

Allelopathic activity of the three strains of Baltic picocyanobacterium *Synechococcus* sp. on selected algae and cyanobacteria

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Abstract: The article describes the identification of human errors in the real work during the repair of an electric motor in power plant. These processes are managed by internal regulations, technological procedures and the implementation step process in which human factor contributes. The partner control method for managed activity has been assessed to observe the principles of work safety by an impartial observer. The aim was to identify human error factor. Coaching and observation were used as tools to identify the deviations from the standard procedure when repairing the electric engine. Human factor errors were identified. The measures for applications of the tools to prevent human errors were set up on retraining and self-perception of the incorrect procedures of the employees due to objective and subjective reasons. The implementation of methods of the effective coaching and the control from the management are the measures in the area of nuclear power stations and, thus, they lead to achieve desirable behaviour change and awareness of the personal responsibility for quality and safe work performed by the staff. Allelopathic compounds affect competition between species, structure of phytoplankton and may be a strategy for some species that allows them to survive and expand. The main aim of this work was to investigate the allelopathic effect off picocyanobacteria *Synechococcus* strains BA-120, BA-124 and BA-132 on growth, fluorescence parameter: the maximum quantum yield of PSII photochemistry (F_v/F_m) and pigments content: chlorophyll *a* and carotenoids of *Nostoc* sp., *Amphora coffeaeformis* and *Chlorella* sp. The results of this study demonstrated that picocyanobacteria caused allelopathic effects on mentioned species. It was noted that addition of cell-free filtrate from *Synechococcus* strains BA-120, BA-124 and BA-132 decreased the number of cells of *Nostoc* sp., *A. coffeaeformis* and *Chlorella* sp. Furthermore, it was found, that picocyanobacteria significantly decrease fluorescence parameter F_v/F_m and chlorophyll *a* and carotenoid content of these species. Results of this experiment may provide further information about allelopathic interactions between picocyanobacteria and other co-existing phytoplankton species in the Baltic Sea.

Key words: allelopathy; picocyanobacteria; diatom; green alga; cyanobacterium; growth; fluorescence; Baltic Sea

Abstrakt/ Streszczenie: Związki allelopatyczne mogą wpływać na konkurencję między gatunkami, skład fitoplanktonu oraz stanowić strategię niektórych gatunków pozwalającą im na przeżycie lub nawet na masową ekspansję. Głównym celem niniejszej pracy było zbadanie efektu allelopatycznego oddziaływania trzech szczepów pikoplanktonowej sinicy *Synechococcus* sp. na wzrost, maksymalną wydajność kwantową fotosystemu II (F_v/F_m) i zawartość barwników fotosyntetycznych w odniesieniu do sinicy nitkowatej *Nostoc* sp., okrzemki *Amphora coffeaeformis* i zielenicy *Chlorella* sp. Wyniki przedstawione w tej pracy pozwalają stwierdzić, że szczepy pikoplanktonowej sinicy prezentują oddziaływanie allelopatyczne na testowane gatunki. Stwierdzono bowiem, że dodanie przesączu z hodowli szczepów *Synechococcus* sp. BA-120, BA-124 i BA-132 zmniejszyło liczbę komórek w hodowlach *Nostoc* sp., *A. coffeaeformis* i *Chlorella* sp. Ponadto wykazano, że pikoplanktonowe sinice w znaczącym stopniu obniżają wartość parametru fluorescencji F_v/F_m i zawartość chlorofilu *a* oraz barwników karotenoidowych w komórkach testowanych gatunków. Uzyskane wyniki dostarczają nowych informacji na temat allelopatycznych interakcji między pikoplanktonowymi sinicami a innymi gatunkami budującymi fitoplankton w Morzu Bałtyckim.

Klíčové slová/ Słowa kluczowe: allelopatia; pikoplanktonowe sinice; okrzemki; zielenice; wzrost; fluorescencja; Morze Bałtyckie

Introduction

Picoplankton is the smallest size fraction of plankton. The size of organisms of picoplanktonic cell size ranges from 0.2 to 2.0 μm . The group contains hetero- and autotrophic organisms. The latter group contains the following groups: Chlorophyta, Bacillariophyta, and Picobiliphytes, as well as prokaryotic Cyanobacteria (Stockner, 1988; Not et al., 2007; Jakubowska, Szelaż-Wasielewska, 2015; Jasser, Callieri, 2017). The first reports on the occurrence of picoplankton organisms in high concentrations in both seawater (Johnson, Sieburth, 1979; Waterbury et al., 1979) and freshwater reservoirs (Paerl, 1977) were only the beginning of many studies conducted in this field. Currently, significant amounts of picoplanktonic organisms are observed in all oceans of the world (Callieri, 2010; Flombaum et al., 2013; Jasser, Callieri, 2017). There are also many reports about their occurrence mainly in Europe, but also in North America and New Zealand (Beardall, 2008, Worden, Wilken, 2016). Due to the small size of these organisms, their role in aquatic ecosystems was still insufficiently known.

The species diversity of marine planktonic cyanobacteria is low, they are represented mainly by two genus *Synechococcus* and *Prochlorococcus*. Organisms of the genus *Synechococcus* occur throughout the world and are also found in freshwater ecosystems. They are organisms with a significant role in aquatic environments because they occur in high concentrations up to 1.5×10^9 cells per liter of water (Partensky et al., 1999; Jasser, 2006). In the Baltic Sea genus *Synechococcus* is dominant among picoplanktonic organisms. Furthermore, in the Baltic Sea *Synechococcus* group contain klads depending on their pigment composition. That include strains rich in phycoerythrin that can differ in color from orange to red and strains that contain mainly phycocyanin that can occur in different shades of green and blue (Haverkamp, 2009). What is more, strains that mainly contain phycoerythrin can also comprise two different fikobilin pigment; phycoerythrobilin or phycourobilin. In addition, red strains occur mainly in clear oceanic waters and green strains prefer turbid fresh waters both of these strains appear in the Baltic Sea but occupy different ecological niche by occurring on different depths (Vörös et al., 1998; Haverkamp et al., 2009).

Picoplanktonic organisms play an important role as food for many organisms. Due to their size, they constitute the main source of food for ciliates and flagellates and other larger representatives of zooplankton (Jyothibabu et al., 2013). In addition, literature data indicate that picoplankton may account for 98% of the biomass produced by phytoplankton (Sorokin et al., 2004). That is why it can be responsible for the flow of energy to higher trophic levels. Despite such a large role of this group in marine ecosystems, information on phytoplankton physiology is limited and needs further investigation. Their role is even greater due to the fact that some species produce toxic substances and form massive blooms that are potentially dangerous for the functioning of marine ecosystems (Śliwińska-Wilczewska et al., 2018). In this study, we investigated the allelopathic effect of three strains of picocyanobacteria *Synechococcus* sp. BA-120, BA-124 and BA-132 on filamentous cyanobacterium *Nostoc* sp., diatom *Amphora coffeaeformis* (C.Agardh) Kützing and green alga *Chlorella* sp. Furthermore, the influence of picocyanobacteria was examined by adding the cell-free filtrate of *Synechococcus* strains BA-120, BA-124 and BA-132 to studied species.

Material and Methods

The experiments were conducted on the three strains of the picocyanobacteria *Synechococcus* sp. BA-120, BA-124 and BA-132 and filamentous cyanobacterium: *Nostoc* sp. (BA-81), diatom: *Amphora coffeaeformis* (BA-16) and green alga: *Chlorella* sp. (BA-167) (Figs. 1, 2). The strains were isolated from the coastal zone of the Gulf of Gdańsk (southern Baltic Sea) and are maintained as monocultures in the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, University of Gdańsk, Poland (Latała et al., 2006). The tests on the 'batch cultures' were carried out in 25 mL glass Erlenmeyer flasks containing 20 mL sterilised f/2 medium (Guillard, 1975).

The media were prepared from Baltic water with a salinity of 8 psu, which was filtered through Whatman GF/C glass fiber filters, and autoclaved. Analysed organisms were grown 7 days in constant conditions of 18°C and 8 psu, under a 16:8 h light : dark cycle at irradiance of 10 $\mu\text{mol photons (PAR) m}^{-2} \text{ s}^{-1}$ and this were the control treatment conditions. Fluorescent lamps (Cool White 40W, Sylvania, USA) were used as source of irradiance. The intensity of PAR was measured using a LI-COR quantum-meter with a cosine collector. The donor and target organisms were acclimated to these culture condition for 7 days; afterwards, actively growing cultures were used for the establishment of the allelopathic experiment.

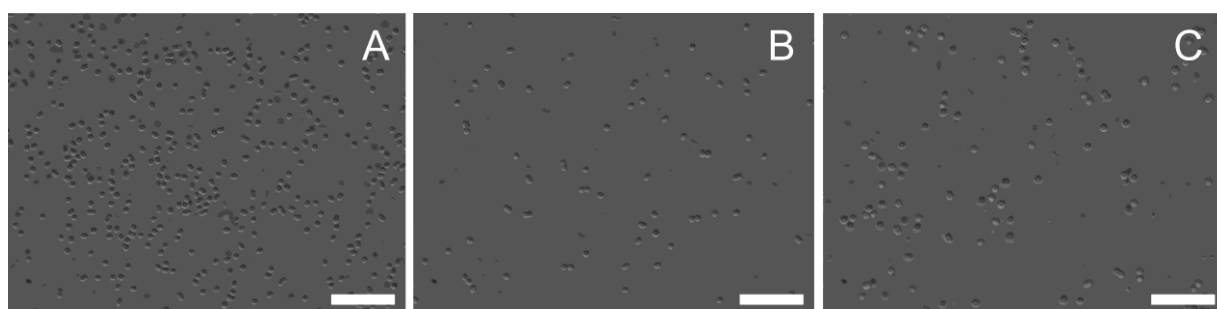


Fig 1 Baltic *Synechococcus* strains of picocyanobacteria used in this study: A – BA-120, B – BA-124 and C – BA-132; scale bars = 10 μm

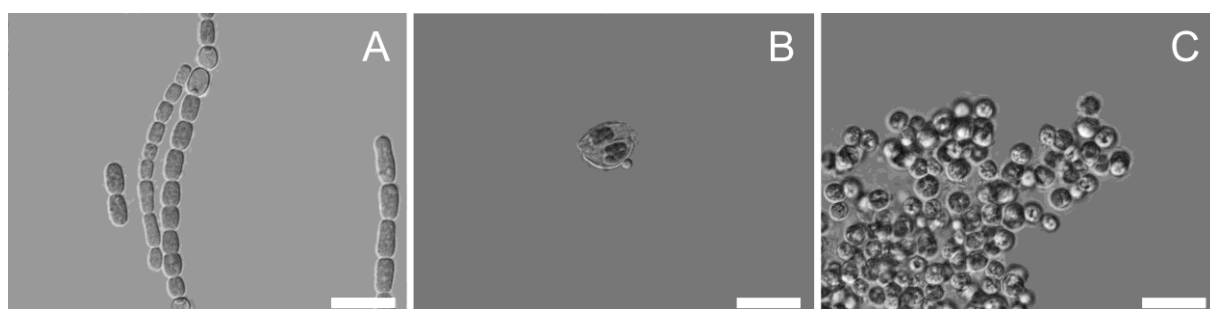


Fig 2 Target species used in this study: A – *Nostoc* sp. BA-81, B – *Amphora coffeaeformis* BA-16 and C – *Chlorella* sp. BA-167; scale bars = 10 μm

Allelopathic interactions in monocultures were determined by using the modified method proposed by Suikkanen et al. (2004). Allelopathic interaction was studied by adding the individual filtrate obtained from picocyanobacterial cultures of *Synechococcus* sp. BA-120, BA-124 and BA-132 to tested filamentous cyanobacterium, diatom and green alga. The cultures

of picocyanobacteria were filtered through 0.45- μm pore size Macherey-Nagel MN GF-5 filters. In all experiments, the ratio of picocyanobacterium to target species in Erlenmeyer flasks was adjusted to 1:1 based on the Chl *a* content (final Chl *a* concentration in the experimental cultures was 0.4 $\mu\text{g chl } a \text{ mL}^{-1}$). The cell-free filtrate ($V = 10 \text{ mL}$) was added to 25 mL Erlenmeyer flasks containing the tested species ($V = 10 \text{ mL}$). Control samples were prepared by adding mineral medium f/2 with a volume equal to the added cell-free filtrate. Tests were conducted in triplicate and all analyzed species were obtained from early exponential growth phase.

The number of cells (N) in cultures was estimated with previously determined linear correlations between cell abundance ($N \text{ mL}^{-1}$) and optical density (OD). N was counted using the BD Accuri™ C6 Plus flow cytometer following a procedure according to Marie et al. (2005) or using a Bürker chamber (48 squares per count) and light microscope (LM) following a procedure according to Guillard and Sieracki (2005) and the OD was measured spectrophotometrically at 750 nm with a Multiskan GO UV-VIS spectrophotometer (Thermo Scientific, Massachusetts, USA). The linear correlation between N and OD for *Synechococcus* sp. BA-120, BA-124, BA-132 as $y = 4.2 \cdot 10^6 x - 3.6 \cdot 10^4$, ($r^2 = 0.97$); $y = 93.0 \cdot 10^6 x - 9.8 \cdot 10^4$, ($r^2 = 0.99$); $y = 139.1 \cdot 10^6 x - 4.4 \cdot 10^4$, ($r^2 = 0.99$), respectively. The linear correlation between N and OD for target species; *Nostoc* sp. (BA-81), *A. coffeaeformis* (BA-16) and *Chlorella* sp. (BA-167) as $y = 39.9 \cdot 10^6 x - 1.2 \cdot 10^4$, ($r^2 = 0.95$); $y = 4.4 \cdot 10^6 x - 1.6 \cdot 10^4$, ($r^2 = 0.98$); $y = 3.7 \cdot 10^6 x - 27.4 \cdot 10^4$, ($r^2 = 0.93$), respectively, where $y = N \text{ (mL}^{-1}\text{)}$ and $x = \text{OD}$. Cell counts and OD measurements were performed on the 0th (1h), 1st, 3rd and 7th days of experiment and controls.

Chlorophyll *a* fluorescence was measured with a Pulse Amplitude Modulation (PAM) fluorometer (FMS1, Hansatech), using a 594 nm amber modulating beam with a 4-step frequency control as a measuring light. Samples were taken for chlorophyll fluorescence analysis after the 1st, 3rd and 7th days of experiment. Samples were filtered through 13-mm glass fiber filters (Whatman GF/C). Before measurement, the filtered sample was kept in the dark for approximately 15 min. The maximum PSII quantum efficiency (F_v/F_m) was calculated (Campbell et al., 1998).

Chlorophyll *a* and carotenoid pigments was measured 7th day of experiment. In the experiment extraction of the material (4 mL) was carried out in experimental flasks in 2 mL of 90% acetone in the dark and at a low temperature of -60°C for about 1 hour. After this time, the extract was centrifuged for 1 min. at $13,000 \text{ rpm min}^{-1}$. Absorbance measurements were then carried out in 1 cm glass cuvettes on a Beckman spectrophotometer model DU 530 at wavelengths (nm): 480, 665 and 750. For the determination of chlorophyll *a* and carotenoid pigments formula as described by Strickland and Parsons (1972):

$$\text{Chlorophyll } a \text{ (}\mu\text{g mL}^{-1}\text{)} = 11.236 \cdot (A_{665} - A_{750}) \cdot V_a/V_b$$

and

$$\text{Carotenoids (}\mu\text{g mL}^{-1}\text{)} = 4 \cdot (A_{480} - A_{750}) \cdot V_a/V_b,$$

where: V_a – volume of extract (mL); V_b – volume of the filtered material (mL); A_n – absorption at a specific wavelength (n) in nm.

Repeated measures ANOVA was used to test the effect of *Synechococcus* strains on the growth and fluorescence of the targeted species during the following days of experiment. A post-hoc Tukey's test was used to determine significant differences between the control and the other treatment levels. What is more, one-way ANOVA was used to test the effect of picocyanobacterial filtrates on the chlorophyll *a* and carotenoid pigments in control and experimental cultures on the seventh day of the experiment. Data are reported as mean \pm standard deviation (SD). Levels of significance were: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. The statistical analyses were performed using the Statistica® 13.1 software.

Results

The effect of the cell-free filtrate addition obtained from individual *Synechococcus* strains BA-120, BA-124 and BA-132 cultures on the growth of analysed target species *Nostoc* sp. (BA-81), *Amphora coffeaeformis* (BA-16) and *Chlorella* sp. (BA-167) after 0 (h), 1, 3 and 7 days of exposition to the filtrates are shown in Fig 3. The results showed that addition of cell-free filtrate from *Synechococcus* strains BA-120, BA-124 and BA-132 decreased after the 7th day of exposition the number of cells of *Nostoc* sp. (ANOVA, $F_{9,32}=283.1$; $p < 0.001$), *A. coffeaeformis* (ANOVA, $F_{9,32}=39.1$; $p < 0.001$) and *Chlorella* sp. (ANOVA, $F_{9,32}=38.5$; $p < 0.001$) compared to control. After the 7th day of the experiment for a filtrate addition of *Synechococcus* strains BA-120, BA-124 and BA-132 growth of *Nostoc* sp. decreased constituted 31% (Tukey HSD, $p < 0.001$), 34% (Tukey HSD, $p < 0.001$) and 33% (Tukey HSD, $p < 0.001$) respectively compared to control. In addition, it was observed that the cell-free filtrate obtained from three strains of *Synechococcus* sp. BA-120, BA-124 and BA-132 decreased the number of cells of *A. coffeaeformis* constituted: 36% (Tukey HSD, $p < 0.001$), 57% (Tukey HSD, $p < 0.001$) and 29% (Tukey HSD, $p < 0.001$) respectively. What is more, filtrate addition of mentioned strains decreased growth of *Chlorella* sp. and constituted: 75% (Tukey HSD, $p < 0.001$), 67% (Tukey HSD, $p < 0.001$) and 63% (Tukey HSD, $p < 0.001$) respectively compared to control.

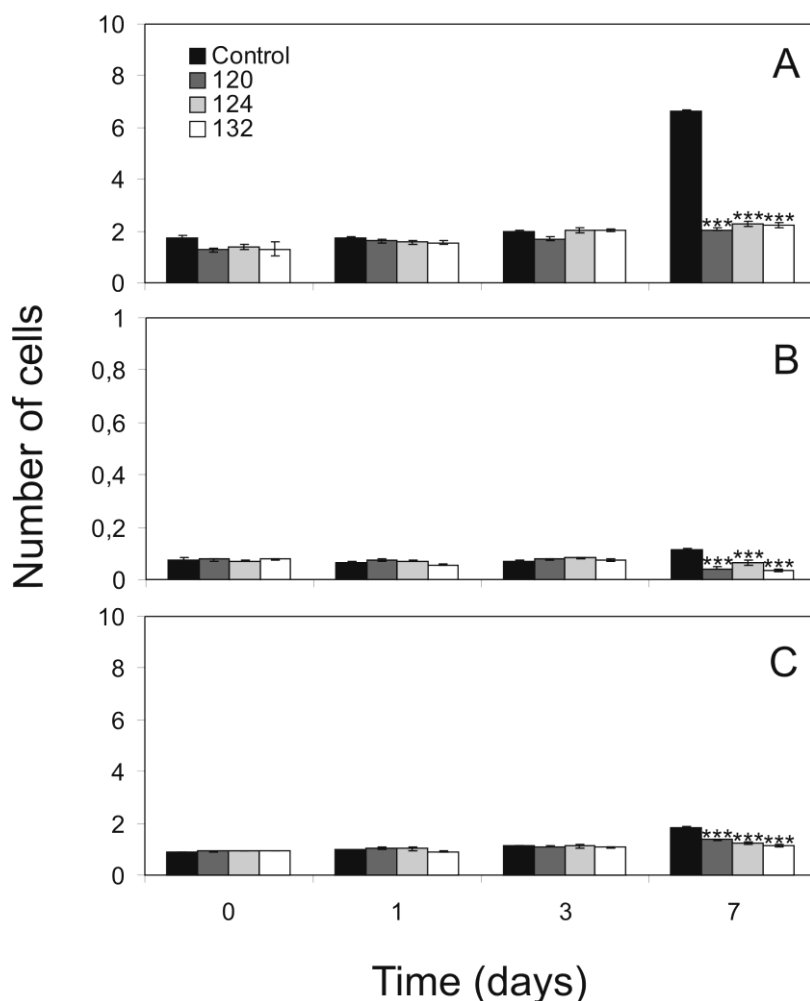


Fig 3 The effect of the addition of cell-free filtrate from *Synechococcus* strains BA-120, BA-124 and BA-132 cultures (referred as: 120, 124 and 132, respectively) on the number of cells (N 10⁶ mL) of *Nostoc* sp. – A, *Amphora coffeaeformis* – B and *Chlorella* sp. – C, after 0th, 1st, 3rd and 7th day of exposition, expressed as a number of cells (N); the values refer to means (n = 3, mean ± SD); asterisk indicates significant difference compared with control * p < 0.05; ** p < 0.01; *** p < 0.001

The effect of the addition of cell-free filtrate from three strains of *Synechococcus* on the fluorescence parameters F_v/F_m of analysed species after 1st, 3rd and 7th day of exposition is showed in Fig 4. The study demonstrated that the addition of cell-free filtrate obtained from *Synechococcus* sp.: BA-120, BA-124 and BA-132 significantly affected the F_v/F_m parameter of *Nostoc* sp. (ANOVA, $F_{6,24}=11.7$; $p < 0.001$). It was noted that the strongest influence on the F_v/F_m value was caused by filtrate obtained from *Synechococcus* sp. BA-132 after 1st, 3rd and 7th day of exposition and constituted: 21% (Tukey HSD, $p < 0.001$), 18% (Tukey HSD, $p < 0.001$) and 29% (Tukey HSD, $p < 0.001$), respectively compared to control. What is more also filtrate obtained from *Synechococcus* sp. BA-120 and BA-124 caused a significant changes of the F_v/F_m value, after 1st day of exposition constituted: 44% (Tukey HSD, $p < 0.001$) and 24% (Tukey HSD, $p < 0.001$), 3rd day of exposition constituted: 52% (Tukey HSD, $p < 0.001$) and 25% (Tukey HSD, $p < 0.001$) and 7th day of exposition constituted: 43% (Tukey HSD, $p < 0.001$) and 27% (Tukey HSD, $p < 0.001$), respectively compared to control.

Based on the results, it was found that the filtrate obtained from *Synechococcus* strains BA-120, BA-124 and BA-132 also caused significant changes of the F_v/F_m value of *A. coffeaeformis* (ANOVA, $F_{6,24}=4.0$; $p < 0.01$). After 3rd day of exposition the strongest influence on the fluorescence parameter F_v/F_m was caused by filtrate obtained from *Synechococcus* strain BA-124 and constituted 47% (Tukey HSD, $p < 0.001$). What is more after 3rd day of exposition also *Synechococcus* strains BA-120 and BA-132 had strong influence on F_v/F_m parameter and constituted 53% (Tukey HSD, $p < 0.001$) and 57% (Tukey HSD, $p < 0.001$) respectively. However it was noted that after 7th day of exposition the strongest influence on F_v/F_m parameter was caused by filtrate obtained from *Synechococcus* strain BA-132 and constituted 64% (Tukey HSD, $p < 0.001$) compared to control. The study showed that also the addition of the filtrate obtained from *Synechococcus* strain BA-124 significantly changed value of F_v/F_m parameter and constituted 67% (Tukey HSD, $p < 0.01$) compared to control.

The study showed that the addition of cell-free filtrate obtained from *Synechococcus* strains BA-120, BA-124 and BA-132 also significantly affected the F_v/F_m of *Chlorella* sp. (ANOVA, $F_{6,24}=359.0$; $p < 0.001$). It was noted that the strongest influence on the F_v/F_m value was caused by filtrate obtained from *Synechococcus* strain BA-120 after 1st day of exposition and constituted 88% (Tukey HSD, $p < 0.001$) compared to control. On that day also *Synechococcus* strains BA-124 and BA-132 had strong influence on F_v/F_m parameter and constituted 90% (Tukey HSD, $p < 0.001$) and 91% (Tukey HSD, $p < 0.001$) respectively. However after 3rd and 7th days of exposition the strongest influence on the F_v/F_m value was caused by *Synechococcus* strain BA-132 and constituted 94% (Tukey HSD, $p < 0.001$) and 84% (Tukey HSD, $p < 0.001$) respectively compared to control. What is more also *Synechococcus* strains BA-120 and BA-124 had considerable influence on F_v/F_m parameter and after 3rd day of exposition constituted: 99% (Tukey HSD, $p < 0.01$), 94% (Tukey HSD, $p < 0.001$) and after 7th day of exposition constituted 95% (Tukey HSD, $p < 0.001$), 87% (Tukey HSD, $p < 0.001$), respectively compared to control.

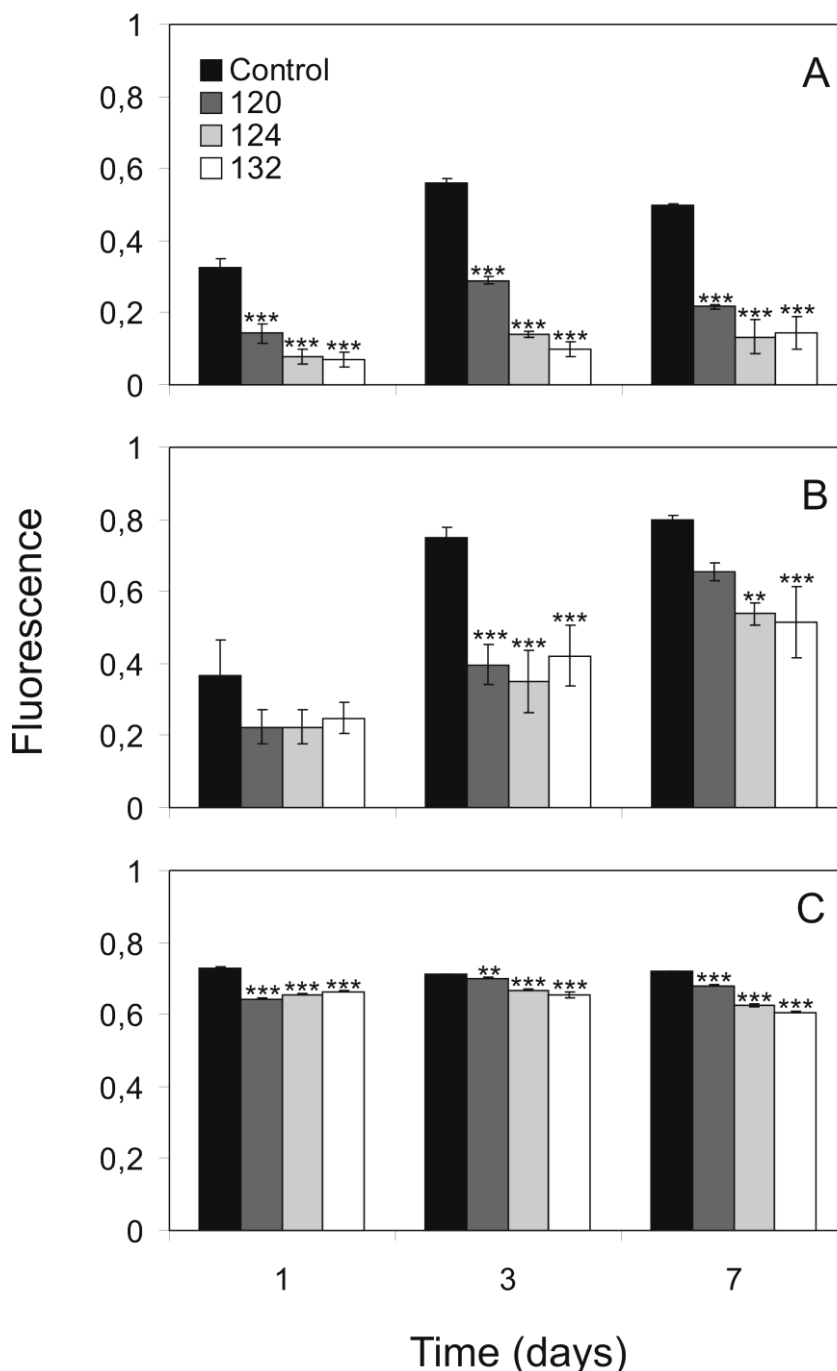


Fig 4 The effect of the addition of cell-free filtrate from *Synechococcus* strains BA-120, BA-124 and BA-132 cultures (referred as: 120, 124 and 132, respectively) on the fluorescence parameters (F_v/F_m) of *Nostoc* sp. – A, *Amphora coffeaeformis* – B and *Chlorella* sp. – C after 1st, 3rd and 7th day of exposition; the values refer to means (n = 3, mean \pm SD); asterisk indicates significant difference, compared with control * p < 0.05; ** p < 0.01; *** p < 0.001

The effect of the addition of cell-free filtrate from three *Synechococcus* strains BA-120, BA-124 and BA-132 on the Chl *a* and Car contents of analyzed species after 7th day of exposition is showed in figure 5. The results showed that addition of cell-free filtrate from *Synechococcus* strains BA-120, BA-124 and BA-132 decreased the content of Chl *a* in *Nostoc* sp. and constituted 47% (one-way ANOVA, p < 0.05), 30% (one-way ANOVA, p < 0.05) and 17% (one-way ANOVA, p < 0.05), respectively compared to control. What is more, filtrate from *Synechococcus* strains BA-120, BA-124 and BA-132 decreased the Car content which

constituted 68% (one-way ANOVA, $p < 0.05$), 45% (one-way ANOVA, $p < 0.01$) and 31% (one-way ANOVA, $p < 0.01$), respectively.

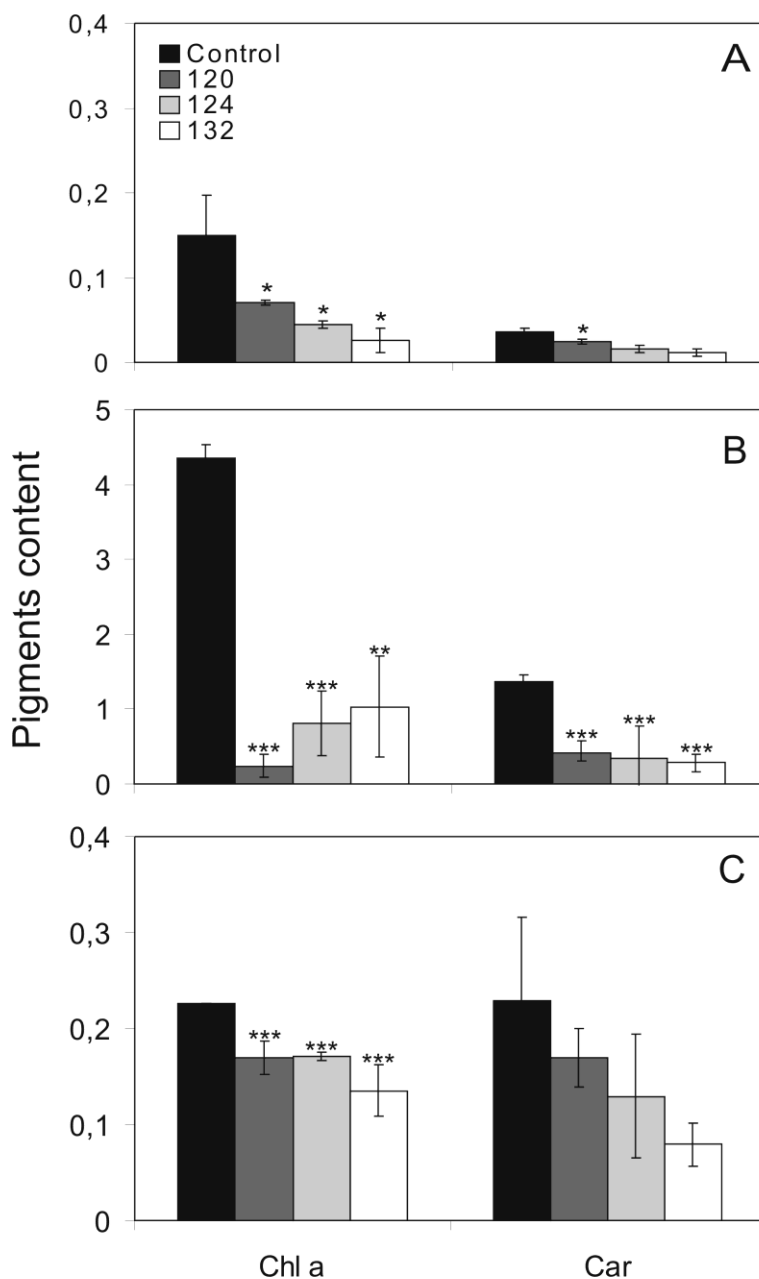


Fig 5 The effect of the addition of cell-free filtrate from *Synechococcus* strains BA-120, BA-124 and BA-132 cultures (referred as: 120, 124 and 132, respectively) on the chlorophyll *a* (Chl *a*) and carotenoid (Car) pigments content (pg cell⁻¹) of *Nostoc* sp. – A, *Amphora coffeaeformis* – B and *Chlorella* sp. – C, after 7th day of exposition; the values refer to means (n = 3, mean ± SD); asterisk indicates significant difference compared with control * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

It was noted that the addition of cell-free filtrate from three *Synechococcus* strains BA-120, BA-124 and BA-132 caused significant changes in the Chl *a* content in *A. coffeaeformis* and constituted 6% (one-way ANOVA, $p < 0.001$), 19% (one-way ANOVA, $p < 0.001$) and 24% (one-way ANOVA, $p < 0.01$), respectively. Furthermore addition of filtrate from *Synechococcus* strains BA-120, BA-124 and BA-132 decreased the Car content and constituted

31% one-way ANOVA, $p < 0.001$), 25% (one-way ANOVA, $p < 0.001$) and 21% (one-way ANOVA, $p < 0.001$) respectively compared to control.

Based on the results, it was found that the filtrate obtained from *Synechococcus* strains BA-120, BA-124 and BA-132 also caused significant changes of the Chl *a* content in *Chlorella* sp. and constituted 78% (one-way ANOVA, $p < 0.001$), 76 % (one-way ANOVA, $p < 0.001$) and 60% (one-way ANOVA, $p < 0.001$), respectively. However, it was found that the filtrate from *Synechococcus* strains BA-120, BA-124 and BA-132 did not statistically affect Car content in *Chlorella* sp. (one-way ANOVA, $p > 0.05$).

The results showed that addition of cell-free filtrate from *Synechococcus* strains BA-120, BA-124 and BA-132 decreased the number of cells as well as the chlorophyll fluorescence, Chl *a* and Car contents of *Nostoc* sp., *A. coffeaeformis* and *Chlorella* sp. Thus, results of this experiment may provide further information about allelopathic interactions between picocyanobacteria and other co-existing phytoplankton species in the Baltic Sea.

Discussion

The allelopathic compounds secreted to the environment by the donor organism can influence the co-occurring organisms in various ways (Granéli and Hansen, 2006). The vast majority of allelopathic compounds produced by cyanobacteria and microalgae affect mainly the physiology of exposed organisms by inhibiting the growth of cells and the activity of photosynthesis (Maxwell, 2000; Leflaive and Ten-Hage, 2007; Machado et al., 2015). Due to an important role of allelopathy in marine environment the main aim of this work was to investigate the allelopathic effect of picocyanobacteria *Synechococcus* strains BA-120, BA-124 and BA-132 on growth, fluorescence parameters and pigments content of *Nostoc* sp., *Amphora coffeaeformis* and *Chlorella* sp.

Only few literature data described the allelopathic effect of cyanobacteria on coexisting phytoplankton species in the Baltic Sea. More detailed data on allelopathic effects of Baltic cyanobacteria on diatoms were presented by Suikkanen et al. (2004). These studies demonstrated the allelopathic effect of *Nodularia spumigena*, *Anabena lemmermannii* and *Aphanizomenon flos-aquae* (L.) Ralfs ex Born. et Flath. on the diatom *Thalassiosira weissflogii* (Grunow) G.Fryxell & Hasle. Research showed that after a single addition of the filtrate, on the first day of the experiment, all these three cyanobacteria had generally negative effect on the tested species. Furthermore, allelopathic interactions of *Synechococcus* sp. on Baltic diatom *Navicula perminuta* Grunow was examined by Śliwińska-Wilczewska et al. (2016). As a result of this work it was noted that addition of the cell-free filtrate obtained from the picocyanobacterium *Synechococcus* sp. had a significant inhibitory effect on coexisting target species which may explain the formation of dense picocyanobacterial blooms during the summer period. Moreover, in studies carried out by Śliwińska-Wilczewska et al. (2017), chlorophyll fluorescence as well as photosynthetic pigments inhibition has been demonstrated after filtrate addition obtained from picocyanobacterium *Synechococcus* sp. The study showed that the addition of cell-free filtrate obtained from *Synechococcus* sp. had significant effects on the values of the F_v/F_m of *A. flos-aquae* and *Phormidium* sp. What is more, it was noted that the addition of filtrate inhibits chlorophyll *a* and carotenoid contents of *Phormidium* sp. and *Rivularia* sp. Some authors noted that changes in both pigments content and chlorophyll fluorescence indicates the activity of the cells defense mechanism and response to stress factors (Machado et al., 2015; Śliwińska-Wilczewska et al., 2017).

Despite the great ecological importance of *Synechococcus* sp. knowledge about their harmful effects on other organisms is still limited. Nevertheless, conducted studies show that picocyanobacteria *Synechococcus* sp. affects respiration rate and can cause balance disorders of invertebrate (Martins et al., 2008). What is more, recent work suggests that picocyanobacteria form the genus *Synechococcus* also contribute to behavioral changes and locomotive disorders in vertebrates. Studies conducted on fish *Embiotoca jacksoni* Agassiz indicated that organisms exposed to direct contact with mentioned picocyanobacteria were characterised by reduced mobility, moving much slower and spending more time immobile. It is suggested that such changes may be the result of harmful substances that are absorbed by the gills and then disrupting the functioning of the nervous system (Hamilton et al., 2014). In addition, previous studies carried out on cyanobacteria isolated from Portugal showed that *Synechococcus* sp. produced toxic substances affecting mammals, marine invertebrates, as well as causing inhibition of growth in Gram-positive bacteria and cytotoxicity in human cell lines (Martins et al., 2007, 2008; Selheim et al., 2005). *Synechococcus* sp. have been rarely studied with respect to their potential as producers of allelochemicals because most of previous works have assumed a lack of toxin production by these picocyanobacteria. However, *Synechococcus* sp. belong to the organisms that produce wide range of secondary metabolites, like microcystins, β -*N*-methylamino-*L*-alanine, 2-methylisoborneol, geosmin, thionsulfolipid and lipopolysaccharides (Śliwińska-Wilczewska et al., 2018).

Inhibition of the growth of some cyanobacteria and microalgae under the influence of substances secreted by co-existing picocyanobacteria in the natural environment may lead to the dominance of some species which, may result in a change in the natural structure of the phytoplankton community, for example during the summer in the Baltic Sea (Suikkanen et al., 2004; 2008). Despite the relatively large number of studies performed to this day, the mechanisms of allelopathic compounds and their chemical composition are not fully investigated and require further analysis because, as already mentioned, they are of great importance for the functioning of aquatic ecosystems.

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