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GROWTH DYNAMICS OF THE BENTHIC DIATOM *ARDISSONEA CRYSTALLINA* (C. AGARDH) GRUNOW, 1880 (BACILLARIOPHYTA) UNDER COPPER IONS EFFECT

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A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation
E-mail: *el_nevrova@mail.ru*

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Increasing anthropogenic load on coastal ecosystems of the Black Sea determines the need for regular assessing the state of planktonic and benthic communities. Planktonic microalgae contributing up to 20–25 % of global primary production are traditionally used as test objects; however, the contribution of microphytobenthos is comparable to that of phytoplankton. Benthic diatoms are close-associated with bottom substrate, and most of them are highly sensitive to the effect of technogenic pollutants accumulating in sediments. The changes in physiological indicators of benthic Bacillariophyta may objectively reflect the negative effect of various toxicants; accordingly, benthic diatoms can be used as test objects in the indirect assessment of the marine environment quality. We aimed to study the growth dynamics of abundance of clonal strain cells for a new biotesting object – the diatom *Ardissonea crystallina* (C. Agardh) Grunow, 1880 (Bacillariophyta) – under the effect of various $CuSO_4 \cdot 5H_2O$ concentrations during 10-day laboratory experiments. This species is widespread in the Black Sea sublittoral and highly sensitive to the effect of different technogenic pollutants, *inter alia* heavy metals. As shown, at copper ions concentrations of 32–128 $\mu g \cdot L^{-1}$, *A. crystallina* growth dynamics generally corresponds to the dose–response curve in a toxicological experiment. The correlation was found between a decrease in intensity of the culture growth and increase in toxicant concentration in the experimental medium. At copper ions concentration of 256–320 $\mu g \cdot L^{-1}$, the ratio of alive cells in the clonal strain decreases gradually from 62–66 % (the 1st day) to 34–37 % (the 10th day); the indicators of an increase in cell abundance in the clonal strain are characterized by a negative trend – from –0.01 (on the 2nd day) to –0.34 (on the 10th day). At Cu^{2+} concentrations of 384 $\mu g \cdot L^{-1}$ and higher, drastic inhibition and subsequent death of *A. crystallina* cells were revealed. At 448–1,024 $\mu g \cdot L^{-1}$, complete cell mortality was registered already on the 3rd day of the experiment. Statistical comparison of the ratio variability of *A. crystallina* alive cells and the specific growth in their abundance for the control and Cu^{2+} concentrations of 64–128 $\mu g \cdot L^{-1}$ showed as follows: at 32–128 $\mu g \cdot L^{-1}$, the differences between the mean values of the test indicators were significant ($P = 0.002 \ldots 0.020$). At 256 $\mu g \cdot L^{-1}$, the changes in total abundance and alive cells ratio in the test culture significantly differ ($P = 0.002 \ldots 0.014$) from those both at lower and higher copper concentrations. This fact allows to consider the toxicant level of 256 $\mu g \cdot L^{-1}$ as a critical one for *A. crystallina*: its exceeding will result in a sharp increase in cell mortality. Based on the results obtained, this benthic diatom can be recommended for use as a suitable test object in toxicological experiments, as well as for monitoring and indirect environmental assessment of coastal water areas subjected to technogenic pollution.

Keywords: toxicological experiment, copper ions, clonal strain, cell abundance, benthic diatom algae, Black Sea

Increasing anthropogenic load on the Black Sea ecosystem, especially manifested in coastal water area, including off the Crimean coast, determines the need for a regular assessment of current state of planktonic and benthic communities. As one of the objects for biotesting and bioindication, planktonic microalgae are traditionally used, due to relative simplicity of their cultivation and accounting during experiments (Ekologo-toksikologicheskie aspekty, 1985 ; Gelashvili et al., 2015 ; Nevrova, 2015 ; Spirkina et al., 2014). Importantly, the contribution of microphytobenthos (with its up to 99 % abundance and species richness being contributed by Bacillariophyta representatives) to the sea and ocean primary production is comparable to that of phytoplankton which determines up to 20–25 % of global production (Diatoms: Fundamentals and Applications, 2019 ; Kumar et al., 2015 ; The Diatom World, 2011). Along with a high rate of reproduction, benthic diatoms are characterized by adherence to certain microbiotopes and sensitivity to the effect of adverse environmental factors (Markina, 2009 ; Nevrova et al., 2015 ; Romanova et al., 2017). Therefore, a change in physiological parameters of benthic diatoms (cell growth, reproduction, and abundance) with a greater objectivity (compared to planktonic species) reflects the effect of various pollutants, and this allows to use benthic diatoms as convenient test objects in the indirect assessment of the marine environment quality (Anantharaj et al., 2011 ; Florence & Stauber, 1986 ; Gelashvili et al., 2015 ; Markina, 2009 ; Markina & Aizdaicher, 2006, 2007, 2011, 2019 ; Rijstenbil & Gerringa, 2002 ; Romanova et al., 2017 ; The Diatom World, 2011 ; Yan et al., 2014).

Approaches to using benthic diatoms for monitoring coastal water areas are developed insufficiently (Anantharaj et al., 2011 ; Leung et al., 2017 ; Nagajoti et al., 2010) since there are certain difficulties in their clone isolation, cultivation, and accounting during experiments (Nevrova et al., 2015 ; Petrov & Nevrova, 2020 ; Romanova et al., 2017). Researchers obtain new data on tolerance ranges of different marine diatom species when exposed to various toxicants (copper ions, surfactants, pesticides, *etc.*) (Aizdaicher & Reunova, 2002 ; Markina, 2009 ; Markina & Aizdaicher, 2007) and also develop methodological issues. Those include the investigation of clone cultivation peculiarities, determination of criteria for defining alive cells during visual assessment of morphological alterations and photographing, estimation of increase in cell abundance, ratio of alive and dead cells of a test object under different toxicant concentrations in a cultural medium, analysis of absorption of heavy metals by cells, *etc.* (Ahalya et al., 2003 ; Anantharaj et al., 2011 ; Leung et al., 2017 ; Spirkina et al., 2014). Of key importance is also expansion of knowledge on biology in terms of Bacillariophyta taxa development.

Copper sulfate is chosen as a model toxicant for experiments due to significance of copper compounds both in the biogeochemical cycle and hydrobiont metabolism. Copper is an essential trace element; it is actively involved in physiological processes – nitrogen metabolism, antioxidant protection (Cu/Zn superoxide dismutase), electron transfer in the mitochondrial respiratory chain of eucaryotes (cytochrome c oxidase), *etc.* (Gelashvili et al., 2015 ; Miazek et al., 2015 ; Smolyakov et al., 2010). Copper compounds are found in the Earth's crust in mass and form about 250 minerals; those are the most common technogenic pollutant both in aquatic environment and bottom sediments (Gelashvili et al., 2015 ; Smolyakov et al., 2010), including in the Black Sea coastal waters (Nevrova et al., 2015). Along with mercury ions, copper ions (Cu^{2+}) belong to the most environmentally hazardous substances; in increased concentrations, those become acutely toxic for most marine and freshwater hydrobionts (Ekologo-toksikologicheskie aspekty, 1985 ; Gelashvili et al., 2015).

An investigation of toxicological impact of Cu^{2+} in $CuSO_4$ on aquatic plants and planktonic Bacillariophyta revealed a growth inhibition at a concentration of copper compounds of about 0.1 $\mu g \cdot L^{-1}$ (Gelashvili et al., 2015). Moreover, the effect was studied of copper sulfate at different concentrations on cells of planktonic forms of *Cylindrotheca closterium* (Ehrenberg) Reimann et Lewin, 1964, *Ditylum brightwellii* (West) Grunow ex Van Heurck emend. Dzhafarova, 1984, *Phaeodactylum tricornutum* Bohlin, 1897, and *Thalassiosira oceanica* Hasle, 1983 (Ahalya et al., 2003 ; Cid et al., 1995 ; Florence & Stauber, 1986 ; Kim & Price, 2017 ; Markina & Aizdaicher, 2006, 2011, 2019 ; Rijstenbil & Gerringa, 2002 ; Yan et al., 2014). Obtained results allowed suggesting a pronounced species-specific threshold resistance of diatom algae to copper ions.

Importantly, the value of copper maximum permissible concentration accepted for seawater is 5 $\mu g \cdot L^{-1}$ despite the fact that copper content in marine coastal areas can reach 50–100 $\mu g \cdot L^{-1}$ (Markina & Aizdaicher, 2019). In our opinion, for benthic diatoms, copper content in a water column is less ecologically significant than in bottom sediments: pollutants are accumulated in them while microalgae cells inhabit the surface of substrate particles in motile or attached form. As known, in silty sediments of the Black Sea coastal water areas, copper content can be of 0.4–11.2 $\mu g \cdot g^{-1}$ (Ovsyaniy et al., 2003); in technogenically polluted bays, concentrations can reach 20 and even 37 $\mu g \cdot g^{-1}$ of dry sediment (Burgess et al., 2009 ; Petrov & Nevrova, 2003 ; Petrov et al., 2005). As found, copper ions, along with ions of other heavy metals accumulated in soft bottom sediments, are the key factors for heavily polluted biotopes and affect both taxocene structure and spatial distribution of benthic diatoms (Petrov & Nevrova, 2003, 2004 ; Petrov et al., 2005).

Ardissonea crystallina (C. Agardh) Grunow, 1880 was chosen as a model object for the experiment for several reasons. The species cells are large, and this facilitates both their accounting during photoregistering and assessment of a vital state. Moreover, the species is easy to cultivate and is characterized by high vegetative reproduction rate and ability to form attached colonies. Earlier, *A. crystallina* was transferred from the class Fragilariophyceae to Coscinodiscophycea, and then to Mediophyceae; based on the results of molecular genetic analysis and sexual reproduction experiments, the taxonomic position of the species has been questioned. Apparently, *Ardissonea* (and other Toxariales representatives) can form a unique evolutionary group which is separated from other pennate diatoms and is characterized by an unusual way of sexual reproduction (Davidovich et al., 2017). Recently, some *Ardissonea* species were transferred into other genera; specifically, *Ardissonea crystallina* was transferred to *Synedrosphenia crystallina* (C. Agardh) Lobban & Ashworth comb. nov. (genus *Synedrosphenia* (H. Peragallo ex H. Peragallo et M. Peragallo, 1897–1908) Azpeitia, 1911, emend. Lobban & Ashworth) (Lobban et al., 2022). Nevertheless, taking into account the stick-shaped valve and the ability to form bunch-like colonies attached to substrate, as well as reckoning with the taxonomic system (Round et al., 1990), for our aims, we consider this species as belonging to Fragilariophyceae (until recently, it was referred to this class). Importantly, the present study continues a series of experiments focused on revealing species-specific tolerance of benthic diatoms representing three different Bacillariophyta classes – with their inherent morphological peculiarities and various lifestyle forms. Previously, we statistically confirmed the significance of selective assessment of cell distribution of diatom species belonging to three classes, including *A. crystallina*, in an experimental vessel (Petrov & Nevrova, 2020).

The aim of the work is to reveal the dynamics of growth and cell mortality of marine benthic diatom *Ardissonea crystallina* during 10-day experiments under a wide range of toxicant (copper ions) concentrations in the cultural medium and to evaluate the applicability of this species as a test object new for ecotoxicology.

MATERIAL AND METHODS

Study object. As a test object, *A. crystallina* clonal strain (Bacillariophyta) was used. The diatom cells were sampled from phytoperiphyton of an artificial substrate sampled in the Kazachya Bay (Sevastopol water area) in November 2018 at a 5-m depth. To obtain a clone line, a single cell was isolated with a micropipette under an MBS-10 binocular at ×40 magnification and rinsed 7 times with the medium (Gaisina et al., 2008 ; Petrov & Nevrova, 2020 ; Romanova et al., 2017). This marine benthic species occurs frequently in coastal areas; its cells are attached to substrate surface and form bunch-like colonies of 4–30 cells (Petrov & Nevrova, 2020). Valves are narrow-linear, 410 μm long, and 18 μm wide (see 1–6 in Fig. 1). Cell sizes are indicated as at the time of the beginning of cultivation.

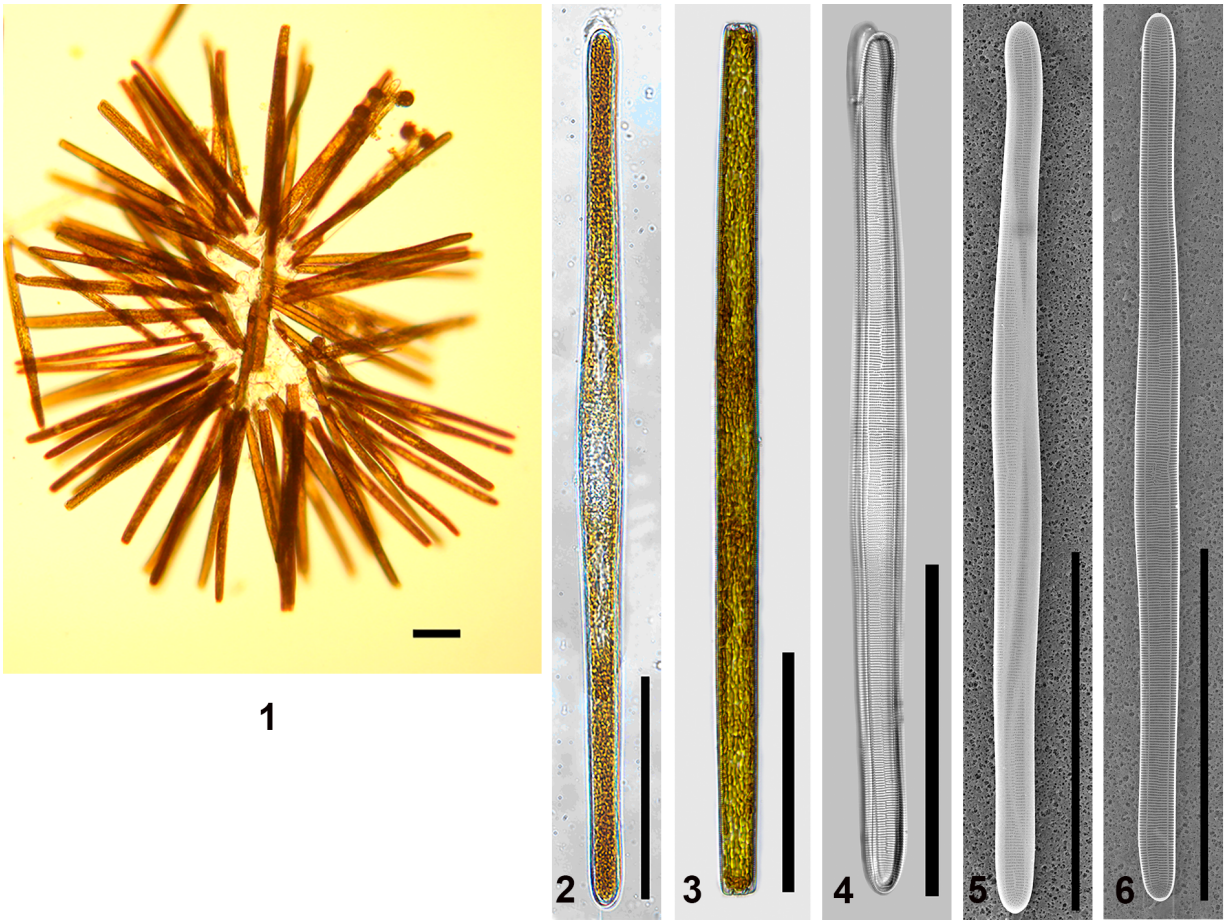

Fig. 1. Marine benthic diatom *Ardissonea crystallina* used in the experiment as a new test object: 1, colony of alive cells (LM, ×10, scale bar 10 μm); 2, alive cell, valve view (LM, ×60, scale bar 100 μm); 3, alive cell, band view (LM, ×60, scale bar 100 μm); 4, valve external view (LM, ×100, scale bar 100 μm); 5, valve external view (SEM, ×550, scale bar 100 μm); 6, valve internal view (SEM, ×550, scale bar 100 μm) (LM denotes light microscope; SEM, scanning electron microscope)

Cultivation. *A. crystallina* clone strain was cultured on a natural seawater medium by Goldberg (Andersen et al., 2005) modified for marine benthic diatoms (Petrov & Nevrova, 2020), at a constant temperature of (15 ± 2) °C, under scattered natural light. Seawater for the medium was sampled in a 12-mile zone off Crimean coast during research cruises of the RV "Professor Vodyanitsky", filtered through a 0.45-μm filter, and pasteurized three times at +75 °C; then, the medium was enriched with nutrients according to the protocol (Petrov & Nevrova, 2020).

Microphotography. During the experiment, alive cells were photoregistered with a Carl Zeiss Axiostar Plus light microscope equipped with an Achroplan ×10 objective and Canon PowerShot A640 camera (IBSS benthos ecology department). For taxonomic identification, micrographs of alive and cleaned diatom valves were taken under an inverted Nikon Eclipse Ts2R light microscope equipped with a Plan Fluor ×60 OFN25 DIC objective and an Infinity3-6UR camera, under a Carl Zeiss Primostar Plus light microscope with an N-Achroplan ×100 objective and an integrated camera (IBSS laboratory of biodiversity and functional genomics of the World Ocean), and under a Hitachi SU3500 scanning electron microscope. The species was identified according to the guide books (Guslyakov et al., 1992 ; Witkowski et al., 2000).

Experimental design. A stock solution was prepared with $CuSO_4 \cdot 5H_2O$ concentration of 40,000 $\mu g \cdot L^{-1}$ (10,240 $\mu g \cdot L^{-1}$ in terms of Cu^{2+} ions). For the experiments, a certain volume of the natural seawater medium, $CuSO_4 \cdot 5H_2O$ stock solution, and 1 mL of *A. crystallina* clonal strain inoculum were added to each 90-mm Petri dish with a micropipette dispenser so that the total volume of liquid in each dish was of 30 mL. To obtain test solutions with increasing copper ions concentrations (from 32 to 1,024 $\mu g \cdot L^{-1}$), different aliquots of stock solution (from 0.09 to 3 mL) were added to Petri dishes (Table 1). The effect of each copper ions concentration was analyzed in triplicate. All the experiments lasted for 10 days. Petri dishes were sealed with Parafilm® to avoid contamination or evaporation of the test solution.

Table 1. Design of the experimental study of copper sulfate ($CuSO_4 \cdot 5H_2O$) effect on the growth of *A. crystallina* clonal strain

$CuSO_4 \cdot 5H_2O$ concentration in the test solution, $\mu g \cdot L^{-1}$	Cu^{2+} concentration in the test solution, $\mu g \cdot L^{-1}$	Volume of the added stock solution, mL	Volume of the medium, mL	Volume of the inoculum, mL
Control	0	0	29.00	1
125	32	0.09	28.91	1
250	64	0.19	28.81	1
500	128	0.38	28.62	1
1,000	256	0.75	28.25	1
1,250	320	0.94	28.06	1
1,500	384	1.13	27.87	1
1,750	448	1.31	27.69	1
2,000	512	1.50	27.50	1
4,000	1,024	3.00	26.00	1

We do not know any data on *A. crystallina* use in biotesting practice, peculiarities of a long-term cultivation, levels of resistance of cells of this species to copper impact, and maximum permissible concentrations of copper and other metals for marine bottom sediments. Therefore, to reveal the ranges

of critical Cu^{2+} concentration, the experiment was carried out in two successive stages. At the first stage, in addition to the control (the natural seawater medium by Goldberg with no toxicant), *A. crystallina* was tested by doubly increasing toxicant concentrations – 32, 64, 128, 256, 512, and 1,024 μg·L^{-1} (in terms of Cu^{2+} ions).

According to results of the first stage, threshold concentrations of copper ions, at which a sharp cell elimination in the clonal strain begins, are in the range of 256–512 μg·L^{-1}. In this regard, the second stage of the experiment was carried out: intermediate Cu^{2+} concentrations (320, 384, and 448 μg·L^{-1}) were tested. Response to the toxicant effect on the clonal strain was assessed by the change in a ratio of alive cells (%). All the experiments were carried out in triplicate for each concentration and exposure time (in 1, 3, 5, 7, and 10 days). Cells were counted, and their vital state was assessed visually, on micrographs. A cell was defined as an alive one by valve shape and integrity, invariability of chloroplast structure and color, and divergence of cells after vegetative division. In case of cytoplasmic lysis, sharp chloroplast darkening, and valve destruction, a cell was defined as a dead one. Abundance of alive and dead cells at each exposure time was calculated from averaged data obtained in 12–16 random viewing fields which were photographed in experimental Petri dishes with a bottom area of about 5,700 mm^2. For all tested copper ions concentrations, both ratio of alive cells and growth (mortality) rate in cell abundance (cells·day^{-1}) were determined; in toxicology, the second indicator is one of the basic since it allows assessing the state of microalgae populations (Filenko et al., 2006 ; Markina & Aizdaicher, 2007, 2019 ; Spirkina et al., 2014). The rate of cell growth for the culture was determined by a number of cell divisions (ν) *per* day and calculated by the formula (Schlegel, 1987):

$$\nu = \frac{\ln N_{(t+\Delta t)} - \ln N_t}{\Delta t \times \ln 2}, \quad (1)$$

where N_t denotes mean cell abundance in the culture at time t (the 1st day of the experiment);

$N_{(t+\Delta_t)}$ denotes mean cell abundance in the culture at time t + Δt (the 3rd, 5th, 7th, and 10th days);

Δt denotes exposure time (days).

Statistical data processing. The experimental results were statistically processed using standard routines of parametric and rank analysis of the statistical software package SigmaPlot 11.5 (2021).

For three independent replications for each toxicant concentration, variances were compared by the Fisher's test (ANOVA) for a significance level $P = 0.05$. Significance of differences in mean values of abundance and ratio of alive cells, as well as rate of cell growth at different exposure time were compared by Student's *t*-test – in case of normality of distribution mode and variances equality. To compare independent samples, with distribution mode differing from normal, the nonparametric Mann–Whitney *U* test, Holm–Šidák test (for equal sample sizes), and Dunn's test (for different sample sizes) were applied (SigmaPlot NG, 2021). The mean values of the indicators and standard errors of the sample (*SE*) are given in Fig. 2 and in Tables 2 and 3.

RESULTS AND DISCUSSION

At copper concentrations in experimental dishes from 32 to 128 μg·L^{-1} (in terms of Cu^{2+} ions), the change in the ratio of alive cells and an increase in *A. crystallina* abundance generally correspond to the dose–response curve in a toxicological experiment (Gelashvili et al., 2015). On the 1st day, for dishes with various Cu^{2+} ions concentrations, there were no statistically significant

differences (P = 0.30…0.39) between the mean values of the indicator (the ratio of alive cells in 12–16 viewing fields amounted to 56–60 %). After the 1st day of the experiment, for copper ions concentrations of 32–128 μg·L^{-1}, a short period of cell adaptation was observed (for the control and at minimum Cu^{2+} concentration of 32 μg·L^{-1}), or the lack of a lag phase was registered. On the 3rd–7th days, an increase in the indicator values and reaching a plateau were revealed; on the 7th–10th days, a decrease in the values was recorded (Fig. 2, Table 2). From the 3rd to the 7th day (according to the mean values from viewing fields), no statistically significant effect (P = 0.18…0.93) of various toxicant concentrations was registered on an increase in abundance and ratio of alive cells. Significant decrease (P = 0.002…0.020) in the values of test indicators was noted on the 7th–10th days of the experiment; this can be directly caused by the negative effect of high toxicant concentrations in the experimental medium.

Table 2. Ratio (%) of *A. crystallina* alive cells (mean ± *SE*) at different toxicant concentrations (in terms of Cu^{2+} ions) and exposure time (results of the first and second stages are combined)

Cu^{2+} concentration, μg·L^{-1}	1st day	3rd day	5th day	7th day	10th day	P (7th day *vs.* 10th day)
Control	63 ± 3	67 ± 2	85 ± 2	84 ± 2	76 ± 3	0.071
32	58 ± 1	75 ± 4	86 ± 4	84 ± 1	81 ± 3	0.248
64	60 ± 6	79 ± 2	86 ± 1	80 ± 1	67 ± 4	**0.002**
128	60 ± 2	83 ± 2	83 ± 2	79 ± 4	65 ± 10	**0.003**
256	56 ± 2	77 ± 6	79 ± 1	77 ± 3	60 ± 1	**0.017**
320	58 ± 2	49 ± 1	47 ± 2	44 ± 1	46 ± 5	0.398
384	30 ± 5	22 ± 3	8 ± 1	0.8 ± 0.8	0.6 ± 0.6	0.412
448	21 ± 5	0.6 ± 0.4	0	1.0 ± 0.6	0.5 ± 0.5	–
512	13 ± 3	0.3 ± 0.1	0	0	0	–
1,024	3.6 ± 1	0.2 ± 0.1	0	0	0	–

Note: P is significance level of no differences between the mean values of the indicator when comparing the ratio of alive cells on the 7th and 10th days of the experiment. Statistically significant differences are highlighted in bold.

At copper ions concentrations of 256 μg·L^{-1} and higher, the ratio of alive cells in the clonal strain at all the stages of the experiment was significantly lower (P = 0.002…0.014) starting from the 3rd day than at 32–128 μg·L^{-1}. At 256–320 μg·L^{-1}, the ratio of alive cells decreased monotonically from 47–49 % (on the 3rd day) to 34–37 % (on the 10th day) – with no pronounced inflections in the model response curve. The critical toxicant concentration found during the experiments (256 μg·L^{-1}) can be considered as a threshold: upon reaching it, there is a statistically significant inhibition of growth activity and physiological state of the clonal strain.

At copper ions concentrations of 384 μg·L^{-1} and higher, a drastic inhibition of the clonal strain in dishes was observed already on the 1st day of the experiment. On the 5th day, the ratio of alive cells decreased to zero. For concentrations of 448–1,024 μg·L^{-1}, almost complete cell mortality was registered already on the 3rd day of the experiment (see Fig. 2, Table 2).

When determining cells abundance in viewing fields, the data variability differed much during the experiment. Specifically, coefficient of variation in *A. crystallina* samples during the 1st day of the experiment was up to 46 %; in 5 days, the value was 31 %. This fact could be due to uneven

distribution in viewing fields when, along with single cells, there are aggregations of bunch-like colonies attached to dish bottom at one spot (see 1 in Fig. 1). The results of analysis showed that the variances of samples, when comparing three replicates, did not differ statistically: $P = 0.25…0.28$ (in 1 day) and $P = 0.09…0.23$ (in 5 days). All pairwise differences in mean abundance of *A. crystallina* cells between replicates both on the 1st and the 5th day of exposure are insignificant ($P_{exp} >> 0.05$). Apparently, variability of mean cell abundance in different replicates does not exceed the statistical error, and this allows to consider all the replicates (random cell samples) as belonging to one initially taken population with a similar variability of indicators (Petrov & Nevrova, 2020). This fact is of key importance for a valid comparison of differences in absolute cell abundance in dishes at different stages of the experiment and at different toxicant concentrations.

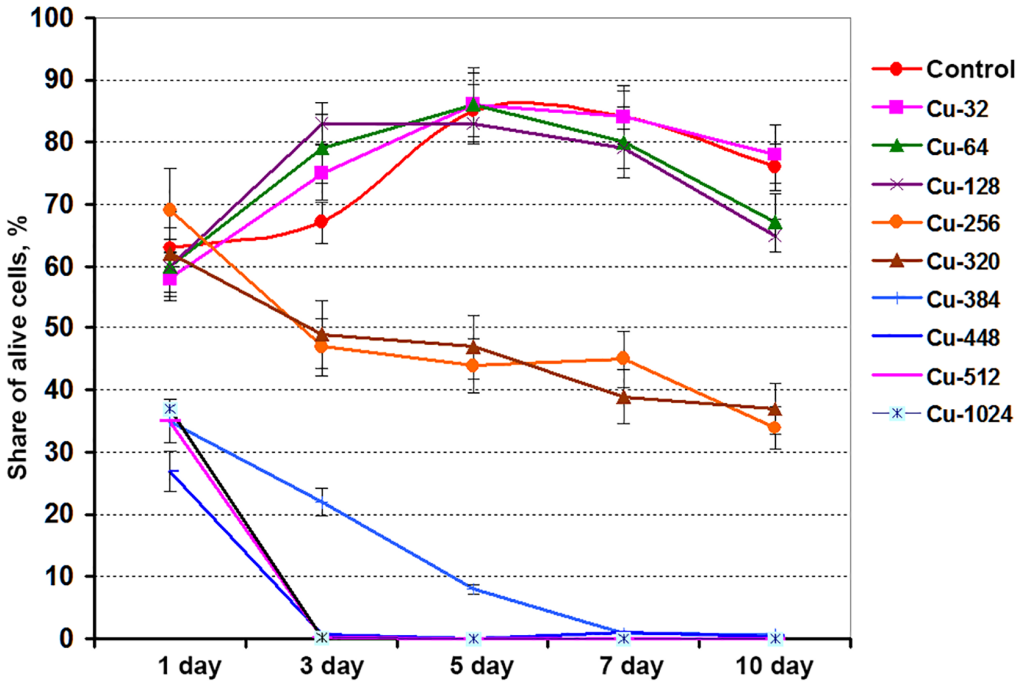

Fig. 2. Changes in the ratio (%) of alive cells (mean ± *SE*) in *A. crystallina* clonal strain during the experiment at different toxicant concentrations (in terms of Cu^{2+} ions)

One more indicator for assessing the toxic effect of copper on *A. crystallina* strain is the mean number of vegetative cell divisions *per* day (v). Comparison of mean values of specific cell growth in clonal strain at different toxicant concentrations is given in Table 3. At the first three days, the culture tends to have a positive increase at 32–128 μg·L^{-1}. Importantly, along with a rise in toxicant concentrations, the daily growth rate rose; the highest specific increase was revealed at 128 μg·L^{-1}. Apparently, at this concentration, primary stimulation by copper ions of cell growth and division occurs (Filenko et al., 2006). Specifically, compared with the control, the rate of *A. crystallina* cell division rose 18 times (from 0.17 to 3.0 divisions *per* day); already at 256 μg·L^{-1}, it slowed down to almost zero. At toxicant concentrations of 320 μg·L^{-1} and higher, an increase in cell abundance became negative, and a rate of cell mortality rose (see Table 3). Importantly, in the control, the specific growth rate first increased (up to 0.42 divisions on the 7th day) and then decreased (down to 0.21 divisions on the 10th day); there was a noticeable

cell mortality in the strain. In general, an increase in cell abundance was recorded up to the 5th day of the experiment at 32–128 μg·L^{-1}. At higher copper ions concentrations and longer exposure time, negative specific growth rate was registered in the test culture.

Our experiments revealed higher resistance of benthic diatom *A. crystallina* to the impact of copper compared to species of planktonic microalgae. Specifically, as recorded in the study on copper chloride exposure on survival and reproduction of *Scenedesmus quadricauda* (Turpin) Brébisson, 1835 (Filenko et al., 2006), a noticeable decrease in total abundance and ratio of alive cells occurs already at copper concentration of 10–100 μg·L^{-1} at the exponential phase (10th–14th days). At the same time, after 14 days of the experiment, the ratio of actively dividing cells did not exceed 10 % already at 1–10 μg·L^{-1}, and the remaining part of strain was at the resting stage and did not affect the growth rate in a test culture. When investigating the effect of copper ions concentrations of 50–100 μg·L^{-1} on another planktonic microalga, *Porphyridium purpureum* (Bory) K. M. Drew & R. Ross, 1965 (Markina & Aizdaicher, 2019), a pronounced inhibition of its growth and a decrease in photosynthetic pigments content as compared to the control were recorded in cells already on the 4th day. As revealed, heavy metals (copper and cadmium) inhibit cell growth and can damage the cell membrane; this results in a significant decrease in chlorophyll pigments in the benthic diatom *Amphora coffeaeformis* (C. Agardh) Kützing, 1844 at copper ions concentrations of 0.02–10 μg·L^{-1} (Anantharaj et al., 2011).

Table 3. Comparison of the specific growth in *A. crystallina* cell abundance (v, cells·day^{-1}) at different stages of experiment and different toxicant concentrations (in terms of Cu^{2+} ions)

Cu^{2+} concentration	Exposure time			
	1st–3rd days	3rd–5th days	5th–7th days	7th–10th days
Control	**0.17 ± 0.04**	**0.27 ± 0.07**	**0.42 ± 0.10**	**0.21 ± 0.06**
32	**1.15 ± 0.11**	**0.30 ± 0.03**	**0.03 ± 0.01**	–0.03
64	**1.60 ± 0.05**	**0.11 ± 0.01**	–0.12	–0.13
128	**3.00 ± 0.32**	**0.06 ± 0.01**	–0.13	–0.11
256	–0.01	–0.10	–0.19	–0.04
320	–0.02	–0.11	–0.25	–0.34
384	–0.04	–0.32	–0.47	
448	–0.48	–0.50		
512	–0.49	–0.50		
1,024	–0.47			

Note: values of positive increase in cell abundance are highlighted in bold.

An inhibition of growth and physiological state of *A. crystallina* and cells of other microalgae species can be associated with the negative effect of copper ions on the photosynthetic apparatus and with damage to chloroplast membranes which are involved in synthesis of amino acids and phytohormones affecting population growth (Kiseleva et al., 2012), as well as with the suppression of vegetative cell reproduction (Filenko et al., 2006). Apparently, higher resistance to the toxic effect of copper on benthic diatom *A. crystallina*, compared to planktonic species, is related to the presence of a thick silicified single-wall valve with pseudosepta; its complex areoles system provides contact with marine environment

but does not allow penetration of microparticles into the cell (Lobban et al., 2022 ; The Diatom World, 2011). Such morphological peculiarities ensure sustainable development of benthic diatoms on soft bottom, with its levels of copper content being much higher than in a water column. Moreover, diatoms have a unique ability to bioaccumulate heavy metals up to values tens of thousands higher than those in the environment. Increased copper content in bottom sediments can also cause additional secretion of polysaccharide mucus in diatoms which is one of universal mechanisms for the detoxification of heavy metals, *inter alia* copper (Crespo et al., 2013 ; Miazek et al., 2015). It seems important to continue research on assessing the state of benthic diatoms to determine species-specific threshold concentrations of toxicants.

Conclusion. During 10-day toxicological experiments with a new test object, marine benthic diatom *Ardissonea crystallina*, various types of response to copper exposure were analyzed (changes in total cell abundance, ratio of alive cells, and specific population growth rate) at copper ions concentrations in the range of 32–1,024 $\mu g \cdot L^{-1}$. At Cu^{2+} concentrations from 32 to 128 $\mu g \cdot L^{-1}$, the dynamics of specific increase in abundance and survival of *A. crystallina* cells generally corresponds to the sigmoid dose–response curve in a toxicological experiment; specifically, there are a minimum period of a lag phase (the 1st day of the experiment), reaching a plateau (on the 3rd–7th days), and pronounced negative specific growth in cell abundance (from 7th to 10th day). As revealed, an increase in toxicant concentration results in a significant decrease in the ratio of alive cells and in specific growth in the test culture. For the first time, the threshold concentration was established (256 $\mu g \cdot L^{-1}$ in terms of Cu^{2+} ions): upon its reaching, a statistically significant inhibition of growth activity and physiological state of the test culture were recorded. This threshold concentration is significantly (3–10 times) higher than experimentally obtained threshold concentrations of copper which are critical for survival and growth of some planktonic microalgae. At 384 $\mu g \cdot L^{-1}$ and higher concentrations, a drastic inhibition and subsequent mortality of *A. crystallina* cells occurred already at the 1st day of the experiment; on the 5th–7th days, cell mortality was of 100 %. At 448–1,024 $\mu g \cdot L^{-1}$, complete cell mortality was registered already on the 3rd day of the experiment.

Higher resistance of *A. crystallina* to the toxic effect of copper compared to planktonic species seems to result from morphological peculiarities of the valve which ensures sustainable development of benthic diatoms on soft bottom where copper content is much higher than in a water column. Obtained results allowed to recommend *A. crystallina* as a new test object for toxicological experiments with heavy metals, as well as for ecological monitoring in coastal marine areas under high technogenic pollution.

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ДИНАМИКА РОСТА БЕНТОСНОЙ ДИАТОМОВОЙ ВОДОРОСЛИ *ARDISSONEA CRYSTALLINA* (C. AGARDH) GRUNOW, 1880 (BACILLARIOPHYTA) ПРИ ВОЗДЕЙСТВИИ ИОНОВ МЕДИ

Е. Л. Неврова, А. Н. Петров

ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН»,
Севастополь, Российская Федерация
E-mail: *el_nevrova@mail.ru*

Увеличение антропогенной нагрузки на прибрежные экосистемы Чёрного моря определяет необходимость постоянной оценки состояния сообществ планктона и бентоса. В качестве тест-объектов традиционно используют планктонные диатомовые микроводоросли, вносящие до 20–25 % глобальной первичной продукции, между тем как вклад микрофитобентоса сопоставим по своей значимости. Диатомовые бентоса обладают высокой чувствительностью к влиянию техногенных поллютантов, накапливающихся в донных отложениях. Изменение физиологических параметров бентосных Bacillariophyta объективно отражает воздействие различных токсикантов, что позволяет применять их как тест-объекты при опосредованной оценке качества морской среды. Целью работы было изучить динамику численности клеток клоновой культуры нового для практики биотестирования вида морской микроводоросли *Ardissonea crystallina* (C. Agardh) Grunow, 1880 (Bacillariophyta) при воздействии разных концентраций $CuSO_4 \cdot 5H_2O$ в течение 10 суток. Данный вид микроводорослей характеризуется широкой встречаемостью в сублиторали Чёрного моря и высокой чувствительностью к различным техногенным поллютантам, включая тяжёлые металлы. Показано, что при концентрациях токсиканта от 32 до 128 мкг·л$^{-1}$ (в пересчёте на ионы Cu^{2+}) динамика роста *A. crystallina* в целом соответствует кривой отклика тест-объекта в токсикологическом эксперименте. Выявлено снижение интенсивности прироста культуры и возрастание концентраций токсиканта в экспериментальной среде. При концентрациях ионов меди в диапазоне от 256 до 320 мкг·л$^{-1}$ доля живых клеток в культуре монотонно уменьшается от 62–66 % (1-е сутки) до 34–37 % (10-е сутки); показатели прироста численности клеток в культуре демонстрируют отрицательную динамику в течение опыта — от –0,01 (на 2-е сутки) до –0,34 (на 10-е сутки). При концентрациях в культуральной среде ионов Cu^{2+} 384 мкг·л$^{-1}$ и выше происходило резкое угнетение и последующее отмирание

клеток *A. crystallina*, а для 448–1024 мкг·л$^{-1}$ отмирание 100 % клеток отмечено уже на 3-и сутки эксперимента. Статистическое сравнение вариативности доли живых клеток *A. crystallina* и показателей удельного прироста их численности для контроля и концентраций ионов меди 64–128 мкг·л$^{-1}$ продемонстрировало, что только на 10-е сутки различия между средними значениями параметров достоверны (P = 0,002…0,020). Изменение общей численности и доли живых клеток в культуре при 256 мкг·л$^{-1}$ достоверно отличается (P = 0,002…0,014) от такового как при меньших, так и при более высоких концентрациях, что позволяет рассматривать этот уровень токсиканта как критический для обитания данного вида диатомовой водоросли: его превышение приводит к резкому усилению процесса отмирания клеток. С учётом полученных результатов вид *A. crystallina* может быть рекомендован для широкого использования в качестве тест-объекта в токсикологических экспериментах, а также при экологическом мониторинге и опосредованной оценке состояния прибрежных морских акваторий, подверженных техногенному загрязнению.

Ключевые слова: токсикологический эксперимент, ионы меди, клоновая культура, численность клеток, бентосные диатомовые водоросли, Чёрное море