the Barents sea were measured. It is shown that under natural conditions the content of hydrogen peroxide and catalase activity decrease in the series *U. obscura* < *P. palmata* < F. vesiculosus; the activity of SOD decreases in the series *P. palmata* < *U. obscura* < *F. vesiculosus*. When diesel fuel is introduced into the habitat of algae, the concentration of hydrogen peroxide in the cells of *U. obscura* and *P. palmata* decreases and in *F. vesiculosus*-increases. Catalase activity in *P. palmata* and *U. obscura* increases and catalase activity is not various in *F. vesiculosus*. The activity of SOD in the prototypes of *G. obscura* and *P. palmata* decreases and it remains unchanged in *F. vesiculosus*. It is shown that the enzyme complex *Ulvaria obscura* can be used to assess the impact of diesel fuel and only SOD can be used in *P. palmata*. No reaction of SOD and CAT to the presence of petroleum products was detected in *F. vesiculosus*.

Keywords: *Palmaria palmata; Fucus vesiculosus, Ulvaria obscura*, antioxidant system, diesel fuel, Barents sea.

Abbreviations: AOS-antioxidant system, SOD-superoxide dismutase, CAT-catalase, CA-catalase activity.

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1. Introduction

Marine coastal ecosystems are constantly experiencing anthropogenic impact and for assessing its impact on biocenoses it is necessary to analyze the state of separate components - plants, animals and microorganisms.

Plants, in particular, algae are rather interesting objects for research. Due to changing morpho-physiological parameters (growth rate, photosynthesis intensity, pigment concentration, etc.) not only the magnitude or strength, but also the duration of anthropogenic impact can be estimated. Macrophyte algae are perspective indicator species for determining the effects of organic and nonorganic pollutants, as they tend to be the most common life forms in the coastal zone. There are several levels of research: phytocenotic level (the change of species composition and change of dominants are considered), physiological level (the growth rate, the intensity of photosynthesis/respiration, enzyme activity are under research and biochemical level (features of the accumulation of substances (amino acids, polysaccharides) are investigated. Currently, a great interest causes the possibility of using enzymes of the antioxidant system (AOS) as markers of the state of living organisms under anthropogenic load [1].

Not only the individual state of the organism can be assessed by the level of enzymes, but also the ecosystem development can be predicted, including its emergency or chronic pollution activity. The most popular and available for measurement are the enzymes AOS: superoxide dismutase (SOD), catalase, glutathione peroxidase and ascorbate peroxidase, the concentration of hydrogen peroxide, also often used as a marker of the presence of stress -- lipid peroxidation or lipid oxidation products (LOPs).

The aim of the research was to investigate the antioxidant systems complex of algae-macrophytes of the Barents Sea for possible use in the system of biomonitoring in contaminated coastal environment with oil products.

2. Methods and Equipment

2.1. Methods

Macrophyte algae species of different systematic groups which are dominant in the littoral zone of the Murmansk coast of the Barents Sea were used as objects of research: *Palmaria palmata* (L.) O. Kuntze; *Fucus vesiculosus* L., *Ulvaria obscura* (Kützing) Gayral ex Bliding.

The research was carried out in the summer of 2016-2019 on the basis of the seasonal field biostation Murmansk Marine Biological Institute Kola Scientific Center RAS in Dalniye Zelentsy -- the Murmansk coast of the Barents Sea.

The research was carried out in 2 stages:

1. Determination of the natural level of enzyme activity. For this purpose algae were gathered on the littoral at low tide, enzyme activity was determined within an hour after plants gathering. For obtaining comparable results algae were gathered at a time in July, as these indicators are variable over time.

2. Determination of changes in the state of algae AOS in response to the introduction of a toxicant (diesel fuel) in the habitant. A number of experiments to solve this problem was carried out. The research was done in a laboratory, in a thermostated room at +10°C air temperature, constant lighting of 150 W / m2 and aeration. The plants that were used for the experiment had previously been acclimated to the laboratory conditions for 7 days.

Diesel fuel at a concentration of 20mkl/l was added in experimental containers with algae and then kept for 14 days. Control containers were in the same conditions. At the end of the experiment, the control and experimental samples were determined by the indicators of the state of the AOS.

2.2. Determination of AOS enzymes activity

Preparation of the extract: algae weighing 150-200 mg were pounded on ice in a mortar with the addition of 2000 µl extraction buffer (K / Na-phosphate buffer). The homogenate was centrifuged 5 min, 12000 g at 4°C. The resulting supernatant was used in further reactions.

Catalase activity (CAT) was measured using a modified spectrophotometric method [2]. In order to do it 2 ml of 0.03% hydrogen peroxide solution was added to 0.1 ml of the extract. 0.1 ml of distilled water was added to the blank sample instead of the supernatant. The reaction was stopped in 10 minutes by adding 1 ml of 4% ammonium molybdate. The intensity of the developing color was measured on a spectrophotometer at a wavelength of 410 nm against a control sample in which 2 ml of water was added instead of hydrogen peroxide.

The activity of superoxide dismutase (SOD) was determined by Giannopolitis [3]. Two identical test tubes with reaction mixture and extract were prepared for each sample. One was placed in darkness, the other in light at an air temperature of 18°C. The control tube contained instead of extract 100 µl K / Na-phosphate buffer. Then 0.5 ml 0.05% p-R

NBT, 20 µl 0.24% p-R Na-EDTA, 0.9 ml K/Na-phosphate buffer were added. For starting the enzymatic reaction, 20 µl of Riboflavin was added into all tubes. The reaction took place within 15 minutes. The optical density of the contents of the tubes was determined at 560 nm on a spectrophotometer.

The content of hydrogen peroxide was determined by a modified spectrophotometric method according to Bellincampi [4]. Algae weighing 150-200 mg were pounded on ice in a mortar with the addition of 2000 µl of filtered seawater. The homogenate was centrifuged 10 min, 12000 g at 4°C. The supernatant was used for analysis, 1.5 ml of supernatant and 20 µl of Xylenol orange reagent were added to the tubes. In the control tube, 1.5 ml of seawater was added instead of the sample. The samples were kept for 45 minutes at room temperature (18C). The reacting mixture was centrifuged for 5 min at 10,000 g. then the optical density was measured at a wavelength of 560nm.

Calculation of enzyme activity (CAT and SOD) was carried out on dry weight.

The dry mass of the samples was determined according to the generally accepted method [5].

All measurements were performed in 3-4 times repetition and are presented as an arithmetic mean with standard deviation. Microsoft Excel 2010 was used for data processing.

3. Results

At the first stage of the work, the natural level of activity of algae enzymes was analyzed. It has been shown that the content of hydrogen peroxide decreases in the series *U. obscura* < *P. palmata* < *F. vesiculosus* (Figure 1, a).

Catalase activity decreases from *U. obscura* < *P. palmata* < *F. vesiculosus* (Figure 1, b). The activity of SOD in the cells of *P. palmata* is maximal and it is minimal in *F. vesiculosus* (Figure 1, c).

Analysis of the measurement results showed that the enzyme activity in *U. obscura* and *P. palmata* changes during the experiment. Differences between control and experimental samples were not recorded in *F. vesiculosus*.

Under the influence of diesel fuel in *U. obscura* and *P. palmata*, the concentration of hydrogen peroxide in cells decreases and the concentration of peroxide increases in *F. vesiculosus* (Fig. 2a)

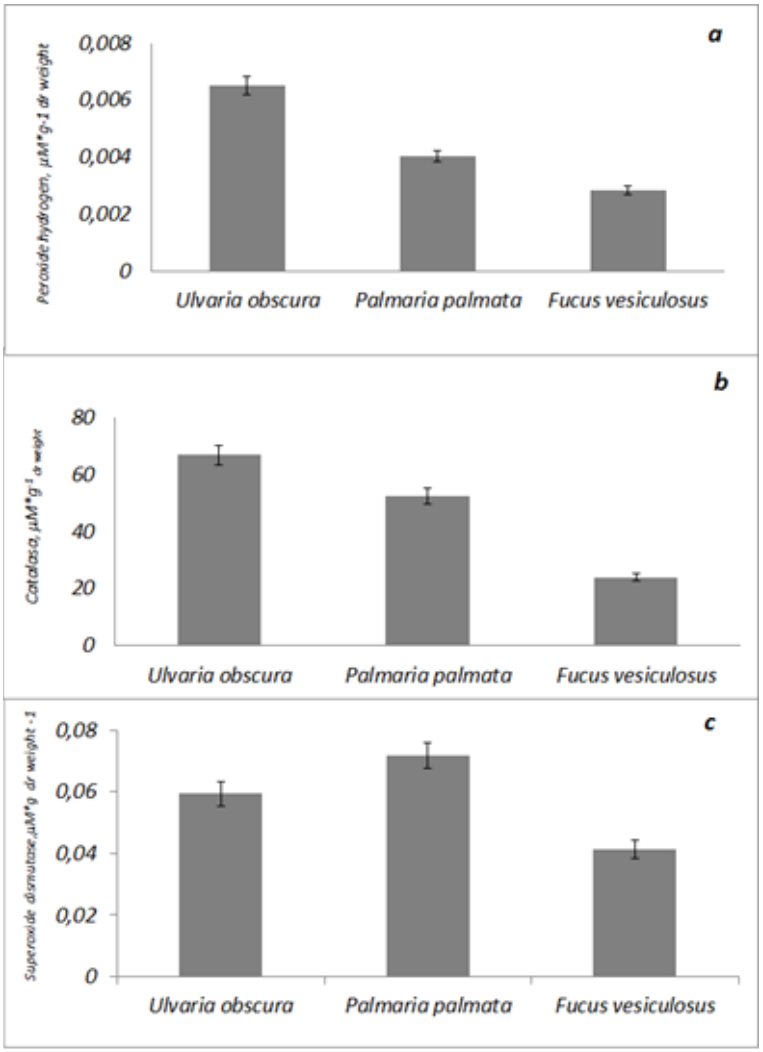

Figure 1: Activity of enzymes AOS algae in nature: A-hydrogen peroxide, B-catalase, C-superoxide dismutase.

Catalase activity in experimental samples of U. obscura increases 2 fold compared to the control sample, it slightly increases in *P. palmata*, control and experimental samples do not differ in enzyme activity in *F. vesiculosus* (Fig 2b).

The activity of SOD in the experimental samples of *U. obscura* and *P. palmata* is 1.5 fold reduced compared to the control samples, no differences in activity were noted for *F. vesiculosus* (Figure 2, c).

4. Discussion

The formation of AFO (active forms of oxygen) in photosynthetic organisms is a mandatory process that accompanies various metabolic processes-photosynthesis, respiration,

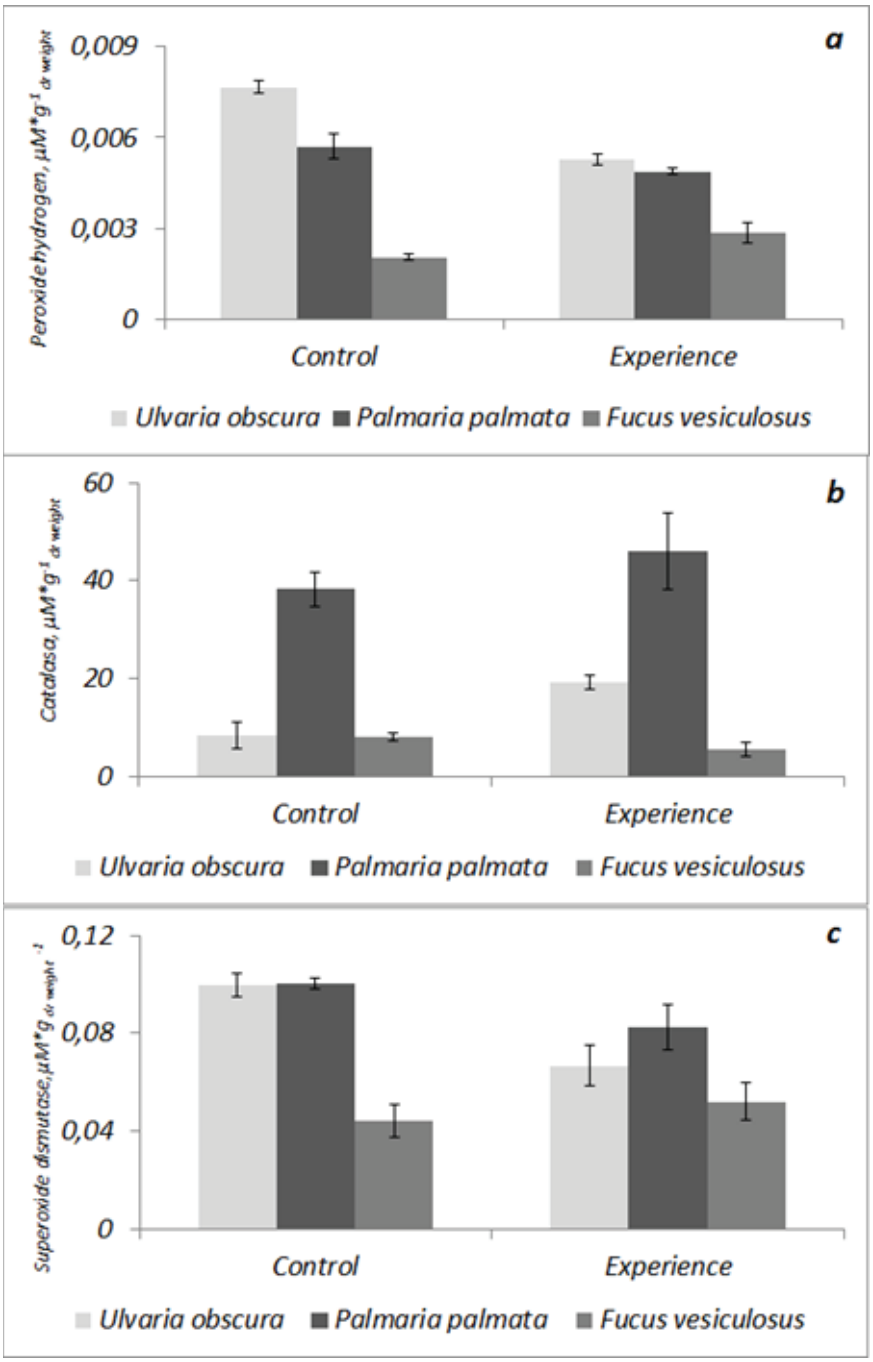

Figure 2: Changes in the activity of AOS enzymes under the influence of diesel fuel: a-hydrogen peroxide, b-catalase, c-superoxide dismutase.

nitrogen fixation, etc. [6], therefore, enzyme and non-enzyme compounds are constantly produced in the body.

The intensity of the enzymes synthesis of the antioxidant system is a species-specific feature. Also, the activity of enzymes can be determined by the origin of the species, for example, it was previously shown that boreal species have a higher antioxidant status

compared to the Arctic (for example, *U. obscura*-boreal, and *F. vesiculosus*-arcto-boreal species).

In summer period the intensity of enzyme synthesis is quite high since maximum physiological activity, growth and synthesis of spare nutrients are characterized for algae at this time of year, especially *for F. vesiculosus* [7]. In addition to the internal characteristics of the body, the activity of enzymes will be influenced by abiotic and anthropogenic factors.

Changing the intensity of their impact causes the formation of stress in the body, the presence of which is manifested in an increase in the concentration of reactive oxygen species in the cells. This, in turn, generates the activation of different groups of enzymes and non-enzymatic components of the antioxidant system. High concentrations of antioxidants will be crucial for regulating the level of photo-oxidative stress caused by reduced energy consumption as a result of different groups of xenobiotics [8].

The main enzymes of AOS are superoxide dismutase, catalase, peroxidase, etc., and a number of non-enzymatic compounds such as carotenoids, flavonoids, phenolic compounds (e.g., phlorotannins). Depending on the type and magnitude of the impact will vary the response of enzymes. For example, for *F. vesiculosus*, which grows in the Arctic, it has been shown that low air temperature conditions and / or a combination of low air temperatures and high light suppress catalase activity, while SOD activity does not change [9]. Both SOD and catalase react to changes in habitat temperature in the red algae *Palmaria palmate* [10].

And if the change in the activity of the enzyme system in response to the action to a number of factors of the abiotic environment (temperature, illumination) is partially revealed, the reaction to petroleum products (in particular, diesel fuel) is studied rather poor.

The results of our research showed that representatives of the green and red algae experience changes in the activity of enzymes catalase and SOD in response to the action of petroleum products, the representative brown -- this system is not sensitive to the toxicant.

Such differences in the reaction of enzymes to the action of xenobiotics can be determined by a number of reasons: the nature of the toxicant, the type of plant, its stage of the life cycle and so on [1]. So, the early stages of development (young seedlings, zygotes, gametes) are more sensitive than adults as the latter are able to adapt to such effects, including through the activation of AOS, heat shock proteins, etc. Researches with the use of chemical dispersants for eliminating oil spills have shown that the synthesis of heat shock proteins in algae increases resistance to crude oil [11]. At the

same time, some concentrations of petroleum products can have a stimulating effect on the body [12].

Also, the degree of toxicity to the body can be determined by the features of the structure of algae. *Fucus* algae are characterized by a complex internal structure with the presence of differentiation of cells into a layer, a relatively dense cuticle layer is formed on the surface of the thallus. *D*ifferent types of bacteria are developed, including those capable of oxidizing petroleum products [13]. Presumably, the penetration of petroleum products to the underlying layers is slowed down precisely due to the presence of this layer and the activity of bacteria that neutralize adsorbed petroleum products [14]. A single-layer plate is characterized for *U. obscura*, it has no protective layer and when it hits the surface of the thallus, the oil product quickly penetrates into the cells. *P. palmata* occupies an intermediate position between them: it has several layers of cells, on the surface there is a small cuticle layer, which slows down the penetration of the toxicant.

Since the algae at the time of the experiments have retained their viability, it can be assumed that they successfully cope with stressful conditions. The SOD -- catalase complex takes part in reducing the level of AFO in green and red algae. Catalase activity does not change in *F. vesiculosus* under the toxicant. A comparison of the enzyme activity of *F. vesiculosus* growing in different polluted places (the amount of petroleum products in the water varied from 0.023 to 0.057 mg/dm^3) showed that the catalase activity did not differ. The absence of catalase response to pollution was noted for the related species *Cystoseira crinita*, which grows in the Black Sea [15]. Unlike catalase SOD of plants from more dirty areas was higher.

Perhaps, when contaminated with oil products, *fucus* algae activate another system responsible for the neutralization of AFO. For example, the second major enzyme in *fucus* and *Palmaria* would be ascorbate peroxidase (APX) which reduces peroxides to H_2O or corresponding alcohols, respectively, using ascorbate as an electron donor [1]. Carotenoids and polyphenols can act as antioxidants. Carotenoids are involved in the stabilization of the lipid fraction of photosynthetic membranes, than preserve their functional integrity, extinguish electron-excited molecules [16], such as O^{2-}, which are capable of inducing DNA destruction [1], often they act in conjunction with polyphenols [17, 18]. Another auxiliary mechanism for H_2O_2 removal is the formation of volatile halocarbons (e.g., bromoform, chloroform, and trichloroethylene) from vanadium-bromoperoxidases. This mechanism is more typical for red algae exposed to both biotic and abiotic stresses [19].

5. Conclusion

The effects of diesel fuel which we are considering is evolutionarily new to organisms, algae differ in resistance to toxicant, they activate different systems of antioxidant protection.

It is shown that *Fucus vesiculosus* in response to environmental pollution with petroleum products did not show a clearly expressed enzyme response, which may be due to the activation of another enzymes complex with neutralize AFO. In *Palmaria palmata*, under the influence of a toxicant, there was a change in the activity of SOD, the enzyme catalase was less sensitive. *Ulvaria obscura* showed a good response of catalase and SOD to the impact of petroleum products, this complex can be used to assess the impact of toxicants on the viability of algae.

Thus, the enzymes catalase and SOD can be used to assess the impact on the body of the toxicant only in green algae *Ulvaria obscura*. For the rest of the studied algae, this group of enzymes will not be indicative and for them it is necessary to conduct additional studies aimed at identifying ways to detoxify ROS.

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Conflict of Interest

The authors have no conflict of interest to declare.

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