Annales Universitatis Paedagogicae Cracoviensis
Studia Naturae, 7: 111–126, 2022, ISSN 2543–8832
DOI: 10.24917/25438832.7.7

Gracjana Budzałek*, Sylwia Śliwińska-Wilczewska
Institute of Oceanography, University of Gdansk, Gdynia, Poland, *gbudzalek@gmail.com

Allelopathic effect of the green macroalga *Ulva intestinalis* (Ulvaceae, Chlorophyta) on selected Baltic cyanobacteria

Introduction

Allelopathy is a unique strategy to deter or eliminate organisms coexisting in the same ecosystem (Molisch, 1937; Śliwińska-Wilczewska et al., 2021). In aquatic ecosystems, allelopathic activity depends on the production and secretion of active allelopathic compounds and their effective dispersal in the environment (Lewis, 1986). The benthic zone is limited compared to the extensive pelagic zone in the sea, so allelopathic interactions in these coastal ecosystems may be stronger. Macroalgae have been found to produce active metabolites that inhibit other organisms that compete with them for light and space (Harlin, 1987; Jeong et al., 2000), but their allelopathic activity on Baltic cyanobacteria is still insufficiently recognised.

Previous studies have confirmed that green algae of the genus *Ulva* L. (Chlorophyta) are capable of allelopathic effects on selected microalgal species (Jin, Dong, 2003; Nan et al., 2004; Jin et al., 2005; Wang et al., 2007; Tang, Gobler, 2011). Jin and Dong (2003) studied the effects of extract, filtrate, and fresh thallus of *Ulva pertusa* Kjellman on *Heterosigma akashiwo* (Y. Hada) Y. Hada ex Y. Hara & M. Chihara and *Alexandrium tamarense* (Lebour) Balech. *H. akashiwo* and *A. tamarense* were strongly inhibited by aqueous extract and fresh tissue from *U. pertusa*. In contrast, the authors showed that the green algae-derived filtrate had no significant effect on the cell counts of the test organisms. A year later, Nan et al. (2004) investigated the effect of fresh tissue and filtrate from *U. pertusa* on the *Tetraselmis subcordiformis* (Wille) Butcher (species form phylum Chlorophyta), the *Heterosigma akashiwo* and *Alexandrium tamarense* (representative of Miozoa), the *Skeletonema costatum* (Greville) Cleve, *Nitzschia Closterium* (Ehrenberg) W. Smith, and *Chaetoceros gracile* F. Schütt (member of Bacillariophyta), the *Chroomonas placoidea* Butcher *ex* G. Novarino & I.A.N. Lucas (species from phylum Cryptophyta), and the *Isochrysis galbana* Parke (representative of Haptophyta). The

growth was significantly inhibited for each tested species. Additionally, the authors' results suggest that allelopathic compounds from *U. pertusa* are highly degradable. The inhibitory effect of extract, filtrate, and fresh thallus from *U. lactuca* was also demonstrated by Tang and Gobler (2011). This green macroalgae adversely affected the cell concentrations of *Aureococcus anophagefferens* Hargraves & Sieburth in Sieburth, P.W. Johnson & Hargreaves and *Chattonella marina* (Subrahmanyan) Y. Hara & M. Chihara (member of Ochrophyta), the *Cochlodinium polykrikoides* Margalef, *Karlodinium veneficum* (D. Ballantine) J. Larsen in Daugbjerg & al., *Karenia brevis* (C.C. Davis) Gert Hansen & Moestrup, and *Prorocentrum minimum* (Pavillard) J. Schiller (species form phylum Miozoa), and *Pseudo-nitzschia multiseries* (Pavillard) J. Schiller (representative of Bacillariophyta). Wang et al. (2007) investigated the inhibitory effect of extract, filtrate, and fresh thallus of *Ulva pertusa* on the growth of two dinoflagellates *H. akashiwo* and *Alexandrium tamarense*. On the other hand, Jin et al. (2005) investigated the inhibitory effect of fresh thallus and extract of *U. pertusa* and *U. linza* L. on the dinoflagellata *Prorocentrum micans* Ehrenberg. A recent work indicates that *Ulva intestinalis* L. also has allelopathic properties (Budzałek et al., 2021a). It was shown that the addition of the cell-free filtrate obtained from *U. intestinalis* significantly inhibited growth and photosynthetic efficiency of filamentous cyanobacteria *Nodularia spumigena* Mertens ex Bornet & Flahault and *Nostoc* sp. Suprysingly, the authors found that the addition of different concentrations of aqueous extract, as well as filtrate, stimulated the *Aphanizomenon* sp.

There is only one report of allelopathic activity of *Spirogyra* sp. (Charophyta) (Irfanullah, Moss, 2005). In their work, they studied the effects of allelopathic compounds secreted by *Spirogyra* sp. on phytoplankton communities. Phytoplankton species dynamics and species composition were apparently not influenced by allelopathy of living or decomposing *Spirogyra* sp. In addition, they investigated the allelopathic effects of filtrate and living thallus of *Spirogyra* sp. on phytoplankton. There was no change in phytoplankton growth and species composition under the allelopathy of living or decomposing *Spirogyra* sp. This study showed that filamentous macroalgae from Charophyta phylum probably cannot control phytoplankton biomass in a nutrient-rich environment by secreting allelopathic compounds.

Allelopathic activity was also confirmed for macroalgae belonging to the genus *Chara* (Charophyta) (Donk, Bund, 2002; Berger, Schagerl, 2003; Mulderij et al., 2003; Pakdel et al., 2013; Mähnert et al., 2017; Złoch et al., 2018). Pakdel et al. (2013) studied the effects of extract, filtrate, and live material from the stonewort – *Chara australis* R. Brown on the cyanobacteria *Anabaena variabilis* Kützing *ex* Bornet & Flahault and the green alga *Scenedesmus quadricauda* (Turpin) Brébisson in Brébisson & Godey. *C. australis* had a highly inhibitory effect on the growth of *A. variabilis*. In contrast, the extract had no significant effect on *S. quadricauda*. Recently, the effect of the extract

of Baltic *C. aspera* Wild., *C. baltica* Bruzelius, and *C. canescens* Loiseleur on the cyanobacteria *Synechococcus* sp. was demonstrated by Złoch et al. (2018). Both inhibition and stimulation of growth and photosynthesis of the tested species were demonstrated. *C. aspera* had both stimulatory and inhibitory effects on the studied cyanobacteria. The other two *Chara* sp. inhibited the growth of cyanobacteria cell numbers. Mähnert et al. (2017) showed inhibitory effects of extracts, as well as fresh thallus of *C. aspera*, *C. globularis*, *C. rudis*, and *C. tomentosa* on the green microalgae *Chlorella vulgaris* L., *Acutodesmus acuminatus* (Lagerheim) P.M. Tsarenko in Tsarenko & Petlovanny, the cyanobacteria *Synechococcus elongatus*, *S. leopoliensis*, and the bacterium *Aliivibrio fischeri* Beijerinck. Strong inhibitory effects of the extract and fresh thallus on cyanobacteria and bacteria were demonstrated. Berger and Schagerl (2003) demonstrated the inhibitory effect of *C. aspera* extract on the growth of the filamentous cyanobacteria *Anabaena cylindrica* Lemmermann. The allelopathic inhibitory effect of *C. aspera* was also studied on the green algae *Scenedesmus acutus* Meyen (Donk, Bund, 2002). On the other hand, Mulderij et al. (2003) studied the effect of filtrate obtained from *Chara globularis* var. *globularis* (Detharding ex Willdenow) R.D. Wood and *C. contraria* var. *contraria* (A. Braun ex Kützing) J.A. Moore on three green algae. They showed growth stimulation of *Selenastrum capricornutum* Printz and *Chlorella minutissima* Fott & Nováková. However, they showed no significant differences for *Scenedesmus obliquus* (Turpin) Kützing.

Toxic cyanobacterial blooms pose a serious threat to the environment and human health, and restoration of affected water bodies can be a challenge. Although cyanobacterial blooms are a common phenomenon already, their occurrence and severity are expected to increase in the future due to climate change (Reichwaldt, Ghadouani, 2012). In fresh and brackish water bodies of toxic cyanobacterial blooms have been recorded for at least 2 millennia (Zillén, Conley, 2010). Massive cyanobacterial blooms appear in the Baltic Sea almost every year during summer, however, it is very difficult to predict their location and intensity of occurrence (Kahru et al., 2020). The genus *Aphanizomenon* sp. commonly dominates the water column biomass during blooms in the Baltic Sea, along with *Nodularia spumigena*, which has consistently caused fatalities of both wild and domesticated animals in the Baltic Sea (Wasmund, 1997). A third common species in this basin is *Nostoc* sp., which also exhibits cytotoxic properties (Surakka et al., 2005).

Thus, the aim of this study was to demonstrate the allelopathic effects of Baltic macroalga *Ulva intestinalis* L. (syn. *Enteromorpha intestinalis* (L.) Nees) thallus on growth and photosynthetic activity of three bloom-forming cyanobacteria *Aphanizomenon* sp., *Nodularia spumigena*, and *Nostoc* sp. These studies help define the role of *U. intestinalis* allelopathy as a biological factor in the distribution of bloom-forming cyanobacteria in the coastal Baltic Sea region.

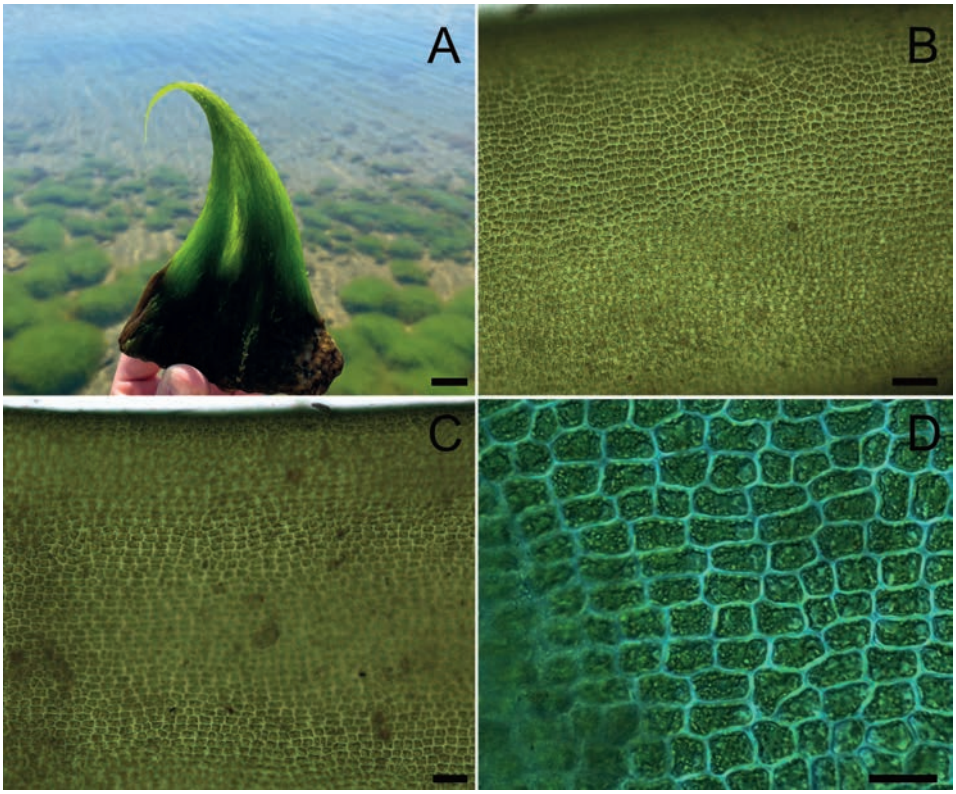

Fig. 1. *Ulva intestinalis* L. thalli from habitat and light micrographs of tubular thallus (young cell arranged in longitudinal rows). Scale bar: 100 mm – A, 60 µm – B, 40 µm – C, 20 µm – D (Photo. G. Budzałek)

Material and methods

Place of sampling and material cultivation

The material used in the experiments consisted of strains of Baltic cyanobacteria *Aphanizomenon* sp. (CCBA-69), *Nodularia spumigena* (CCBA-15), and *Nostoc* sp. (CCBA-81) (so-called target organisms). Strains of cyanobacteria were isolated from the natural phytoplankton communities of the coastal waters of the Gulf of Gdańsk (southern Baltic Sea) (54°30'53.7"N; 18°54'23.5"E) and are maintained in the Culture Collection of Baltic Algae (CCBA) at the Laboratory of Marine Plant Ecophysiology at the University of Gdańsk (Latała et al., 2006). The macroalga *Ulva intestinalis* used in the study (donor organism) was collected from the coastal zones of the southern Baltic Sea region (54°30'08.7"N; 18°33'32.3"E). Determination of *U. intestinalis* (Fig. 1) based on the examination of morphological features (such as the number of pyrenoids and shape of cells) using identification keys was made (Starmach, 1972; Škaloud et al., 2018). The herbarium sheets (voucher number: BA M50; herbarium website: https://

zielnik.ug.edu.pl/en/home/) were prepared in accordance with guidelines in Drobnik (2007) and Rybak (2018) and deposited at the Institute of Oceanography, University of Gdansk (Poland).

The studied macroalga and cyanobacteria were cultured on sterile mineral medium f/2 (Guillard, 1975) prepared with Baltic Sea water filtered through glass fiber filters (Whatman GF/C) and autoclaved. The salinity was 8 PSU as measured with a salinometer (inoLab Cond Level 1, Germany). The *U. intestinalis* and cyanobacterial strains used in the experiments was maintained in 300–mL and 50–mL glass Erlenmeyer flasks, respectively. Donor and target organisms were cultured at a PAR intensity of 10 μmol photons $m^{-2}s^{-1}$ (16:8 h light: dark cycle) and a temperature of 18°C. Photosynthetically active irradiance (PAR) was measured using a quantum meter (LI-COR, USA). The light sources used in the experiment were lamps (Cool White 40W, USA). The cultures were acclimated to these conditions for 2 days, and these growth conditions were used for the experiments.

Determination of the allelopathic effect of *Ulva intestinalis* thallus

The allelopathic effect of *Ulva intestinalis* thallus was tested according to a method proposed by Wang et al. (2007) with modifications. The cyanobacteria monocultures were exposed to three different concentrations of the presence of live thallus of *U. intestinalis*.

Target cyanobacterial strains were maintained in 25–mL Erlenmeyer flasks. In all experiments, the starting concentration of chlorophyll *a* in cyanobacterial cultures was 0.4 μg chl *a* mL^{-1}. The coexistence assays were performed using a mixed culture system of one macroalga and one strain of cyanobacteria. Different initial inoculation concentrations (0.01, 0.05, and 0.1 g wet weight mL^{-1}) of fresh algal thallus were inoculated into 25–mL Erlenmeyer flasks containing 20 mL of the target cyanobacteria strains. Control cultures were prepared analogously, but f/2 medium was added instead of thallus at 0.01, 0.05, and 0.1 mL^{-1}. The concentration of major nutrients in the control and experimental samples were adjusted to the same level as in the f/2 standard. Therefore, the influence of nutrients, micronutrients and vitamins on the experimental result can be excluded. After 7 days of the exposure, before the measurements, the *Ulva* sp. thallus was removed, and the cyanobacterial cells concentration, as well as chlorophyll *a* fluorescence parameters, were determined. All allelopathic tests were conducted in triplicate.

Determination of cyanobacterial number of cells

The number of cells (N) in *Aphanizomenon* sp., *Nodularia spumigena*, and *Nostoc* sp. cultures was estimated with previously determined linear correlations between cell abundance (N mL^{-1}) and optical density (OD). N was counted using a Bürker chamber (48 squares per count) and light microscope 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 mentioned cyanobacteria was described by Budzałek et al. (2021). OD measurements were performed on the 7th days of the experiment. In addition, absorption spectra in the wavelength range from 400 nm to 750 nm with an interval 1 nm was determined for cyanobacterial abundances in controls and allelopathic treatments.

Determination of the chlorophyll a fluorescence

In the conducted experiments, the Pulsed Amplitude Modulation (PAM) method was used to measure the chlorophyll *a* fluorescence (FMS1, Hansatech). This method is widely used to measure chlorophyll *a* fluorescence in cyanobacteria, both in the laboratory and in the natural environment (Schreiber et al., 1995; Campbell et al., 1998). Samples were taken for chlorophyll fluorescence analysis after the 7th days of the experiment. About 5 mL of target cyanobacteria were filtered through 13-mm glass fiber filters (Whatman GF/C). In the next step, the filters were placed in holders. The samples were kept in the dark for 5 min before measurement. In this study, the maximum PSII quantum efficiency (*F*v/*F*m) and the effective quantum yield of PSII photochemistry (ΦPSII) were determined (Campbell et al., 1998).

Statistical analysis

To confirm the allelopathic effect of the extracts obtained from macroalgae on the number of cells and chlorophyll *a* fluorescence parameters of target cyanobacteria, a one-way ANOVA was performed. Data are reported as the means ± standard deviations (SD), where level of significance is $p < 0.05$. Statistical analysis and graphs were performed using Statistica® 13.1 software and OriginPro program, Version 2021 (OriginLab Corporation, Northampton, MA, USA).

Results

Allelopathic effect of *Ulva intestinalis* thallus on cyanobacterial abundances

In this study, the number of cells (N 105 mL^{-1}) of *Aphanizomenon* sp., *Nodularia spumigena*, and *Nostoc* sp. in controls and cultures to which were added: 0.01, 0.05, and 0.1 g wet weight mL^{-1} of fresh thallus obtained from *Ulva intestinalis* after 7 days of the experiment were determined.

It was found that thallus obtained from *U. intestinalis* had no statistically significant effect on the number of cells of the cyanobacterium *Aphanizomenon* sp. at higher concentrations (0.05 and 0.1 g mL^{-1}). On the other hand, it was examined a stimulating effect of 0.01 g mL^{-1} of the fresh thallus on the number of *Aphanizomenon* sp. cells

which constituted 168% (one-way ANOVA, $p < 0.05$), relative to the control treatment (Fig. 2A). However it was also shown that the fresh thallus addition resulted in a decrease in the number of *N. spumigena* cells. For this cyanobacterium, the growth was, relative to the control, 45%, 27%, and 46% (one-way ANOVA, $p < 0.05$, for all), after addition of 0.01, 0.05, and 0.1 g wet weight mL^{-1} of fresh thallus, respectively (Fig. 2B). In experiments with *Nostoc* sp. and *U. intestinalis* thallus addition, the negative effect on cyanobacterial growth was detected on the 7th day of exposure at 0.1 g wet weight mL^{-1} and constituted 97% (one-way ANOVA, $p < 0.05$) of control. In turn, the *U. intestinalis* thallus addition had no significant effect on the growth of *Nostoc* sp. at lower concentrations (0.01 and 0.05 g mL^{-1}) (Fig. 2C).

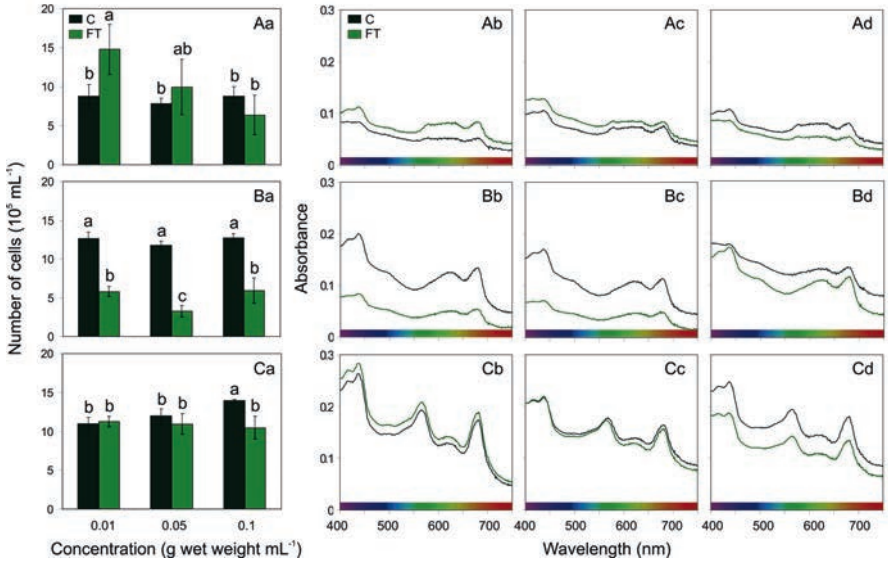

Fig. 2. The number of cells (N 105 mL^{-1}) of *Aphanizomenon* sp. – A, *Nodularia spumigena* Mertens ex Bornet & Flahault – B, and *Nostoc* sp. – C in controls (C) and cultures to which were added different concentrations (g mL^{-1}) of fresh thallus (FT) obtained from *Ulva intestinalis* L. after 7 days of the experiment (a) and PAR absorption spectra determined for this strains at thallus concentrations: 0.01 (b), 0.05 (c), and 0.1 (d). The values shown are mean values (n = 3, mean ± SD). Different letters indicate significant differences between the means of the treatments ($p < 0.05$, one-way ANOVA)

Allelopathic effect of Ulva intestinalis thallus on fluorescence parameters

The values of the fluorescence parameter *Fv*/*Fm* (the maximum PSII quantum efficiency) and ΦPSII (the effective quantum yield of PSII photochemistry) for *Aphanizomenon* sp., *N. spumigena*, and *Nostoc* sp. in control and cultures in the presence of different concentrations (0.01, 0.05, and 0.1 g wet weight mL^{-1}) of fresh thallus obtained from *U. intestinalis* after 7 days of experiment were examined.

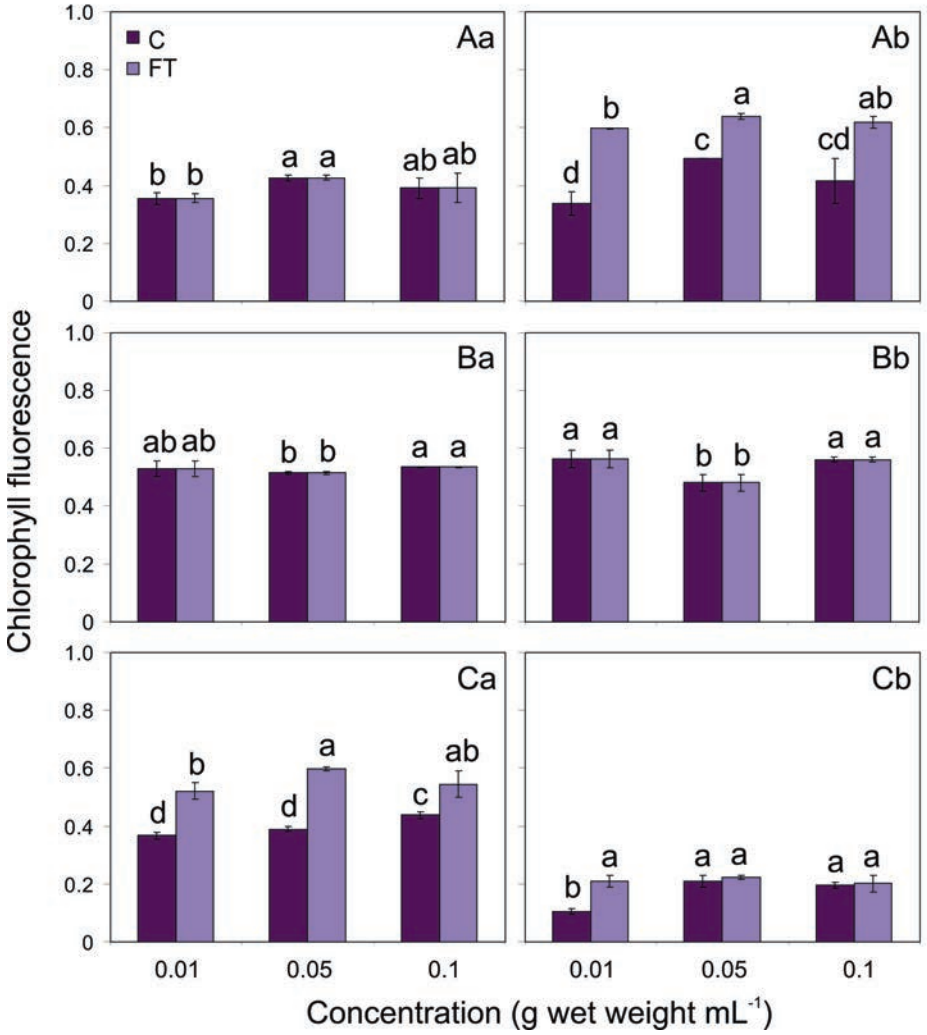

Fig. 3. The values of the fluorescence parameter *Fv*/*Fm* (a) and ΦPSII (b) for *Aphanizomenon* sp. – A, *Nodularia spumigena* Mertens ex Bornet & Flahault – B, and *Nostoc* sp. – C in controls (C) and cultures to which added: 0.01, 0.05, and 0.1 (g mL^{-1}) of fresh thallus (FT) obtained from *Ulva intestinalis* L. after 7 days of the experiment. The values shown are mean values (n = 3, mean ± SD). Different letters indicate significant differences between the means of the treatments ($p < 0.05$, one-way ANOVA)

A stimulating effect of concentrations of 0.01, 0.05, and 0.1 g wet weight mL^{-1} of *U. intestinalis* thallus on the values of ΦPSII of cyanobacteria *Aphanizomenon* sp. were observed and in these conditions these parameters constituted 176%, 129%, and 148% (one-way ANOVA, $p < 0.05$, for all), respectively (Fig. 3Ab). Surprisingly, addition of thallus of *U. intestinalis* did not affect the value of *Fv*/*Fm* of *Aphanizomenon* sp. (Fig. 3Aa). Moreover, the addition of fresh thallus obtained from *U. intestinalis*

had no statistically significant effect on the fluorescence parameters *F*v/*F*m and ΦPSII of *N. spumigena* (Fig. 3Ba–b). In turn, the *F*v/*F*m of *Nostoc* sp. was positively affected after addition of 0.01, 0.05, and 0.1 g wet weight mL^{-1} of thallus, being, relative to the control, 142%, 153%, and 124% (one-way ANOVA, $p < 0.05$, for all), respectively (Fig. 3Ca). Moreover, in the fresh thallus addition experiments, the value of ΦPSII for the mentioned cyanobacterium was significantly different from the control at the concentration of 0.01 g mL^{-1}, when it constituted 202% (Fig. 3Cb).

Discussion

Effects of allelopathic compounds produced by Baltic macroalga *Ulva intestinalis* on cyanobacterial growth

In the present study, the allelopathic effect of Baltic green macroalga *Ulva intestinalis* thallus on growth of three bloom-forming cyanobacteria *Aphanizomenon* sp., *Nodularia spumigena*, and *Nostoc* sp. was investigated. The live thallus had an inhibitory effect on the number of cells of *N. spumigena*, and *Nostoc* sp. in the first week. Whereas cell growth in the *Aphanizomenon* sp. sample was stimulated by *U. intestinalis* thallus at the lowest concentration (0.01 g wet weight mL^{-1}).

In several other works, the authors also showed allelopathic activity of fresh thallus of green algae of the genus *Ulva* on co-occurring phytoplankton species. Wang et al. (2007) noted that *Heterosigma akashiwo* and *Alexandrium tamarense* showed different responses when exposed to live *U. pertusa* thallus. The authors showed that relatively low concentrations had a lethal effect on *H. akashiwo*, while *A. tamarense* cells were not completely degraded even at the highest macroalgal concentration. Differences in the cell surface structure of *H. akashiwo* and *A. tamarense* may account for their different sensitivity to allelopathic compounds (Wang et al., 2007). Kakisawa et al. (1988) suggested that allelopathic compounds produced by macroalgae may be more active against organisms that lack cell walls. In the present study, three species of Baltic cyanobacteria that possess cell walls were used for testing. In addition, cyanobacteria are covered with a mucous envelope of varying thickness, which may also diminish the effect of allelochemicals. This may partly explain the lack of effect of the filtrate and live *U. intestinalis* mollusks on the cell abundance of *Aphanizomenon* sp. It should be noted here that the composition of allelopathic compounds from green macroalge is still not well known. In a review by Budzalek et al. (2021b), known compounds from macroalgae with an active effect on other organisms were listed. Among them, it has been shown that the *U. intestinalis* can produce Penostatins A–H, Cytochalasans, Penochalasins A–H, Chaetoglobosin, and Communesins A–B with cytotoxic activities. However, further research is needed to unequivocally show which compounds are responsible inhibition or stimulation of cyanobacteria in the Baltic ecosystem.

In contrast, Uchida et al. (1995) reported that *Heterocapsa circularisquama* Horiguchi completely annihilated *Gyrodinium instriatum* Freudenthal & J.J. Lee by direct cell contact. In the present study, the possibility of inhibition of cyanobacterial growth by direct contact with *U. intestinalis* was also considered and this possibility was finally ruled out by additionally performing experiments with the addition of the filtrate of this donor green alga alone. Since significant changes in the growth of Baltic cyanobacteria were also observed under the influence of the filtrate, it can be assumed that the secretion of allelopathic compounds by *U. intestinalis* is the most probable explanation for the observed growth inhibition of the organisms studied.

Effects of allelopathic compounds produced by Baltic macroalga *Ulva intestinalis* on photosynthesis performance

In this work, we also investigated the allelopathic effect of fresh thallus obtained from *U. intestinalis* on the maximum PSII quantum efficiency (*F*v/*F*m) and the effective quantum yield of PSII photochemistry (ΦPSII) of some Baltic cyanobacteria. It was found that *U. intestinalis* had no allelopathic effect on fluorescence parameters of tested cyanobacterium *N. spumigena*. On the other hand, all tested concentrations (0.01, 0.05, and 0.1 g wet weight mL^{-1}) of thallus from mentioned green macroalga stimulated the values of *F*v/*F*m or ΦPSII of cyanobacteria *Aphanizomenon* sp. and *Nostoc* sp. compared to the control.

In other works, Budzałek et al. (2018, 2021b) and Złoch et al. (2018) demonstrated that extract and filtrate of *U. intestinalis* containing water-soluble allelochemical(s) is able to influence the fluorescence parameters of some Baltic cyanobacteria. Budzałek et al. (2018) noted that the highest decrease in *F*v/*F*m for *Nostoc* sp. was observed after addition of 100 μL mL^{-1} *U. inestinalis* extract. On the other hand, in some cases, the extract and filtrate from *U. inestinalis* caused the stimulation of fluorescence parameters of *Aphanizomenon* sp. and *Nostoc* sp. (Budzałek et al., 2021). Moreover, extract obtained from *Chara baltica* and *C. canescens* caused an increase in fluorescence parameters for *Synechococcus* sp. after the seventh day of the exposure (Złoch et al., 2018). The low level of these parameters may be evidence of certain disturbances in the photosynthesis process due to allelochemicals (Song et al., 2017). On the other hand, stimulating photosynthesis at the low concentration of thallus may indicate the phenomenon of hormesis (Stebbing, 1982). It is worth mentioned here, that this is the first report of the allelopathic effect of *U. intestinalis* thallus on the maximum PSII quantum efficiency and the effective quantum yield of PSII photochemistry of Baltic cyanobacteria. Therefore, more studies should be done to further investigate the interactions between macroalgae and cyanobacteria in the aquatic reservoirs.

Ecological significance of Ulva intestinalis allelochemicals and possibilities of their application

Direct competitive interaction between species is one of the major causes of plant extinction worldwide (Jarchow, Cook, 2009). Competition for available resources is often considered the main competitive mechanism that influences the success of some organisms. Studies have shown that the green algae *Ulva* sp., which have a high surface area to volume ratio, exhibit high rates of nutrient uptake. However, *Ulva* sp. have limited ability to concentrate and store nitrogen internally (Xu et al., 2012). Furthermore, Tait and Schiel (2011) indicated that light intensity plays an important role in macroalgae productivity. The authors noted that high densities of the brown alga *Sargassum muticum* (Yendo) Fensholt exclude native species and reduce biodiversity by shading the associated microalgae (White, Shurin, 2011). In contrast, Svirski et al. (1993) found that growth inhibition of *Gracilaria* sp. cultured in the presence of *U. lactuca* was not due to shading or nutrient depletion but appeared to be caused by competition for inorganic carbon through the production of allelopathic compounds. Nan et al. (2004) found that it is difficult to distinguish competition for available resources from allelopathic interactions in the natural aquatic habitats.

Therefore, the authors in their study focused on performing experiments with the addition of filtrate obtained from *U. pertusa* to exclude the effect of competition for available nutrients in the culture and the effect of elevated pH. The authors showed that the growth of *H. akashiwo* and *S. costatum* was completely inhibited, even with high nutrient availability. Therefore, competition for nutrients cannot explain the growth inhibition of the tested microalgae by *U. pertusa*. The experiments conducted in the present study were refined to be able to exclude the effect of competition for resources. In the work described above, the experiments were also conducted using mineral medium f/2. Hence, the effect of nutrient depletion as a cause of growth inhibition of the cyanobacteria studied in our work can also be excluded.

Macroalgae are known for their good nutrient uptake capacity (Valiela et al., 1997; Neori et al., 2004; Zertuche-González et al., 2009) and are intentionally used in many parts of the world to reduce nutrient levels in coastal waters (Neori et al., 2004; Carmona et al., 2006; Chopin et al., 2001, 2008). Many bloom-forming species are directly or indirectly promoted by nutrients (Heisler et al., 2008; Anderson et al., 2008), thus macroalgae may reduce the occurrence of these species by decreasing the levels of nutrients available in the ecosystem. However, experiments conducted by Tang and Gobler (2011) showed that the observed negative effects of *U. lactuca* on bloom-forming species were not due to nutrient limitation of microalgae or nutrient competition between micro- and macroalgae. In the present study, the studied cyanobacteria grown on a nutrient-sufficient mineral medium, so it can be concluded that the negative effect of *U. intestinalis* was the result of the allelopathic compounds released into the medium.

Macroalgae belonging to the genus *Ulva* are widely distributed. Harvesting these macroalgae from shorelines provides an easy and environmentally friendly way to potentially control the species responsible for creating massive blooms (Jeong et al., 2000). Our results have shown that *U. intestinalis* can produce and secrete some allelopathic compounds that are able to inhibit the growth of the common, bloom-forming cyanobacteria.

Conflict of interest

The authors declare no conflict of interest related to this article.

Acknowledgements

The authors would like to thank the editor and two anonymous reviewers for their valuable comments and suggestions to improve the quality of the paper. This research was funded by UGrants-start, grant number 533-0C20-GS03-22.

References

Anderson, D.M., Burkholder, J.M., Cochlan, W.P., Glibert, P.M., Gobler, C.J., Heil, C.A., Raphael, M.K., Parsons, M.L., Rensel, J.E., Townsend, D.W., Trainer, V.L., Vargo, G. A. (2008). Harmful algal blooms and eutrophication: examining linkages from selected coastal regions of the United States. *Harmful algae*, *8(1)*, 39–53. https://doi.org/10.1016/j.hal.2008.08.017

Berger, J., Schagerl, M. (2003). Allelopathic activity of *Chara aspera*. *Hydrobiologia*, *501(1)*, 109–115. https://doi.org/10.1023/A:1026263504260

Budzałek, G., Śliwińska-Wilczewska, S., Klin, M., Wiśniewska, K., Latała, A., Wiktor, J.M. (2021a). Changes in growth, photosynthesis performance, pigments, and toxin contents of bloom-forming cyanobacteria after exposure to macroalgal allelochemicals. *Toxins*, *13(8)*, 589. https://doi.org/10.3390/toxins13080589

Budzałek, G., Śliwińska-Wilczewska, S., Wiśniewska, K., Wochna, A., Bubak, I., Latała, A., Wiktor, J.M. (2021b). Macroalgal defense against competitors and herbivores. *International Journal of Molecular Sciences*, *22(15)*, 7865. https://doi.org/10.3390/ijms22157865

Budzałek, G., Śliwińska-Wilczewska, S., Latała, A. (2018). Allelopathic effect of *Ulva intestinalis* L. on the Baltic filamentous cyanobacterium *Nostoc* sp. *Annales Universitatis Paedagogicae Cracoviensis Studia Naturae*, *3*, 80–89. http:doi.org/10.24917/25438832.3.6. ISSN 2543–8832.

Campbell, D., Hurry, V., Clarke, A.K., Gustafsson, P., Öquist, G. (1998). Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. *Microbiology and Molecular Biology Reviews*, *62*, 667–683. https://doi.org/10.1128/mmbr.62.3.667–683.1998

Carmona, R., Kraemer, G.P., Yarish, C. (2006). Exploring Northeast American and Asian species of *Porphyra* for use in an integrated finfish–algal aquaculture system. *Aquaculture*, *252(1)*, 54–65. https://doi.org/10.1016/j.aquaculture.2005.11.049

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-Gonzalez, J., Yarish, C., Neefus, C. (2001). Integrating seaweeds into marine aquaculture systems: a key toward sustainability. *Journal of Phycology*, *37(6)*, 975–986. https://doi.org/10.1046/j.1529–8817.2001.01137.x

Chopin, T., Robinson, S.M.C., Troell, M., Neori, A., Buschmann, A., Fang, J. G. (2008). Ecological engineering: multi-trophic integration for sustainable marine aquaculture. In: *Encyclopedia of Ecology*. Amsterdam: Elsevier.

Drobnik, J. (2007). *Zielnik i zielnikoznastwo (Herbarium and studies of herbarium)*. PWN, Warszawa. 293 pp. [In Polish]

Guillard, R.R., Sieracki, M.S. (2005). Counting cells in cultures with the light microscope. *Algal Culturing Techniques*, 239–252. https://doi.org/10.1016/B978-012088426-1%2F50017-2

Guillard, R.R.L. (1975). Culture of phytoplankton for feeding marine invertebrates. In: W.L. Smith, M.H. Chanley (eds.), *Culture of Marine Invertebrate Animals*. New York, USA: Plenum Press, 26–60. https://doi.org/10.1007/978-1-4615-8714-9_3.

Harlin, M.M., Rice, E.L. (1987). Allelochemistry in marine macroalgae. *Critical Reviews in Plant Sciences*, *5(3)*, 237–249. https://doi.org/10.1080/07352688709382241

Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W.C., Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H.G., Seller, K., Stockwell, D.A., Stoecker, D.K., Suddleson, M. (2008). Eutrophication and harmful algal blooms: a scientific consensus. *Harmful algae*, *8(1)*, 3–13. https://doi.org/10.1016/j.hal.2008.08.006

Irfanullah, H.M., Moss, B. (2005). Allelopathy of filamentous green algae. *Hydrobiologia*, *543(1)*, 169–179. https://doi.org/10.1007/s10750-004-6955-8

Jarchow, M.E., Cook, B.J. (2009). Allelopathy as a mechanism for the invasion of *Typha angustifolia*. *Plant Ecology*, *204(1)*, 113–124. https://doi.org/10.1007/s11258-009-9573-8

Jeong, J.H., Jin, H.J., Sohn, C.H., Suh, K.H., Hong, Y.K. (2000). Algicidal activity of the seaweed *Corallina pilulifera* against red tide microalgae. *Journal of applied Phycology*, *12(1)*, 37–43. https://doi.org/10.1023/A:1008139129057

Jin, Q., Dong, S. (2003). Comparative studies on the allelopathic effects of two different strains of *Ulva pertusa* on *Heterosigma akashiwo* and *Alexandrium tamarense*. *Journal of Experimental Marine Biology and Ecology*, *293(1)*, 41–55. http://dx.doi.org/10.1016/S0022-0981(03)00214–4

Jin, Q., Dong, S., Wang, C. (2005). Allelopathic growth inhibition of *Prorocentrum micans* (Dinophyta) by *Ulva pertusa* and *Ulva linza* (Chlorophyta) in laboratory cultures. *European Journal of Phycology*, *40(1)*, 31–37. https://doi.org/10.1080/09670260400019741

Kahru, M., Elmgren, R., Kaiser, J., Wasmund, N., Savchuk, O. (2020). Cyanobacterial blooms in the Baltic Sea: Correlations with environmental factors. *Harmful Algae*, *92*, 101739. https://doi.org/10.1016/j.hal.2019.101739

Kakisawa, H., Asari, F., Kusumi, T., Toma, T., Sakurai, T., Oohusa, T., Hara, Y., Chiharai, M. (1988). An allelopathic fatty acid from the brown alga *Cladosiphon okamuranus*. *Phytochemistry*, *27*, 731–735. https://doi.org/10.1016/0031–9422(88)84084–6

Latała, A., Jodłowska, S., Pniewski, F. (2006). Culture Collection of Baltic Algae (CCBA) and characteristic of some strains by factorial experiment approach. *Algological Studies/Archiv für Hydrobiologie*, *122*, 137–154. http://dx.doi.org/10.1127/1864–1318/2006/0122–0137

Lewis Jr, W.M., (1986). Evolutionary interpretations of allelochemical interactions in phytoplankton algae. *The American Naturalist*, *127*, 184–194.

Maehnert, B., Schagerl, M., Krenn, L. (2017). Allelopathic potential of stoneworts. Fottea, *Olomouc*, *17(2)*, 137–149. http://dx.doi.org/10.5507/fot.2016.024

Molisch, H. (1937). *Der Einfluss einer Pflanze auf die andere, Allelopathie* (*The influence of one plant on the other, allelopathy*). Jena: Gustav Fischer. https://doi.org/10.1038/141493a0

Mulderij, G., Van Donk, E., Roelofs, J.G.M. (2003). Differential sensitivity of green algae to allelopathic substances from *Chara*. *Hydrobiologia*, *491(1–3)*, 261–271. https://doi.org/10.1023/A:1024483704903

Nan, C., Zhang, H., Zhao, G. (2004). Allelopathic interactions between the macroalga *Ulva pertusa* and eight microalgal species. *Journal of Sea Research*, *52(4)*, 259–268. https://doi.org/10.1016/j.seares.2004.04.001

Neori, A., Chopin, T., Troell, M., Buschmann, A. H., Kraemer, G. P., Halling, C., Shpigel, M., Yarish, C. (2004). Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture*, *231(1–4)*, 361–391. https://doi.org/10.1016/j.aquaculture.2003.11.015

Pakdel, F.M., Sim, L., Beardall, J., Davis, J. (2013). Allelopathic inhibition of microalgae by the freshwater stonewort, *Chara australis*, and a submerged angiosperm, *Potamogeton crispus*. *Aquatic Botany*, *110*, 24–30. https://doi.org/10.1016/j.aquabot.2013.04.005

Reichwaldt, E.S., Ghadouani, A. (2012). Effects of rainfall patterns on toxic cyanobacterial blooms in a changing climate: between simplistic scenarios and complex dynamics. *Water Research*, *46(5)*, 1372–1393. https://doi.org/10.1016/j.watres.2011.11.052

Rybak, A.S. (2018). The *Ulva flexuosa* complex (Ulvaceae, Chlorophyta): An updated identification key with special reference to the freshwater and hyperhaline taxa. *Phytotaxa, 345(2)*, 83–103.

Schreiber, U., Endo, T., Mi, H., Asada, K. (1995). Quenching analysis of chlorophyll fluorescence by the saturation pulse method: particular aspects relating to the study of eukaryotic algae and cyanobacteria. *Plant and Cell Physiology*, *36*, 873–882.

Škaloud, P., Rindi, F., Boedeker, C., Leliaert, F. (2018). *Freshwater Flora of Central Europe*, Vol 13: Chlorophyta: Ulvophyceae (Süßwasserflora von Mitteleuropa, Bd. 13: Chlorophyta: Ulvophyceae). Springer Berlin Heidelberg, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-55495-1

Song, H., Lavoie, M., Fan, X., Tan, H., Liu, G., Xu, P., Fu, Z., Paerl, H.W., Qian, H. (2017). Allelopathic interactions of linoleic acid and nitric oxide increase the competitive ability of *Microcystis aeruginosa*. *The ISME Journal*, *11*, 1865–1876. https://doi.org/10.1038/ismej.2017.45

Starmach, K., 1972. *Filamentous green algae*. Freshwater flora of Poland, PWN, Warszawa-Kraków, 163 pp.

Stebbing, A.R.D. (1982). Hormesis – the stimulation of growth by low levels of inhibitors. *Science of the Total Environment*, *22*, 213–234. https://doi.org/10.1016/0048–9697(82)90066–3

Surakka, A., Sihvonen, L.M., Lehtimäki, J.M., Wahlsten, M., Vuorela, P., Sivonen, K. (2005). Benthic cyanobacteria from the Baltic Sea contain cytotoxic *Anabaena, Nodularia*, and *Nostoc* strains and an apoptosis-inducing *Phormidium* strain. *Environmental Toxicology: An International Journal*, *20(3)*, 285–292. https://doi.org/10.1002/tox.20119

Svirski, E., Beer, S., Friedlander, M. (1993). *Gracilaria conferta* and its epiphytes:(2) Interrelationship between the red seaweed and *Ulva* cf. *lactuca*. *Hydrobiologia*, *260(1)*, 391–396. https://doi.org/10.1007/BF00049046

Śliwińska-Wilczewska, S., Wiśniewska, K., Konarzewska, Z., Cieszyńska, A., Felpeto, A., Lewandowska, A.U., Latała, A. (2021). The current state of knowledge on taxonomy, modulating factors, ecological roles, and mode of action of phytoplankton allelochemicals. *Science of the Total Environment*, *773*, 145681. https://doi.org/10.1016/j.scitotenv.2021.145681

Tait, L.W., Schiel, D.R. (2011). Dynamics of productivity in naturally structured macroalgal assemblages: importance of canopy structure on light-use efficiency. *Marine Ecology Progress Series*, *421*, 97–107.

Tang, Y.Z., Gobler, C.J. (2011). The green macroalga, *Ulva lactuca*, inhibits the growth of seven common harmful algal bloom species via allelopathy. *Harmful Algae*, *10(5)*, 480–488. https://doi.org/10.1016/j.hal.2011.03.003

Uchida, M., Aoyama, Y., Eda, N., Ohta, A. (1995). Investigation of the microstructure in the catalyst layer and effects of both perfluorosulfonate ionomer and PTFE-loaded carbon on the catalyst layer of polymer electrolyte fuel cells. *Journal of the Electrochemical Society*, *142(12)*, 4143. https://doi.org/10.1149/1.2048477

Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D., Foreman, K. (1997). Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography*, *42(5part2)*, 1105–1118. https://doi.org/10.4319/lo.1997.42.5_part_2.1105

Van Donk, E., van de Bund, W.J. (2002). Impact of submerged macrophytes including charophytes on phyto-and zooplankton communities: allelopathy versus other mechanisms. *Aquatic Botany*, *72(3–4)*, 261–274. https://doi.org/10.1016/S0304-3770(01)00205–4

Wang, R., Xiao, H., Zhang, P., Qu, L., Cai, H., Tang, X. (2007). Allelopathic effects of *Ulva pertusa*, *Corallina pilulifera* and *Sargassum thunbergii* on the growth of the dinoflagellates *Heterosigma akashiwo* and *Alexandrium tamarense*. *Journal of Applied Phycology*, *19*, 109–121. https://dx.doi.org/10.1007%2Fs10811-006-9117-8

Wasmund, N. (1997). Occurrence of cyanobacterial blooms in the Baltic Sea in relation to environmental conditions. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, *82(2)*, 169–184. https://doi.org/10.1002/iroh.19970820205

White, L. F., Shurin, J. B. (2011). Density dependent effects of an exotic marine macroalga on native community diversity. *Journal of Experimental Marine Biology and Ecology, 405(1–2)*, 111–119. http://dx.doi.org/10.1016/j.jembe.2011.05.024

Xu, D., Gao, Z., Zhang, X., Fan, X., Wang, Y., Li, D., Wei, W., Zhimeng, Z., Ye, N. (2012). Allelopathic interactions between the opportunistic species *Ulva prolifera* and the native macroalga *Gracilaria lichvoides*. *PlosOne*, *7(4)*. https://doi.org/10.1371/journal.pone.0033648

Zertuche-González, J.A., Camacho-Ibar, V.F., Pacheco-Ruíz, I., Cabello-Pasini, A., Galindo-Bect, L.A., Guzmán-Calderón, J.M., Carranza-Macias, V., Espinoza-Avalos, J. (2009). The role of *Ulva* spp. as a temporary nutrient sink in a coastal lagoon with oyster cultivation and upwelling influence. *Journal of Applied Phycology*, *21(6)*, 729. https://doi.org/10.1007/s10811-009-9408-y

Zillén, L., Conley, D.J. (2010). Hypoxia and cyanobacterial blooms are not natural features of the Baltic Sea. *Biogeosciences Discussions*, *7(2)*, 1783–1812. http://dx.doi.org/10.5194/bgd-7-1783-2010

Złoch, I., Śliwińska-Wilczewska, S., Kucharska, M., Kozłowska, W. (2018). Allelopathic effects of *Chara species* (*C. aspera*, *C. baltica*, and *C. canescens*) on the bloom-forming picocyanobacterium *Synechococcus* sp. *Environmental Science and Pollution Research*, *25*, 36403–36411. https://doi.org/10.1007/s11356-018-3579-5

Abstract

Macroalgae have been found to produce active allelochemicals that inhibit the growth of other organisms that compete with them for light and space. However, their allelopathic activity on Baltic cyanobacteria is still insufficiently recognised. Therefore, this study aimed to demonstrate the allelopathic effects of Baltic macroalga thallus (*Ulva intestinalis*) on the growth and photosynthetic activity of three bloom-forming cyanobacteria: *Aphanizomenon* sp., *Nodularia spumigena*, and *Nostoc* sp. This study investigated the cell count of the analysed cyanobacteria (N 105 mL^{-1}), the maximum quantum yield of the second photosystem (PSII) in the dark (Fv/Fm), and the real quantum yield of PSII in the light (ΦPSII) (in the control and the experiments). After 7 days of exposure, the following samples were added: 0.01, 0.05, and 0.1 g mL^{-1} of *U. intestinalis* fresh thallus. It was found that thallus obtained from *U. intestinalis* had no statistically significant effect on the number of cells of the cyanobacterium *Aphanizomenon* sp. (at 0.05 and 0.1 g mL^{-1}) and *Nostoc* sp. (at concentrations of 0.01 and 0.05 g mL^{-1}). On the other hand, it was examined a stimulating effect of 0.01 g mL^{-1} of the fresh thallus on the number of *Aphanizomenon* sp. cells which constituted 168%, relative to the control. It was shown that the fresh thallus addition resulted in a decrease in the number of *N. spumigena* cells (45%, 27%, and 46% after addition of 0.01, 0.05, and 0.1 g wet weight mL^{-1} of fresh thallus, respectively). In experiments with *Nostoc* sp., the addition of *U. intestinalis* thallus caused a negative effect on cyanobacterial growth at 0.1 g mL^{-1} and constituted 97% of control. It was also found, that *U. intestinalis* had no allelopathic effect on fluorescence parameters of *N. spumigena*. All tested concentrations of thallus *U. intestinalis* (0.01, 0.05, and 0.1 g wet weight mL^{-1}) stimulated the values of Fv/Fm or ΦPSII of cyanobacteria *Aphanizomenon* sp. and *Nostoc* sp. compared to the control. These studies help define the role of *U. intestinalis* allelopathy as a biological factor in the distribution of bloom-forming cyanobacteria in the coastal Baltic Sea region.

Key words: allelopathy, cyanobacteria, green macroalgae, growth, fluorescence, thallus

Received: [2021.11.05]

Accepted: [2022.03.22]

Wpływ allelopatyczny *Ulva intestinalis* na wybrane gatunki bałtyckich sinic

Streszczenie

Makroglony mają zdolność do produkowania aktywnych związków allelopatycznych, które wpływają na inne organizmy, konkurujące z nimi o światło i przestrzeń. Jednakże ich działanie allelopatyczne na bałtyckie sinice jest wciąż niedostatecznie rozpoznane. Dlatego głównym celem niniejszej pracy było wykazanie aktywności allelopatycznej plechy bałtyckiej zielenicy, ulwa (lub taśma czy błonica) kiszkowata – *Ulva intestinalis*, na wzrost i aktywność fotosyntetyczną trzech sinic bałtyckich, tworzących masowe zakwity: *Aphanizomenon* sp., *Nodularia spumigena* oraz *Nostoc* sp. W niniejszej pracy badano liczebność komórek analizowanych sinic (N 105 mL^{-1}), maksymalną wydajność kwantową drugiego fotosystemu (PSII) w ciemności (*Fv*/*F*m) oraz rzeczywistą wydajność kwantową PSII w świetle (ΦPSII) (w kontroli oraz w eksperymentach). Po 7 dniach ekspozycji, dodawano do nich: 0,01, 0,05, i 0,1 g mL^{-1} świeżej plechy *U. intestinalis*. Wykazano, że obecność plech *U. intestinalis* nie miała istotnego wpływu na liczebność komórek *Aphanizomenon* sp. w ilości (0,05 i 0,1 g mL^{-1}) oraz *Nostoc* sp. w ilości plechy (0,01 i 0,05 g mL^{-1}). Wykazano także stymulujący wpływ plechy dodawanej w najmniejszej ilości 0,01 g mL^{-1} na liczebność komórek *Aphanizomenon* sp., która wynosiła 168%, w stosunku do kontroli. Dodanie 0,01, 0,05 oraz 0,1 g mL^{-1} świeżej plechy powodowało obniżenie liczebności komórek *N. spumigena* (o odpowiednio: 45%, 27%, i 46%, w stosunku do kontroli). Dodatkowo, dodanie 0,1 g mL^{-1} plechy *U. intestinalis* powodowało zahamowanie wzrostu *Nostoc* sp. o 97%. W pracy wykazano także, że plecha *U. intestinalis* nie miała wpływu na wartość parametrów fluorescencji u *N. spumigena*. Plecha tego gatunku (w każdym testowanym stężeniu: 0,01, 0,05 oraz 0,1 g mL^{-1}) stymulowała wartość parametrów *Fv*/*F*m lub ΦPSII u sinic *Aphanizomenon* sp. i *Nostoc* sp. Wyniki uzyskane w niniejszej pracy definiują rolę allelopatii *U. intestinalis* jako ważnego czynnika biologicznego, wpływającego na występowanie zakwitów sinic w przybrzeżnych rejonach Morza Bałtyckiego.

Słowa kluczowe: allelopatia, sinice, zielenice, wzrost, fluorescencja, plecha

Information on the authors

Gracjana Budzałek

The field of her interest is allelopathic interactions between macroalgae and other phytoplankton species. In her research she focuses mostly on Baltic species from Puck Bay. She invastigates what influence of allelopathic compounds produced by macroalgae have on bloom-forming cyanobacteria and microalgae.

Sylwia Śliwińska-Wilczewska https://orcid.org/0000-0002-3147-6605

She is interested in allelopathy of cyanobacteria and microalgae; in particular of picocyanobacteria *Synechococcus* sp. Allelopathy plays an important role in interspecific competition and contributes to cyanobacterial bloom maintenance. In her study, the influence of allelochemicals on the growth, chlorophyll fluorescence and photosynthesis irradiance curves of different phytoplankton species was investigated. She also invastigates what influences have environmental factors on produced allelopathic compounds on algae and cyanobacteria.