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Effect of barium chloride on growth and oxidative stress of saltwater algae

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ABSTRACT

Algae have the ability to detoxify the environment by binding heavy metals. They are also used as natural biosensors. In this study, the effect of barium chloride on saltwater algae was examined. Direct influence on the development of the test organism was visualized by means of infrared spectroscopy. The direct effect of barium chloride describes the amount of polyphenols and chlorophyll content in the culture. Work indicates the toxicity scale of a relatively small amount of toxic factor. The results were compared with the effects for a non-barium chloride culture.

***Keywords*:** algae, Nannochloropsis, toxity, barium chloride, FTIR, IR spectroscopy, TPC, polyphenolic

1. INTRODUCTION

The tested species - *Nannochloropsis* sp. are spherical shaped, 2-4 micrometers in diameter single celled heterotrophic algae. Thanks to the high protein content and ability of omega 3 fatty acids synthesis they are widely used both in freshwater and saltwater

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aquaculture. There are currently 6 known species in a Genus *Nannochloropsis*. They are so closely related that they can only be distinguished by costly rbcL gene and 18S rDNA sequence analysis. *Nannochloropsis* sp. lacks of chlorophyll b [1], yet in some articles there are information about determination this fraction [2]. This organism is used in the process of biofuels production, it has the ability to accumulate heavy metals, hence the interest in measuring bar toxicity for living cells. Algae remove harmful metals such as Ni, Cr, Cu, Pb, Zn, Hg or Co. Such systems have a potential to be an important element in removing harmful industrial residues [3]. In many water reservoirs where the content of barium is recorded, macroalgae, including brown algae, accumulate it in their cells, however, the subject of barium chloride influence on marine organisms is rarely mentioned in the literature, nor is toxicity related to phytoplankton in the safety data sheet of this compound. Barium is not found in nature in the free state, but in the earth's crust it is 17th in terms of prevalence, counting weight percentages, while in the oceans per 1000 kilogram of water there is 20 mg of pure barium [4].

2. EXPERIMENTAL

2. 1. Test organism

Pure culture of *Nannochloropsis* sp. was obtained from Laboratory of Marine Organisms Reproduction in West Pomeranian University of Technology in Szczecin.

2. 2. Algae culturing

Picture 1. Photobioreactors in Laboratory of Marine Organisms Reproduction in West Pomeranian University of Technology in Szczecin, Poland

Algae were cultured in semiprofessional, acrylic photobioreactors (further reflected as PBR Picture 1.) lit by a 10 W LED spotlight from the top, and 5050 red and blue GROW LED strip from the back. Culturing started with cleaning the reactor well with alcohol, rinsing

with water from reverse osmosis filter (RO). In the next step artificial saltwater was prepared by adding Aquaforest Sea Salt to RO water. Target specific gravity 1.017 was checked with calibrated H_2O refractometer. Clean reactors were filled with 12 liters of salt water previously prepared. Each reactor was inoculated with 1 liter of clean algae culture and f/2 Guillard medium was added in amount of 2 ml per 1 l of culture 26 ml in total per reactor). Aeration was provided 24h and photoperiod was 16h light, 8h dark. It took seven days for the culture to be ready to harvest. Every two days each culture was examined under the microscope with a view to any undesirable organisms namely fungus or other algae species.

2. 3. Chemicals

F/2 Guillard medium and barium chloride were used to algae breeding and creating a test environment. Methanol – water 1:1 solution were used to extraction to TPC test.

2. 4. Cultivation in harmful conditions

The organism used in the test was saltwater unicellular algae *Nannochloropsis* sp. It was treated with 1:10 solution of 2.5% $BaCl_2$* $2H_2O$ (40 mM). Five research variants and one control sample were determined. The cultures were subjected to constant mixing and placed in a sunny place. Growth substances ensured F/2 medium was added to the culture at a ratio of 1 ml per 1l of culture. The algae used in the study were stored in salt water. Six measurements were taken every 24 hours.

2. 5. Optical density measurement

The OD culture density was measured at 600 nm using a spectrophotometer (Thermo Fisher Scientific, USA). For measurement purposes 1 ml of each sample was taken.

2. 6. Measurement amount of chlorophyll

The productivity of algae, expressed as the amount of chlorophyll produced is described according to methodology of Inskeep and Bloom [5]. For this purpose, spectrophotometric measurements were also carried out with wavelengths of 663 and 645 nm. Contents of the chlorophyll fraction and its total content were calculated from the formulas.
Total Chlorophyll = $(8.02 + A_{663})$-$(20.20 + _{A645})$.

2. 7. IR spectroscopy

A FTIR infrared spectroscopy analysis was carried out on the Perkin Elmer spectroscope of the untreated culture spectra and treated with barium chloride. Samples from the cultures were dried in 100 °C using a weighting dryer (RadWag, Polska), before the measurement. Spectra were normalized and developed using the SPECTRUM software.

2. 8. Determination of the total phenolics content (TPC)

Samples of each culture were taken and centrifuged for 10 minutes at 2500 rpm. The pellet was covered with 2 ml of water, methanol 1:1 , and sonicated for 6 minutes. Tests were centrifuged again at 4000 rpm for 5 minutes. The Total phenolic content was determined by the Folin-Ciocalteu reaction according to Łopusiewicz [6], using gallic acid as a standard. Polyphenols were determined in the supernatant, which (20 µl) was mixed with 1.58 ml of

deionized water and 100 µl of the Folin-Ciocalteu reagent and 300 µl of saturated solution of Na_2CO_3. The samples was stored for 30 minut to 40 °C in the dark. Absorbance was measured at 765 nm and TPC was calculated as miligrams of gallic acid equivalents (GAE)/gram.

3. RESULTS AND DISCUSSION

Results of the test samples were averaged and compared to the results of the control sample. All cultures started the experiment with the same OD level that corresponds to standard 0.5 on the McFarland scale. Already after 24 hours, there were clear differences between two cultures. The one with barium chloride was developing at a slower rate and with a distinctly long phase of stagnation, on the fifth day of the measurement the culture began to die off. The measurement was also difficult due to the increased sludge cell deposit building at the bottom of the culture vessels. The last measurements are below the standard 0.5 for the test culture and the standard number 1 for the control breeding.

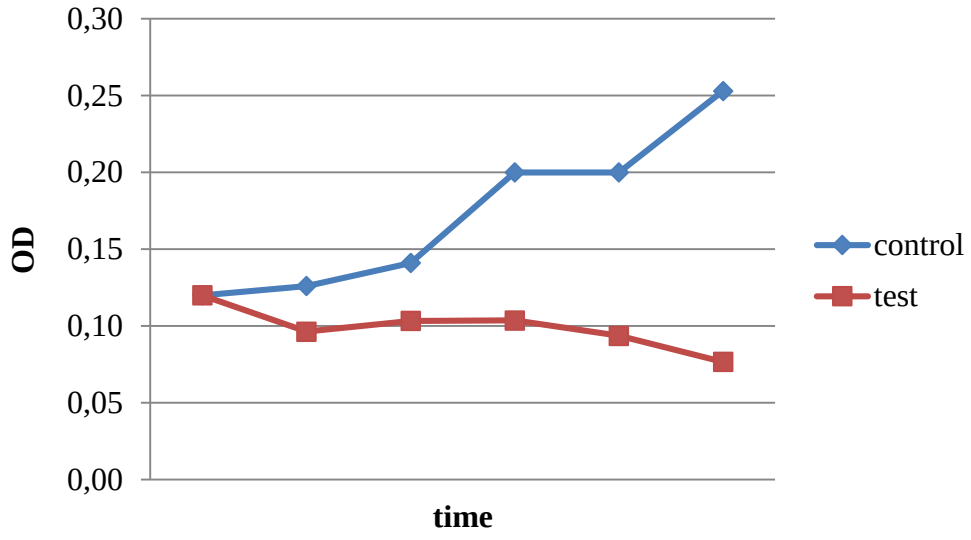

Figure 1. Results of measurement optical density

Chlorophyll measurements were made in order to determine the productivity of the culture. After determining content of individual chlorophyll fractions, the total chlorophyll content was determined. Despite the decrease in optical density, the amount of chlorophyll in cultures with barium chloride was constantly increasing. This means that the algae were still productive despite the toxic effects of barium chloride. A smaller amount of chlorophyll in cells treated with barium chloride than in a rotary culture is caused by the elution of chlorophyll by a toxic substance as described in case of plants. The density of culture in the middle with barium chloride clearly decreases after the 5th day of measurement. Distinct phase of stagnation could also be observed. The results for the test trials, although initially representing the same level of algae, are very different. After a high dose of barium chloride, the OD has dropped considerably and remained at a low level. Nevertheless, the results are on the same level of McFarland's scale.

The mappings of the optical density results are measurements of the amount of chlorophyll. According to research conducted on plants, barium chloride reduces the content of chlorophyll. Concentrations from 2 mM to 10mM were used in the cited studies and resulted in removal of 63% chlorophyll [7]. In this work, the concentration used was 40 mM and after the first measurement a similar chlorophyll loss was noted. However, it can be presumed that with the use of such concentrations as in the aforementioned work, the loss of chlorophyll could be smaller.

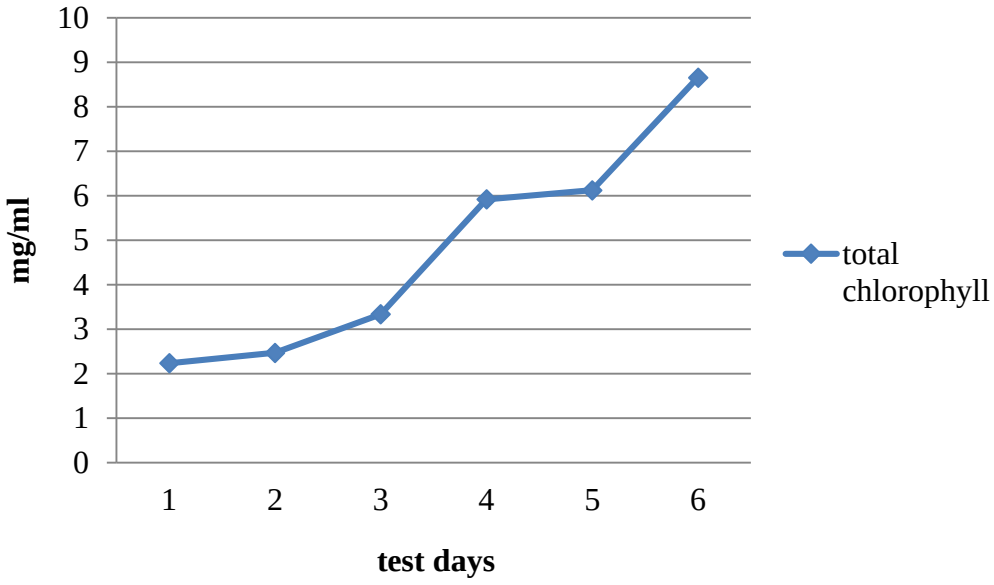

Figure 2. Results of measurement total chlorophyll in control trial

As it can be seen in the culture with the addition of barium chloride there was a growth stopped after the third day of the test. After the fifth day there was a clear drop. The whole culture was less productive in chlorophyll compared to the control. The results obtained for the research sample are averaged over five replications. The standard deviation was arranged on the graph in the form of bars. The rebound fell with the course of the experiment, because all the trials were approaching the point at which the breeding slowly died out.

The TPC results clearly show the oxidative stress caused by the presence of barium chloride in the breeding environment. The polyphenol content of the control culture is 2.4 mg GAE / g, for *Nannochloropsis occulata* should be about 2.04 mg GAE/g according to the literature [8]. Which means that the result is in the standard. The TPC for the barium cultivated crop was 3.7 mg GEA/g.

The production of polyphenols is the cell response to a stress factor, in this case barium chloride in the environment [9]. In other studies on the stress caused by the use of barium chloride, a significant increase in another factor associated with the antioxidant system such as the reactive oxygen forms has been noticed [10]. The barium chloride causes a constantly increasing response from the side of the system corresponding to the cell death.

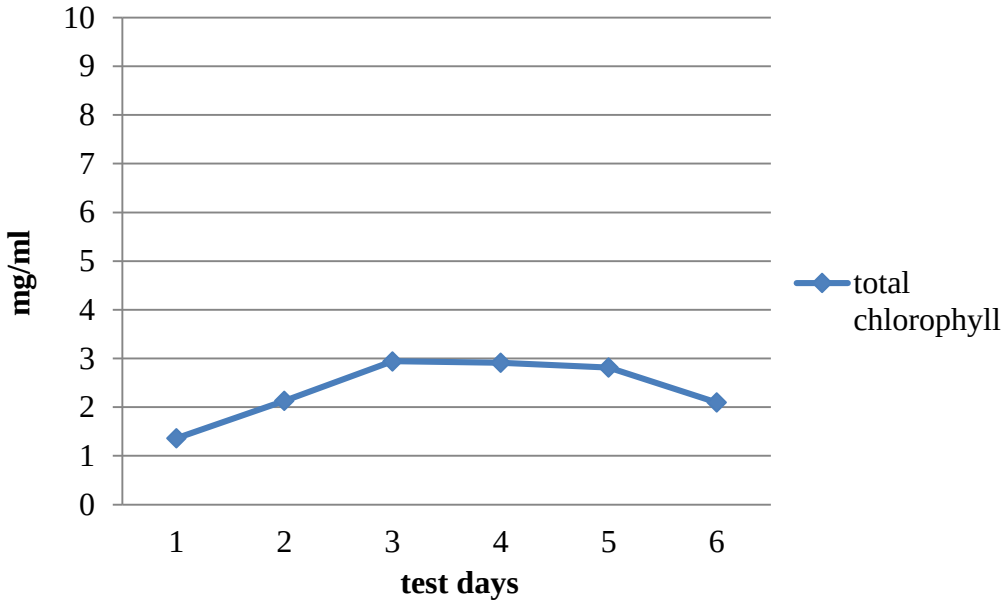

Figure 3. Results of measurement total chlorophyll in test trial

Tabele 1. TPC test results with standard deviation included

Trial	Results	Standard deviation
toxity test	3,7 mg GEA/g	0,010
control	2,4 mgGAE/g	0,003

In order to check the effect of barium chloride on algal cells, an FTIR sample image was taken. The individual files shown in the image correspond to the chemical group. According to the literature about the correct growth should indicate changes in the area 1230-1250 cm^{-1}, because they are vibrations corresponding to phosphorylated protein and nucleic acid [11].

The graph shows an increase in the area of 1300 cm^{-1}, however, it may be the effect of overlapping or shifting the peaks, the increase itself is quite small. After 24 hours the absorbance of both samples was similar, however, changes in the area of 1096-1060 cm^{-1} can be noticed. It is believed that they can advocate for binding in cellular carbohydrates and nucleic acids.

Bands in this respect are also attributed to correlations with changes in DNA. Therefore, it can be presumed that this region plays a special role in the metal's absurdity [12]. This clearly indicates that after 24 hours clerk bar caused stress changes in the cells that may be effect their subsequent metabolism.

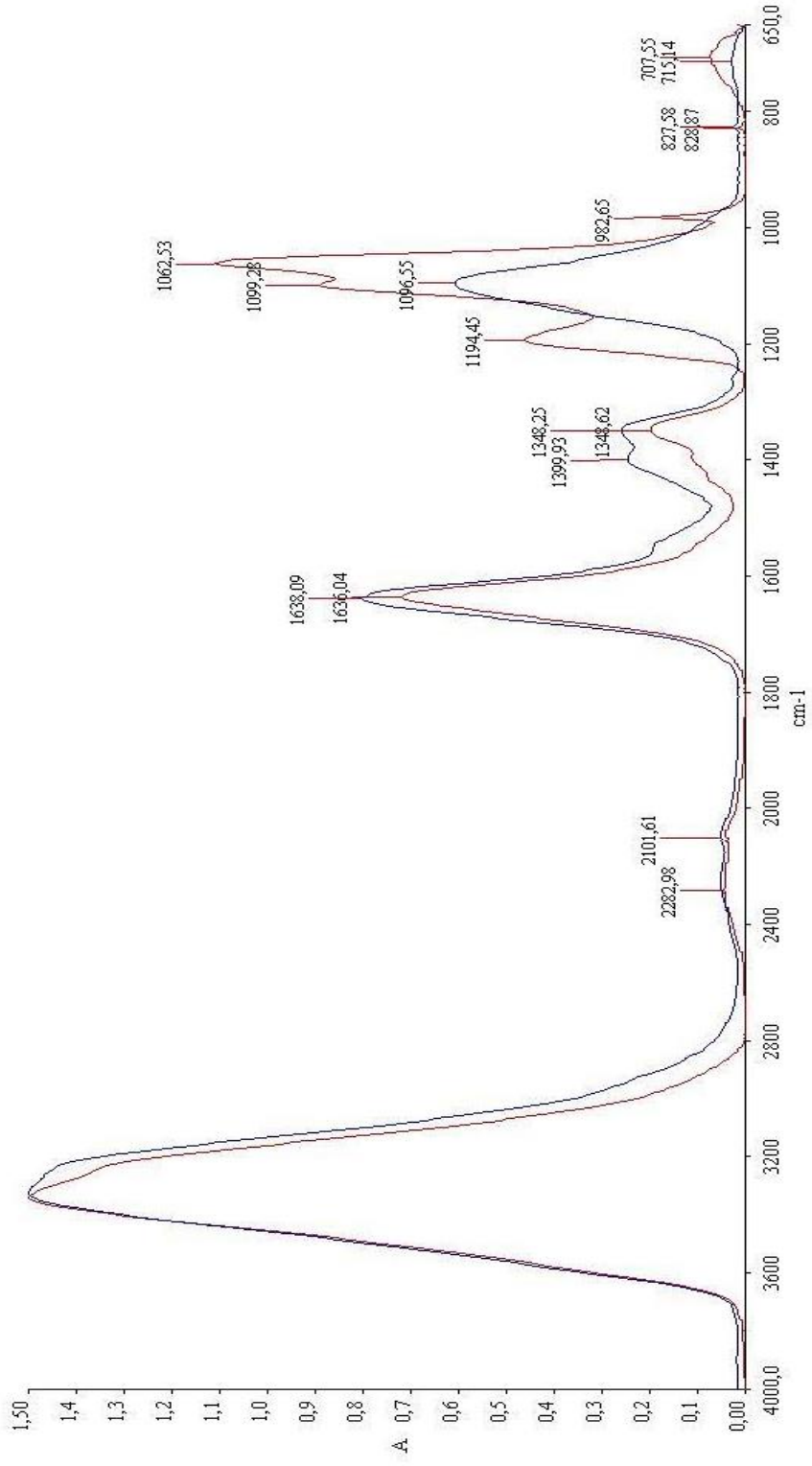

Figure 4. FTIR Analyses model of control trial and test trial. Blue line is control trial, red is test trial.

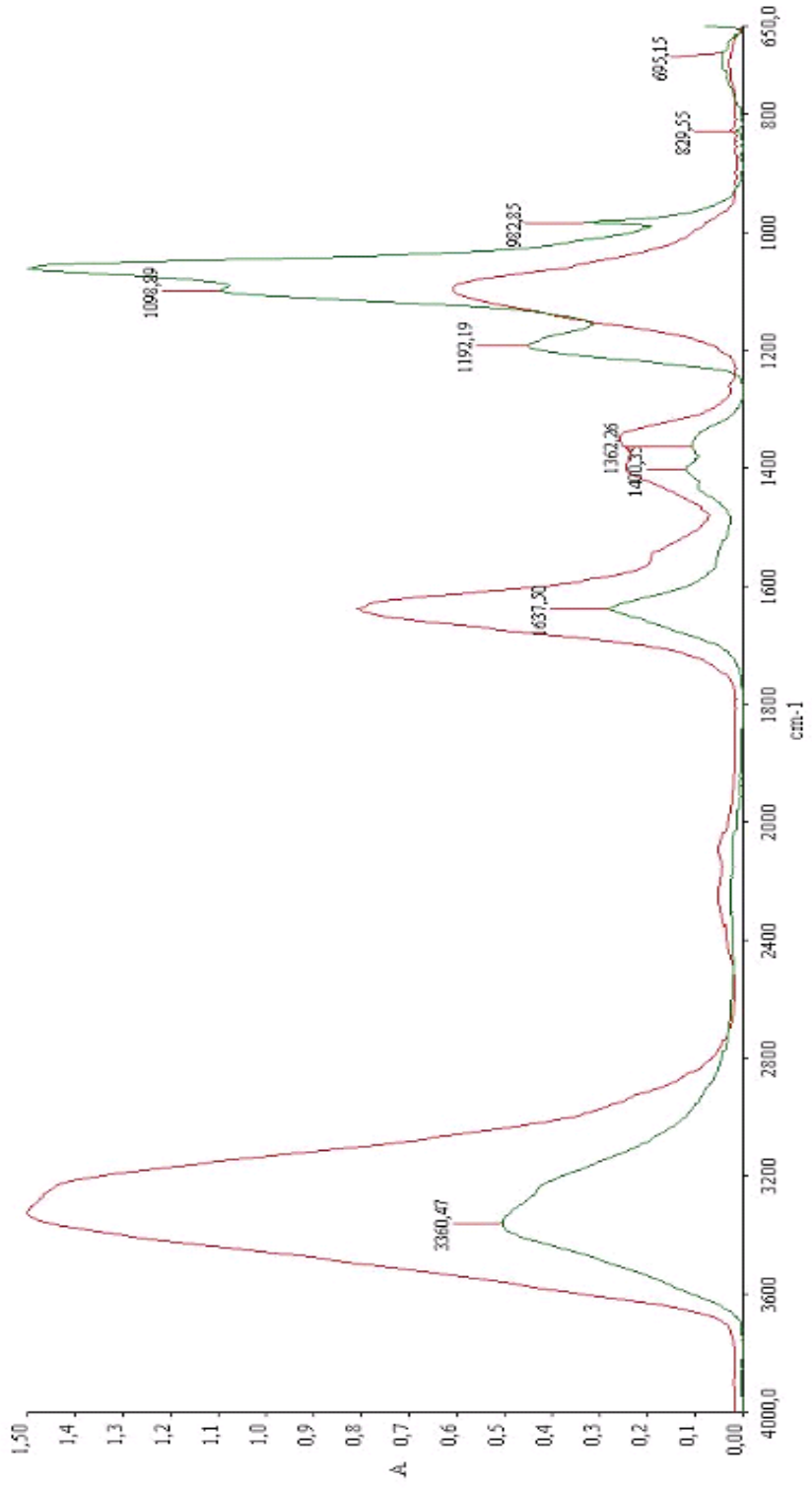

Figure 5. FTIR Analyses model of control trial and test trial after 6 day. Blue line is control trial, red is test trial

After the 6th measurement, the FTIR spectrum showed a marked inhibition of growth relative to the control culture. The intensity of the peaks also increased in the area where changes were noticed after the first measurement. Region from 1600 to 1500 cm^{-1} is specific for amide-II bands, after measurement 6 you can see a clear drop in the peak in this region [12]. This indicates the partial inhibition of protein growth content. A similar effect can be observed in the region of 1400 cm^{-1} and the probable shift towards 1300 cm^{-1} in a farm with the addition of barium chloride. It is interesting, however, that the mechanism of action of barium chloride on live algae includes mainly changes in one part of the spectrum. The mechanism of bar toxicity is not fully understood. It is reported that it impairs the metabolism. According to the study, its action on plant organisms is based on the induction of oxidative stress and the inhibition of photosynthetic dyes production. These observations can be related to algae due to their similarity to plants. As mentioned earlier, the most-changed area is identical to nucleic acids, which can also be condemned to the bar genotoxicity of living organisms. The genotoxicity of barium sulphate in studies using fibroblasts has been described so far [13].

4. CONCLUSIONS

This study shows that barium chloride shows high toxicity to aquatic organisms such as algae. Although in the aquatic environment the bar occurs in the form of compounds such as barite and hematite [14], aquatic organisms are not resistant to water-soluble compounds such as barium chloride. As it can be seen, it subjugates chlorotrophyll degradation, diminishing the density of the cultivation, the growth of polyphenols as a response to the stress factor and changes in the structure that can be attributed to the genotoxic effect. These effects can be attributed to the accumulation of barium in *Nannochloropsis* sp. cells. According to research, after 10 days algae is able to bind over 80% of the bar from the environment [10]. Owing to such properties of algae as the accumulation of antiferogenic impurities in water, there arises interest in the subject of the influence of heavy metals on their development, because as phytoplankton they are food for other marine organisms and are referred to as superfood and sold as a food additive.

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