ORIGINAL RESEARCH ARTICLE

Effects of oxytetracycline on growth and chlorophyll *a* fluorescence in green algae (*Chlorella vulgaris*), diatom (*Phaeodactylum tricornutum*) and cyanobacteria (*Microcystis aeruginosa* and *Nodularia spumigena*)

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Summary The study aimed at measuring the influence of a wide range of oxytetracycline concentrations, with particular attention to the low levels of the antibiotic on cyanobacteria *Microcystis aeruginosa* and *Nodularia spumigena*, diatom *Phaeodactylum tricornutum* and the model green algae *Chlorella vulgaris* by conducting prolonged toxicity tests (lasting 10 days). Standard measurements (cell number, optical density, chlorophyll *a* concentration) were combined with photosynthetic parameters measurements. The obtained results show that concentrations of oxytetracycline present in the environment can affect tested microorganisms. It was found to decrease photosystem II efficiency and disrupt the photosynthesis process. A careful interpretation of photosynthetic parameters allowed a better understanding of the mode of action of oxytetracycline in relation to non-target photoautotrophic organisms like cyanobac-

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teria and microalgae. In conclusion, it would appear that the use of standard chronic toxicity tests (72 h) does not allow to accurately and reliably assess the chronic impact of bioactive compounds including drugs and their metabolites on water organisms. On this basis, we recommend the application of extended duration tests.

1. Introduction

The consumption of pharmaceuticals in terms of quantity and expenditure is increasing globally (Van Boeckel et al., 2014; Xu et al., 2014). Antibiotics have been broadly used in human and veterinary medicine to prevent and treat infections, as well as for other purposes including growth promotion in livestock production, prevention of bacteria-induced crop damage and as feed additives in aquacultures (Boxall et al., 2004). The most important pathways of antibiotic dissemination into the aquatic environment are wastewater effluents, manure application, aquacultures discharge and surface runoff (Kümmerer, 2009; McArdell et al., 2003). The majority of studies focused on the prevalence of antibiotics in freshwater bodies (lakes, rivers, groundwater). However several studies reported their presence in coastal or estuarine marine systems (e.g. Björlenius et al., 2018; Borecka et al., 2013, 2015, Gulkowska et al., 2007; Nödler et al., 2014; Pazdro et al., 2016; Siedlewicz et al., 2014, 2016, 2018; Wille et al., 2010, Yang 2011). The constant release of antibiotics, their bioactive properties, and continuous presence even at relatively low environmental concentrations ranging from ng dm^{-3} to μg dm^{-3} in surface waters and from ng g^{-1} to μg g^{-1} in sediments have caused major concerns about potential risks to aquatic ecosystems (Hernando et al., 2006; Ji et al., 2012; Kümmerer, 2010). Although the major environmental issue is associated with the development of resistance mechanisms in bacteria, the toxicity of antibiotics to non-target organisms also raises serious concerns (González-Pleiter et al., 2013). Phytoplankton species forming the basis of all marine food webs are extremely important as primary producers and shall be included in the risk group. For this reason, data from ecotoxicity tests with different phytoplankton species and the most commonly detected antibiotics is of great importance to illustrate the potential adverse effects. Nevertheless, most of the numerous available reports on the ecotoxicity of pharmaceuticals deal with freshwater organisms and acute ecotoxicity studies still predominate in comparison to the chronic ones (Arnold et al., 2014; Santos et al., 2010). It may also contribute to the increase of inaccuracy of the estimations of risk assessments often relayed on recalculations based on different backgrounds and/or trophic levels (EC, 2003).

Oxytetracycline is a bacteriostatic drug belonging to a group of tetracyclines being a class of inexpensive, broad-spectrum antimicrobial substances used in human and veterinary medicine. Antibiotics from this group are commonly used worldwide including all Baltic Sea countries (Schmidt et al., 2000; Wang et al. 2010). Oxytetracycline does not undergo metabolism and is renally and biliary excreted in unchanged form (Agwuh and MacGowan, 2006). In the eu-

photic zone of marine waters, oxytetracycline seems to easily photodegrade (Leal et al., 2016) which corresponds with the results from laboratory tests (Jiao et al., 2008; Pereira et al., 2011). Despite this, oxytetracycline is very often detected in the marine environment (Capone et al. 1996; Chen et al., 2015; Lalumera et al., 2004). Moreover, existing data suggest a long persistence of oxytetracycline in the aquatic sediments (Wang et al., 2010). In our previous studies concerning the Baltic Sea sediments, oxytetracycline concentration up to 1.515 μg g^{-1} d.w. was reported, which was the highest value of all analyzed antibiotics in this region (Siedlewicz et al., 2018). Moreover, except results described by Ando et al. (2007), Guo et al. (2016) and Holten Lützhøft et al. (1999) most of existing ecotoxicological tests applying algae and cyanobacteria typically do not exceed the exposure period of 96 hours (Fu et al., 2017; Kolar et al., 2014; Kołodziejska et al., 2013). Therefore the full impact of oxytetracycline on aquatic phototrophic organisms and their ability to effectively carry out photosynthesis remains highly ambiguous. Furthermore, it can be assumed that oxytetracycline is continuously introduced to the environment so it is worth to know, how or if at all, it can affect phototrophic microorganisms in longer than reported in the literature period (up to 7 days) used in chronic tests (Dewez et al., 2018; Halling-Sørensen, 2000).

The photosynthetic apparatus efficiency has been reported to be the most sensitive factor for evaluating the degree of antibiotic-related stress damage in higher plants (Rachmilevitch et al., 2006). Recently, changes in the chlorophyll *a* fluorescence transient (OJIP transient) have been used to evaluate the extent of damage to the photosynthetic apparatus under several abiotic stresses. The OJIP transient is defined by O, J, I, and P steps, corresponding to the redox states of photosystem II (PSII) and photosystem I (PSI) and the efficiencies of electron transfer through the intersystem chain to the end electron acceptors at the PSI acceptor side (Strasser et al., 2000, 2004). Besides, the OJIP transient approach has useful and practical advantages: it is non-destructive, relatively easy to perform and allows rapid testing of any type of chlorophyll-containing sample regardless of its form (Strasser et al., 2000, 2004; Zushi et al., 2012). As it has been previously reported in the literature, the measurement of chlorophyll *a* fluorescence is a sensitive and reliable method of detection and quantification of organic pollution stress-induced changes in the PSII and PSI of plants (Chen et al., 2017; Gao and Tam, 2011; Pan et al. 2008; Żak et al. 2012). Despite this, so far only a few studies have been conducted on the impact of oxytetracycline or tetracycline on the photosynthesis system of marine autotrophic microorganisms (Guo et al. 2016; Van der Grinten et al., 2010; Xu et al. 2019).

The study aimed at measuring the influence of a wide range of oxytetracycline concentrations, with particular attention to levels of the antibiotic occurring in the Baltic Sea sediments, on selected algae and cyanobacteria by performing long-term exposure toxicity tests (10—11 days) lasting longer than standard toxicity tests. The extending of the toxicity test period may help to assess their utility in the estimation of the impact of contaminants continuously introduced to the environment. Microalgae and cyanobacteria have been selected due to their ecological relevance, sensitivity and because they are easily cultivated in the laboratory. *Chlorella vulgaris* Beijerink is a cosmopolitan species inhabiting fresh, brackish and marine waters and is commonly used as a reference organism in ecotoxicity tests (Borecka et al., 2016). *Phaeodactylum tricornutum* Bohlin is a model diatom representing a heterogeneous group of photosynthetic eukaryotes prevalent in freshwater and marine habitats. *Nodularia spumigena* Mertens ex Bornet & Flahault is a bloom-forming, filamentous cyanobacterium occurring in many brackish and estuarine waters worldwide. *Microcystis aeruginosa* Kützing is another marine/freshwater widespread cyanobacteria, which can form harmful blooms and is characterized by small cells usually organized into colonies. These four species were selected based on their occurrence in the Baltic Sea as well as their ubiquitous presence in aquatic ecosystems. In presented investigations standard measurements (optical density, chlorophyll *a* concentration) were combined with OJIP fluorescence transitions measurements, therefore results should also help in understanding the impact of oxytetracycline on photosynthesis apparatus in different species of autotrophic microorganisms.

2. Material and methods

2.1. Algal and cyanobacterial monocultures

As target species, we used: (1) green algae A1-76 *C. vulgaris* (Culture Collection IO PAN, Sopot) isolated from the Baltic Sea; (2) diatom *P. tricornutum* SAG 1090-1a (Culture Collection of Algae, University of Göttingen, Germany); (3) cyanobacteria PCC-7820 *M. aeruginosa* purchased from Pasteur Culture Collection of Cyanobacteria (Pasteur Institute, France) and CCNP1401 *N. spumigena* obtained from Division of Marine Biotechnology, University of Gdańsk. All species were grown on a liquid medium in the flasks (batch cultures) at 22°C ± 1, at continuous light (80 μmol photons m^{-2} s^{-1}). Cyanobacterial species were cultivated on: *M. aeruginosa* — 616 medium and *N. spumigena* — BG11 medium in salinity 7‰ (Stanier et al., 1971). For the culture of green algae *C. vulgaris* BBM medium was used (Bischoff and Bold, 1963) and for diatom *P. tricornutum* f/2 medium (salinity 7 ‰) (Guillard, 1975) prepared based on artificial seawater (Lyman and Fleming, 1940) was applied. Cultures used as inocula were in the logarithmic phase of growth.

2.2. Growth assay

Oxytetracycline standards were purchased from Sigma-Aldrich (Germany). Standard stock solutions were prepared in Milli-Q water before the start of the experiments.

Four experiments were performed, each on different species of microorganisms. Sterile cell-free medium with oxytetracycline was added to flasks containing target species *C. vulgaris*, *P. tricornutum*, *M. aeruginosa*, and *N. spumigena*. The total volume of medium in the flasks was 49 cm^3 and the inoculum of *C. vulgaris* corresponded to 4×10^5 cells cm^{-3}; initial optical densities (OD, $\lambda=680$) of *P. tricornutum*, *M. aeruginosa*, and *N. spumigena* equalled to 0.08, 0.06 and 0.06 respectively. The oxytetracycline was added to the flasks with green algae, diatom and cyanobacteria in the volume of 1 cm^3 reaching the five final concentrations: 0.01 μg cm^{-3}, 0.1 μg cm^{-3}, 1 μg cm^{-3}, 4 μg cm^{-3}, 8 μg cm^{-3}. Control samples were prepared by adding 1 cm^3 of Mili-Q water to 49 cm^3 of fresh medium with tested species. All variants of the assay were performed in three independent experiments. To be able to determine a real effect of the chronic exposition on the growth and physiology of the microorganism even in low doses of antibiotics, experiments were carried out for 10/11 days. Subsamples were collected for measurements on days 3, 5, 7, 9, 11 (the end of the experiment) and in case of *P. tricornutum* on days 2, 4, 8, 10 (the end of the experiment). The *C. vulgaris* and *P. tricornutum* cells were counted with a Bürker chamber under a light microscope. Samples were diluted if needed. OD measurements were performed using HITACHI U-2800 UV-VIS spectrophotometer for all species: *C. vulgaris*, *M. aeruginosa*, *N. spumigena* and *P. tricornutum*. Simultaneously, oxytetracycline stability (nominal versus measured concentration) was monitored. Samples with medium and oxytetracycline at concentrations: 1 μg cm^{-3}, 4 μg cm^{-3}, 8 μg cm^{-3} were incubated applying the same experimental conditions as described earlier, and as a control — medium kept in the dark for the same period was used. Samples were analysed by absorbance measurements using HITACHI U-2800 UV-VIS spectrophotometer ($\lambda=355$). The growth rate was calculated based on OD value on the equation given by Karło et al. (2015) and Krzemińska et al. (2014).

2.3. Chlorophyll *a* and pheophytin concentration

Chlorophyll *a* and pheophytin concentration was determined using the spectrophotometric method according to Strickland and Parsons (1968, 1972) and Edler (1979). Aliquots of culture medium with live cells were filtered through the glass-fibre filters (GF/C Whatman). Next, filters were homogenised and extracted in 90% acetone for 2 h at 4°C in the dark. After extraction samples were centrifuged (3800g, 20 min) and the supernatant was collected. In order to estimate the concentration of pheophytin, the measurements were performed after 2 min following the addition of 60 μl of 1M HCl to 5 cm^3 of supernatant. The absorbance measurements for chlorophyll *a* and pheophytin were recorded by HITACHI U-2800 UV-VIS spectrophotometer at wavelengths: 750, 665, 645, 630 nm and 750, 665 nm respectively. Concentrations of both chlorophyll *a* and pheophytin were calculated according to Lorenzen (1967).

2.4. Fluorescence measurements

All fluorescence measurements were made using ultra-high sensitive (up to 0.5 μg chl dm^{-3}) AquaPen Ap-100C handheld

Table 1 Explanation of chlorophyll *a* fluorescence transient (OJIP transient) parameters. Formulas derived from Strasser et al., 2000.

Formula abbreviation	Formula explanation
F_0	$F_0 = F_{50} \mu s$, fluorescence intensity at 50 μs
F_J	F_J = fluorescence intensity at J-step (at 2 ms)
F_M	F_M = maximal fluorescence intensity
F_V	$F_V = F_M - F_0$ (maximal variable fluorescence)
V_J	$V_J = (F_J - F_0)/(F_M - F_0)$
F_M/F_0	Ratio
F_V/F_0	Ratio
F_V/F_M	Maximum quantum efficiency of PS II
M_0 or $(dV/dt)_0$	$M_0 = TR_0/RC - ET_0/RC = 4 (F300 - F_0)/(F_M - F_0)$
Phi_P0	$Phi\_P_0 = 1 - (F_0/F_M)$ (or F_V/F_M)
Psi_0	$Psi\_0 = 1 - V_J$
Phi_D0	$Phi\_D_0 = 1 - Phi\_P_0 - (F_0/F_M)$
ABS/RC	$ABS/RC = M_0. (1/V_J). (1/Phi\_P_0)$
TR_0/RC	$TR_0/RC = M_0. (1/V_J)$
ET_0/RC	$ET_0/RC = M_0. (1/V_J). Psi\_0$
DI_0/RC	$DI_0/RC = (ABS/RC) - (TR_0/RC)$

fluorometer (Photon Systems Instruments, Czech Republic). Both LED blue and red emitters (blue excitation light 455 nm; red excitation light 620 nm) were used throughout the experiments. The blue emitter is suitable for measuring fluorescence in algal suspensions, while the red emitter is applicable for measuring fluorescence in cyanobacterial cultures. All measurements were carried out in single-use polystyrene cuvettes (maximum volume 4 cm^3, optical length 1 cm) on dark-adapted samples. QY (Quantum Yield) and OJIP (Chlorophyll Fluorescence Induction Kinetics) parameters were quantified according to the formulas described by Strasser et al. (2000) (Table 1). QY is a measure of the photosystem II efficiency. In a dark-adapted sample, it is equivalent to F_V/F_M.

2.5. Data analysis

All results are presented as means ± standard deviation (SD) of three replicates (n = 3). Statistical analysis and comparison of group data were performed with one-way ANOVA and Dunnett's multiple comparison post-hoc test with GraphPad Prism software. Relationships were considered significant when $p < 0.05$.

3. Results

The stability test of oxytetracycline level in medium showed that light conditions used in the experiment did not increase the degradation of the antibiotic to a significant extent. Difference between control kept in the dark and samples did not exceed 10%, furthermore a decrease from an initial con-

centration of oxytetracycline to the end of the experiment did not exceed 32% (Fig. S1) Therefore it can be assumed that during the experiment organisms were constantly exposed to oxytetracycline.

The main part of the work was related to the change in growth, chlorophyll synthesis and photosynthetic processes efficiency to comprehensively assess a physiological state of four investigated microorganisms exposed to oxytetracycline.

The influence of oxytetracycline at given concentration (0.01 μg cm^{-3}, 0.1 μg cm^{-3}, 1 μg cm^{-3}, 4 μg cm^{-3}, 8 μg cm^{-3}) on the growth of *Chlorella vulgaris*, *Phaeodactylum tricornutum*, *Nodularia spumigena*, and *Microcystis aeruginosa*, expressed as a change of the OD values (λ=680 nm) during incubation time, is presented in Fig. 1.

When considering the results of these studies, it could be assumed that concentrations of 8 μg cm^{-3} and 4 μg cm^{-3} would affect analysed microorganisms. On the other hand, concentrations corresponding to 1 μg cm^{-3}, 0.1 μg cm^{-3} and 0.01 μg cm^{-3}, similar to those detected in marine sediments, would be expected to have no or little impact. Indeed, as shown in Fig. 1 the growth of all tested microorganisms at oxytetracycline concentrations of 8 μg cm^{-3}, 4 μg cm^{-3} was significantly reduced after the 5th day of the experiment. On the last day of the measurements, growth of *C. vulgaris*, *P. tricornutum*, *N. spumigena*, *M. aeruginosa* dropped by 78%, 89%, 95%, 93% (at concentration of 8 μg cm^{-3}) and by 52%, 85%, 67%, 92% (at concentration of 4 μg cm^{-3}). No, statistically significant differences of the growth rate compared to the control sample were found for most of the 0.01—0.1 μg cm^{-3} concentrations of oxytetracycline, except the decline of growth of *C. vulgaris* by 13—17% at a concentration of 0.01 μg cm^{-3} on the 5th and 7th day of the experiment. The oxytetracycline concentration equalled to 1 μg cm^{-3} strongly affected the growth of *C. vulgaris* (83%, 78% decline after fifth and seventh day respectively) and *P. tricornutum* (51% decline after 10 days) (Fig. 1). The growth rate for control was on the levels of 0.29—0.20 day^{-1} for *C. vulgaris,* 0.23-0.02 day^{-1} for *P. tricornutum*, 0.38—0.29 day^{-1} for *M. aeruginosa* and 0.35—0.24 day^{-1} for *N. spumigena*. After five days to the end of experiment, for all organisms, in concentration 4 and 8 μg cm^{-3} grow rate was lower than in the control sample and was on the level of 0.19—0.12 day^{-1} for *C. vulgaris*, 0.03—0.00 day^{-1} for *P. tricornutum*, 0.21—0.00 day^{-1} for *M. aeruginosa* and 0.02—0.00 day^{-1} for *N. spumigena*.

A similar tendency was observed for chlorophyll *a* concentration analysed at the end of the experiment (Table 2). The decrease in chlorophyll *a* content was noticed for all studied organisms exposed to concentrations of 8 μg cm^{-3} and 4 μg cm^{-3}: *C. vulgaris* (97% and 79 % decline respectively), *P. tricornutum* (95% and 94%), *N. spumigena* (100% and 98%) and *M. aeruginosa* (85% and 49 %). The significant influence of 1μg cm^{-3} and 0.1μg cm^{-3} oxytetracycline concentrations was observed on *C. vulgaris* (36% and 30% decline respectively) and *N. spumigena* (36%, 23%) and 1μg cm^{-3} on *P. tricornutum* (67%). Changes of phaeopigment and chlorophyll *a* content per cell also show a decrease of the pigments content with increasing oxytetracycline concentration (Table 2). However, in the case of *C. vulgaris* concentration of 0.1 μg cm^{-3} appeared to have a higher impact on chlorophyll *a* per cell value than that of 1 μg cm^{-3}
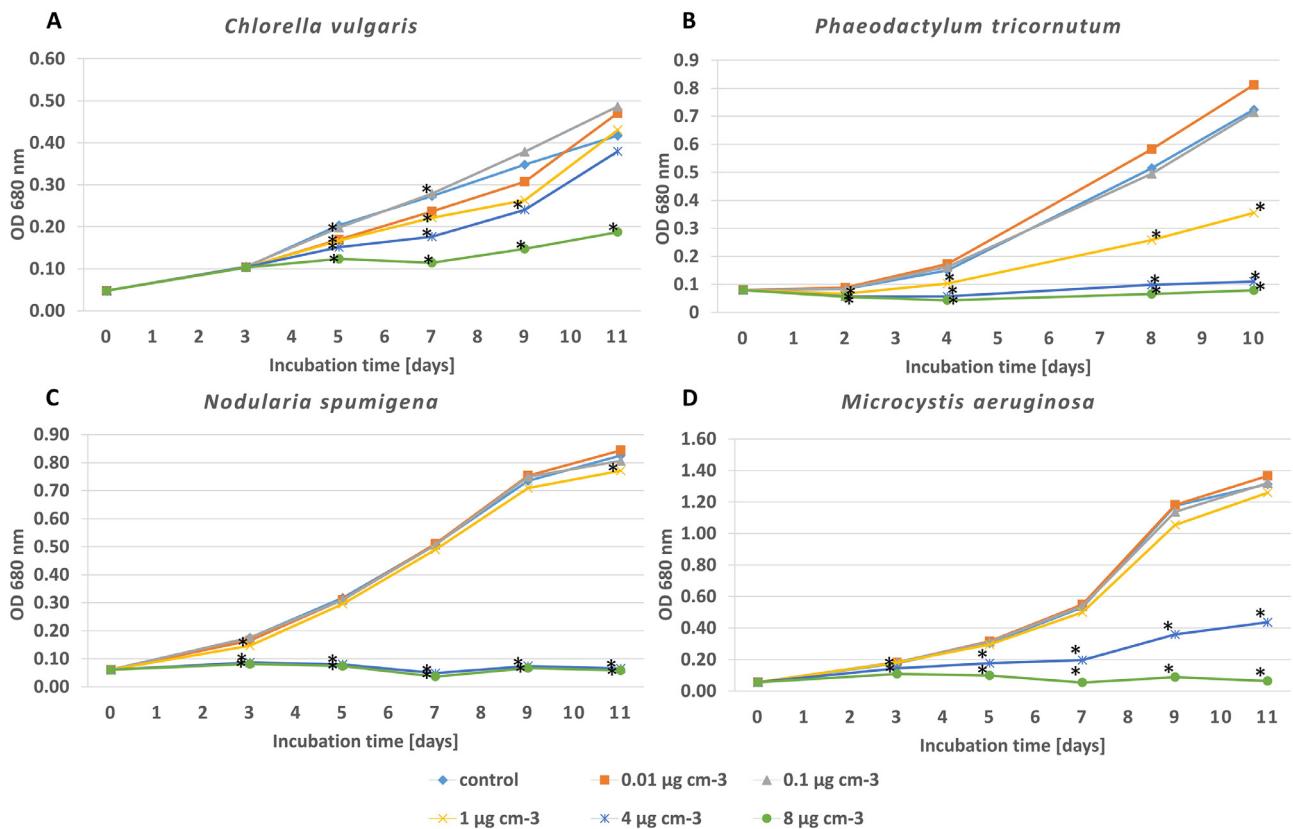
Figure 1 The influence of oxytetracycline on the growth of *Chlorella vulgaris* (A), *Phaeodactylum tricornutum* (B), *Nodularia spumigena* (C) and *Microcystis aeruginosa* (D) expressed as a change of the OD values (λ=680 nm) during incubation time. * differences statistically significant in comparision to the control.

(57% versus 80% of control respectively). This phenomenon was presumably caused by a higher presence of dead cells in $1\mu g$ cm^{-3} oxytetracycline concentration variant. That directly correlates with phaeopigment concentration and is a likely reason for overestimation.

The effect of oxytetracycline on photosynthetic apparatus and photochemistry of the photosystem II was investigated by a fluorescence transient (OJIP) analysis. The measured photosynthetic-fluorescence parameters are presented in Figs. 2, 3, 4 and in supplementary materials (Fig. S2, S3, S4). Changes in parameters in comparison to control were observed already after 72—96 h. However, a longer period of observation (7 and 10—11 days), shows some interesting phenomena about the physiology of the tested microorganisms. It should be mentioned that, after the 7th day of the experiment and/or for concentrations 4 and 8 μg cm^{-3}, in some cases, relatively high errors were observed in measurements. It can be caused by some damage to PSII, and these results are hard to interpret.

One of the most important parameters is F_V/F_M representing the maximum quantum yield of the photosystem II and can be treated as the efficiency of PSII (Fig. 2, Fig. S2). For all investigated organisms, the presence of oxytetracycline in concentrations above 1 μg cm^{-3} caused a significant decrease of F_V/F_M values. In the case of 0.1 μg cm^{-3}, the decrease was observed for *P. tricornutum* during the whole period of the experiment, and for both cyanobacteria in the

early stages of the experiment. Exposure to higher concentrations of oxytetracycline (8 μg cm^{-3} and 4 μg cm^{-3}) resulted in a reduction of F_V/F_M values from 26% to 100% in relation to control, thereby indicating a reduction of PSII ability to carry on photochemical reactions.

The ratio of the harvesting complex absorption per reaction centre (ABS/RC) presented a significant increase (Fig. 3 and S3). The trends in the increase of ABS/RC was observed for all investigated organisms in concentration from 1 μg cm^{-3} and, in case of cyanobacteria, even from 0.01 μg cm^{-3}. However, a statistically significant increase was observed mainly for higher concentrations (4 and 8 μg cm^{-3}) for all tested organisms as well as in the initial phase of an experiment for *P. tricornutum* and *N. spumigena* in concentrations 0.1 and 1 μg cm^{-3}. An interesting observed phenomenon was an intensive increase of ABS/RC value for 4 or/and 8 μg cm^{-3} on a 4—5th day for all species. In the case of *N. spumigena*, the value approached an even 1000% rise. Subsequently, ABS/RC values decreased and stabilized at the end of the experiment. Higher concentrations of oxytetracycline (4 and 8 μg cm^{-3}) have been observed to have a high influence on the effective energy dissipation of an active reaction centre (DI$_0$/RC) (Fig. S5). The increase of ABS/RC and DI$_0$/RC values was detected up to the 5th day of the test, followed by a steady decrease till the eleventh day, when the subsequent values of those parameters were even five-time lower compared to the 5th day of exposure.

Table 2 The influence of oxytetracycline on chlorophyll a and pheopigment concentration in *Chlorella vulgaris*, *Phaeodacty-lum tricornutum*, *Nodularia spumigena*, and *Microcystis aeruginosa* cultures (pigment concentrations measured at the end of the experiment). Data are given as a mean ± SD (n = 3) and % of control (C). Bold values indicate statistically significant differences in comparision to the control.

Oxytetracycline concentration [μg cm^{-3}]	pigments concentration [μg dm^{-3}]				pigment concentration per cell [pg cell^{-1}]	
	active chlorophyll *a*		pheopigment		chlorophyll *a*	
	Average± SD	%C	Average± SD		Average	%C
Chlorella vulgaris						
Control [0]	3743 ± 511	100	656 ± 152		2.43	100
0.01	2723 ± 159	73 ± 4	364 ± 169		1.75	72
0.1	**2618 ± 555**	**70 ± 15**	553 ± 74		**1.39**	57
1	**2387 ± 204**	**64 ± 5**	342 ± 76		**1.94**	80
4	**769 ± 68**	**21 ± 2**	126 ± 33		**1.19**	49
8	**130 ± 20**	**3 ± 1**	69 ± 24		**0.65**	27
Phaeodactylum tricornutum						
Control [0]	1228 ± 125	100	81 ± 51		5.27	100
0.01	1185 ± 113	96 ± 9	84 ± 14		4.43	84
0.1	1092 ± 56	88 ± 4	31 ± 1		2.83	54
1	**409 ± 54**	**33 ± 5**	17 ± 17		**2.92**	55
4	**82 ± 20**	**6 ± 1**	7 ± 8		**1.88**	36
8	**61 ± 3**	**5 ± 0.2**	3 ± 5		**1.82**	34
Nodularia spumigena						
Control [0]	2195 ± 98	100	107 ± 10			
0.01	1992 ± 159	91 ± 7	122 ± 15			
0.1	**1698 ± 0**	**77 ± 0**	104 ± 8			
1	**1396 ± 33**	**64 ± 2**	100 ± 14			
4	**49 ± 2**	**2 ± 1**	10 ± 1			
8	**0 ± 0**	**0 ± 0**	6 ± 0			
Microcystis aeruginosa						
Control [0]	310 ± 52	100	1 ± 1			
0.01	315 ± 14	101 ± 5	5 ± 3			
0.1	320 ± 2	103 ± 1	10 ± 3			
1	260 ± 5	84 ± 1	5 ± 4			
4	**158 ± 166**	**51 ± 54**	6 ± 6			
8	**46 ± 7**	**15 ± 2**	8 ± 1			

Additionally, the capacity of PSII electron transport per active reaction centre (ET$_0$/ RC) was analysed (Fig. 4). This value shows an effective energy move from the reaction centre to the electron transport chain and corresponds to the amount of transported energy that can be used in the photosynthesis process. In general, in the case of samples where the effect on the electron transport chain was observed, the ET$_0$/RC values decreased in comparison to control. However, in the case of *N. spumigena* exposed to 4 μg cm^{-3} and *P.tricornutum* exposed to 1, 4, 8 μg cm^{-3} the significant increase of the ET$_0$/ RC were observed.

4. Discussion

Microalgae and cyanobacteria have been widely recommended as test organisms because of their ecological relevance, sensitivity and owing to the fact that they are easily cultivated in the laboratory (Seoane et al., 2014). For these reasons, *Chlorella vulgaris*, *Phaeodactylum tricornutum*, *Nodularia spumigena*, *Microcystis aeruginosa* have been selected for evaluating the effects of oxytetracycline in the

current ecotoxicological study. Moreover, those species are ubiquitous in brackish and estuarine ecosystems including the Baltic Sea waters. Our results demonstrated the negative impact of high concentrations of oxytetracycline on all tested organisms in terms of growth, chlorophyll *a* concentration and photosynthetic-fluorescence parameters (F$_V$/F$_M$, ABS/RC, ET$_0$/RC). Furthermore, the present findings indicate that lower concentrations can also adversely affect studied organisms. According to the literature, the EC$_{50}$ values obtained for oxytetracycline in the case of *C. vulgaris* are in the range from <3.58 to 7.05 μg cm^{-3} (Eguchi et al. 2004; Kołodziejska et al. 2013). The EC$_{50}$ value for *P. tricornutum* equals to 1.73 μg cm^{-3} (following International Standard ISO 10253:1995) (De Orte et al. 2013). In case of investigated cyanobacteria, EC$_{50}$ values have been reported only for *M. aeruginosa* in 6—7 day tests and were placed in the range from 0.207 μg cm^{-3} to 0.23 μg cm^{-3} (Ando et al., 2007; Holten Lützhøft et al., 1999). When considering the results of these studies, it could be assumed that concentrations of 8 μg cm^{-3} and 4 μg cm^{-3} would affect analysed microorganisms. On the other hand, concentrations corresponding to 1 μg cm^{-3}, 0.1 μg cm^{-3} and
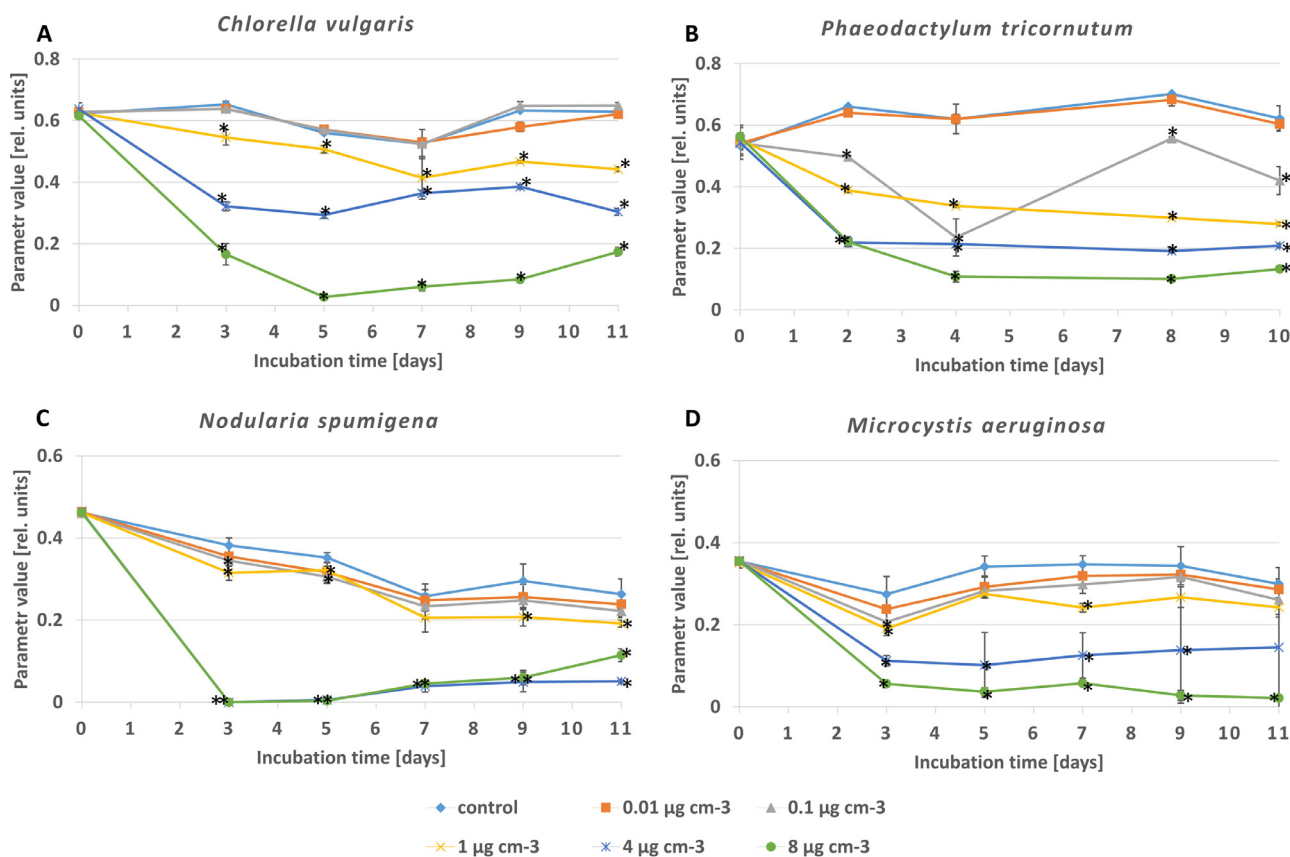
Figure 2   Photochemical efficiency of PSII (F_V/F_M) changes for the *Chlorella vulgaris* (A), *Phaeodactylum tricornutum* (B) and *Nodularia spumigena* (C) and *Microcystis aeruginosa* (D); .* differences statistically significant in comparision to the control.

0.01 μg cm^{-3}, similar to those detected in marine sediments, would be expected to have no or little impact. Eguchi et al. (2004) demonstrated that oxytetracycline exerted influence on the growth of algae in the early stages of the incubation period and consequently suggested that the probability it would cause damage to algae when released continuously into the environment is high. Impact of oxytetracycline on the growth of cyanobacteria and algae was also confirmed by Ando et al. (2007) and Holten Lützhøft et al. (1999) in 6—7 days tests. The values showed by these authors (EC$_{50}$= 0.2 μg cm^{-3}) were lower than used in this study. Our investigations also revealed that oxytetracycline inhibits pigment synthesis. Similar results were described by Seoane et al. (2014); the cells exposed to oxytetracycline were characterized by lower chlorophyll *a* content. The authors suggest that a significant decrease of pigments in cultures exposed to antibiotics can result from the inhibition of the increase of active cell volume compared to the control cultures. Guo et al. (2016), Tsiaka et al. (2013) and Zhang et al. (2012) also demonstrated that some compounds such as carbamazepine, tylosin, lincomycin, and trimethoprim can inhibit the synthesis of chlorophyll *a*. Moreover, the decline of pigments levels in cells could be caused by the enhancement of oxidative stress-related effects (Tsiaka et al., 2013). As it was mentioned before, in the case of *C. vulgaris* oxytetracycline concentration of 0.1 μg cm^{-3} had a higher impact on chlorophyll *a* per cell value than 1 μg cm^{-3}. Unfortunately, we do not have yet enough data to fully investigate this phenomenon. Differences in chlorophyll *a* concentration can be caused by the cell cycle. Cultures incubated with different oxytetracycline concentration variants were not harmonised, so cells could have been in a different growth phase. Cells before division are bigger and have more chlorophyll *a* per cell than cells after division. However, the growth curve (Fig. 1A) shows that 1 μg cm^{-3} seems to be one of the limit concentrations and can significantly impact the growth of the culture. Based on that, we assume it directly contributes to decreasing in overall cell divisions, while maintaining still quite high chlorophyll *a* production.

In the present studies, classical measurements were supported by photosynthetic-fluorescence parameters analysis. Results show that those parameters seem to be reliable biomarkers of oxytetracycline toxicity on tested organisms. Similar conclusions were reported by van der Grinten et al. (2010) in case of F_V/F_M parameter and by Dewez et al. (2018), where those parameters, among others, were used as markers of the global physiological state of the *Lemna gibba* L. affected by silver nanoparticles. We believe that findings reported in our studies can help in understanding how oxytetracycline impacts the PSII of listed species. It has been shown that in most cases concentrations of 1, 4 and 8 μg cm^{-3} caused a high increase of harvesting complex absorption of an active reaction centre (ABS/RC) and effective energy dissipation of an active RC emitted as a heat (DI$_0$/RC). It did not necessarily correlate with efficient usage of energy bound to the reaction centre and transported
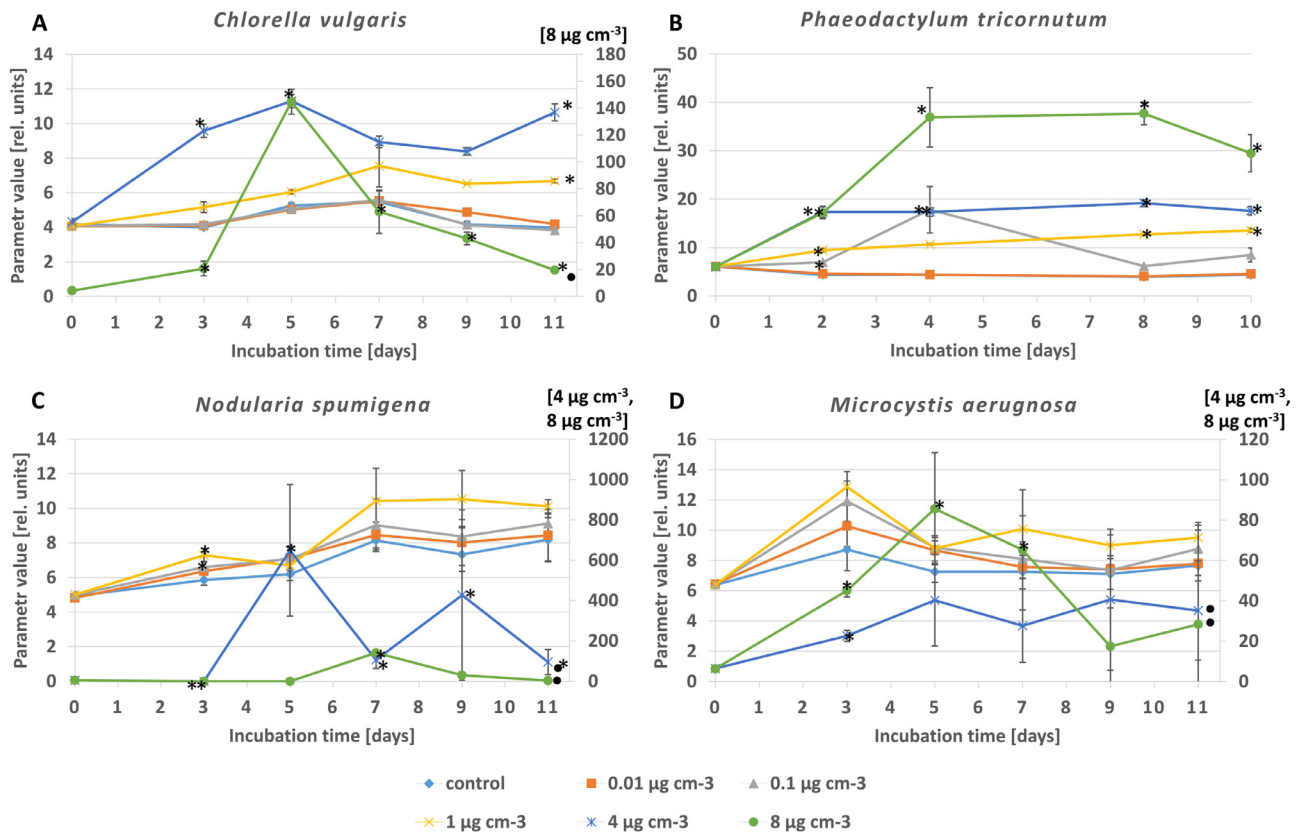
Figure 3 ABS/RC changes for the *Chlorella vulgaris* (A), *Phaeodactylum tricornutum* (B) and *Nodularia spumigena* (C) and *Microcystis aeruginosa* (D). * differences statistically significant in comparision to the control; • data series related to the auxiliary (right side) axis.

to be used in the photosynthesis (ET_0/RC), but always significantly corresponded with the decrease of photosystem II efficiency (F_V/F_M). The exposure of tested organisms to oxytetracycline in concentrations of 1, 4, 8 $\mu g\ cm^{-3}$ affects by some means PSII that leads to an increase of effective antenna size (directly correlated to chlorophyll *a* concentration) necessary to keep energy assimilation on the acceptable level to maintain effective photosynthesis. Unfortunately, the attempt was not successful as antennas' effectiveness in energy assimilation decreased and remaining energy was radiated as heat (DI_0/RC). We speculate that it can be attributed to some defence mechanisms involved in the protection of the reaction centre from destruction. Therefore that may be an explanation why in some cases (8 $\mu g\ cm^{-3}$) ET_0/RC value decreased and the electron started to move to the electron transport chain after a few days of the adaptation period. Simultaneously, the decrease of photosystem II efficiency (F_V/F_M) and in some cases decline of a number of cells in the samples were noticeable. A similar pattern was observed by Oh et al. (2005) for seaweed (*Porphyra yezoensis* Ueda) exposed to oxytetracycline. Also, Chen et al. (2017) showed that oxytetracycline (in concentrations exceeding 10 $\mu g\ g^{-1}$) diminished the photosynthetic capacity of rape (*Brassica campestris* L.). Moreover, van der Grinten et al. (2010) reported EC_{50} values for the oxytetracycline inhibition of the photosynthetic yield (Fv/F_M) for *M. aeruginosa* (5.4 $\mu g\ cm^{-3}$) and *P. subcapitata* (0.6 $\mu g\ cm^{-3}$), which in case of cyanobacteria are higher than it

would be concluded from our data. Pan et al. (2008) also observed toxic inhibitory effects of amoxicillin on the donor side, electron transfer and the acceptor side of PSII. The decrease of active centre resulted in reduced electron transfer (ET_0/RC) and increased dissipation of heat at the level of the antenna (DI_0/RC). Our results are also consistent with previous studies described by Xu et al. (2019) for tetracycline and its degradation products, where authors observed the impact on the growth, cell structure and algal cell oxidative stress of *C. vulgaris* during the 96h incubation period. Those studies showed that concentration of tetracycline > 5 $\mu g\ cm^{-3}$ caused oxidative stress, structural changes in the cells such as plasmolysis and starch granule deposition appeared, the thylakoid lamellae in the chloroplasts became blurred and deformed, and the vacuoles became larger.

The reported results also show differences between tested species in response to stress caused by oxytetracycline. Cyanobacteria are closer in taxonomy to bacteria when compared to green algae so the predicted impact of oxytetracycline on those microorganisms is expected to be higher. The results demonstrated this assumption is true for some parameters like growth or photosynthetic parameters (F_V/F_M, ABS/RC, ET_0/RC), but no for chlorophyll *a* concentration. The impact on *C. vulgaris* and *N. spumigena* was moderately higher than on *M. aeruginosa*. Higher resistance of *M. aeruginosa* to oxytetracycline was also described by van der Grinten et al. (2010). Of course, this issue needs
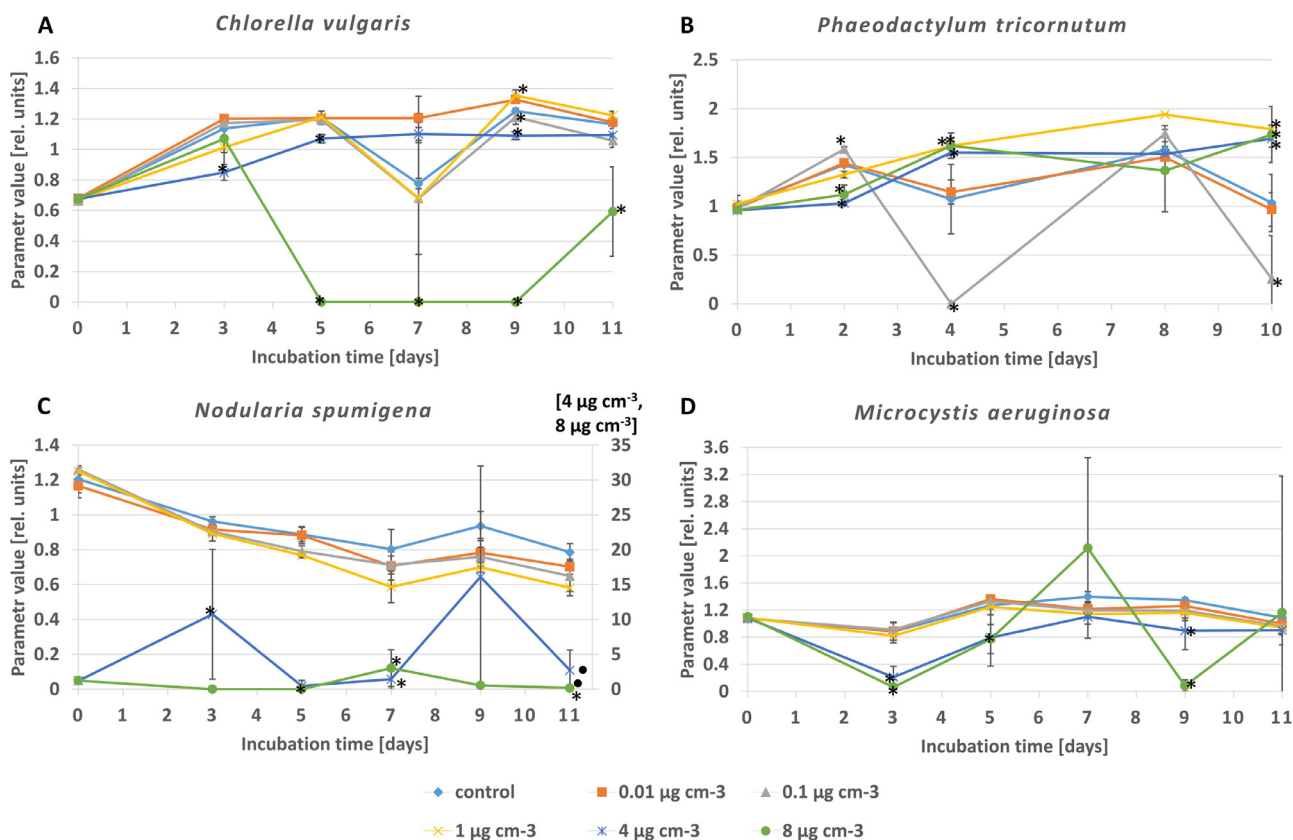
Figure 4   ET$_0$/ RC changes for the *Chlorella vulgaris* (A), *Phaeodactylum tricornutum* (B) and *Nodularia spumigena* (C) and *Microcystis aeruginosa* (D). * differences statistically significant in comparision to the control; • data series related to the auxiliary (right side) axis.

further investigations but authors speculate that it can be due to differences in cell and or exocellular polymeric matrix structures. Moreover, it may indicate that factors other than taxonomical similarities can be critical for the non-target organism.

The chronic character of the presented studies can be useful in risk assessment estimations. To make data more useful the obtained results were compared with no observed effect concentration (NOEC) values presented in the literature. Eguchi et al. (2004) reported NOEC for *C. vulgaris* equals 3.58 μg cm^{-3} which is higher than statistically significant concentration affecting organisms reported in our studies (0.1 μg cm^{-3}). Likewise, Ando et al. (2007) reported NOEC for *Nostoc* sp. at the level of 0.78 μg cm^{-3}. In this study, minimal concentration affecting *N. spumigena* of the same order (Nostocales),was equal to 0.1 μg cm^{-3}. However, in the same publication, Ando et al. (2007) reported NOEC for *M. aeruginosa* as 0.031 μg cm^{-3} which is lower than minimal concentration affecting this cyanobacterium presented in the current study.

Our results show that the changes in measured parameters started to disclose after the second-third day of the experiment. This allows the conclusion that standard 48—72 h chronic tests should be enough to observe the effect of the antibiotic on investigated organisms but the nature of changes can be hard to identify and interpret. On the other hand tests longer than 10 days do not seem to be too long

and labour-intensive compared to the obtained results. In our opinion test from 7 to 10 days should be enough to observe the chronic effect and help to understand the potential mechanism of the negative impact of oxytetracycline on the non-target microorganism.

5. Conclusion

In summary, our results show that oxytetracycline at concentration levels reported in the marine environment can potentially affect photoautotrophic microorganism. The photosynthetic parameters applied along with growth and pigment analysis can be a very sensitive tool for examining the influence of various antibiotics on autotrophic microorganisms. Furthermore, the present findings provide additional information about the mode of action of oxytetracycline concerning non-target organisms like cyanobacteria, diatoms, and microalgae. This allows the conclusion that the use of standard toxicity tests (72 h) does not allow to accurately and reliably assess the chronic impact of bioactive compounds including drugs and their metabolites on water organisms. To improve our knowledge of the emerging risks to aquatic species and to better understand the interactions between those compounds in the environment, more studies should be carried out, focusing particularly on chronic toxicity and toxicity of the mixtures of antibiotics.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:https://doi.org/10.1016/j.oceano.2019.12.002.

References

Agwuh, K.N., MacGowan, A., 2006. Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. J. Antimicrob. Chemother. 58 (2), 256—265, https://doi.org/10.1093/jac/dkl224.

Ando, T., Nagase, H., Eguchi, K., Hirooka, T., Nakamura, T., Miyamoto, K., Hirata, K., 2007. A novel method using cyanobacteria for ecotoxicity test of veterinary antimicrobial agents. Environ. Toxicol. Chem. 26 (4), 601—606.

Arnold, K.E., Brown, A.R., Ankley, T.G., Sumpter, J.P., 2014. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystem. Philos. Trans. R. Soc. B. Biol. Sci. 369 (1656), 1—11, http://dx.doi.org/10.1098/rstb.2013.0569.

Bischoff, H.W., Bold, H.C., 1963. Phycological Studies IV. Some Soil Algae from Enchanted Rock and Related Algal Species. Univ. Texas Publ. 6318, 1—95.

Björlenius, B., Ripszám, M., Haglund, P., Lindberg, R.H., Tysklind, M., Fick, J., 2018. Pharmaceutical residues are widespread in Baltic Sea coastal and offshore waters — Screening for pharmaceuticals and modelling of environmental concentrations of carbamazepine. Sci. Total. Environ. 633, 1496—1509, https://doi.org/10.1016/j.scitotenv.2018.03.276.

Borecka, M., Białk-Bielińska, A., Siedlewicz, G., Kornowska, K., Kumirska, J., Stepnowski, P., Pazdro, K., 2013. A new approach for the estimation of expanded uncertainty of results of an analytical method developed for determining antibiotics in seawater using solid-phase extraction disks and liquid chromatography coupled with tandem mass spectrometry technique. J. Chrom. A. 1304, 138—146, https://doi.org/10.1016/j.chroma.2013.07.018.

Borecka, M., Siedlewicz, G., Haliński, Ł.P., Sikora, K., Pazdro, K., Stepnowski, P., Białk-Bielińska, A., 2015. Contamination of the southern Baltic Sea waters by the residues of selected pharmaceuticals: method development and field studies. Mar. Pollut. Bull. 94, 62—71, https://doi.org/10.1016/j.marpolbul.2015.03.008.

Borecka, M., Białk-Bielińska, A., Haliński, Ł, Pazdro, K., Stepnowski, P., Stolte, S., 2016. The influence of salinity on the toxicity of selected sulfonamides andtrimethoprim towards the green algae Chlorella vulgaris. J Haz. Mat. 308, 179—186, http://dx.doi.org/10.1016/j.jhazmat.2016.01.041.

Boxall, A.B.A., Fogg, L.A., Blackwell, P.A., Kay, P., Pemberton, E.J., Croxford, A., 2004. Veterinary medicines in the environment. Rev. Environ. Contam. Toxicol. 180, 1—91.

Capone, D.G., Weston, D.P., Millera, V., Shoemakera, C., 1996. Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. Aquaculture 145, 55—75, https://doi.org/10.1016/S0044-8486(96)01330-0.

Chen, H., Liu, S., Xu, X.R., Zhou, G.J., Liu, S.S., Yue, W.Z., Sun, K.F., Ying, G.G., 2015. Antibiotics in the coastal environment of the Hailing Bay region, South China Sea: spatial distribution, source analysis and ecological risks. Mar. Pollut. Bull. 95 (1), 365—373, https://doi.org/10.1016/j.marpolbul.2015.04.025.

Chen, Y., Wang, Z., Shen, Z., Ou, Z., Xu, D., Yuan, Z., Zhou, S., 2017. Effects of Oxytetracycline on Growth and Chlorophyll Fluorescence in Rape (Brassica campestris L.). Pol. J. Environ. Stud. 26 (3), 995—1001, https://doi.org/10.15244/pjoes/67575.

de Orte, M.R., Carballeira, C., Viana, I.G., Carballeira, A., 2013. Assessing the toxicity of chemical compounds associated with marine land-based fish farms: The use of mini-scale microalgal toxicity tests Chem. Ecol. 29 (6), 554—563, https://doi.org/10.1080/02757540.2013.790381.

Dewez, D., Goltsev, V., Kalaji, H.M., Oukarroum, A., 2018. Inhibitory effects of silver nanoparticles on photosystem II performance in Lemna gibba probed by chlorophyll fluorescence. Curr. Plant. Biol. 16, 15—21, https://doi.org/10.1016/j.cpb.2018.11.006.

EC, 2003 European Commission Technical Guidance Document in Support of Commission Directive 93//67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substance, Part II Retrieved 10 July, 2019, from https://echa.europa.eu/documents/10162/16960216/tgdpart2_2ed_en.pdf

Edler, L., 1979. Recommendations for marine biological studies in the Baltic Sea: Phytoplankton and chlorophyll. Baltic Mar. Biol. Publ. 5, 1—38.

Eguchi, K., Nagase, H., Ozawa, M., Endoh, Y.S., Got, K., Hirata, K., Miyamoto, K., Yoshimura, H., 2004. Evaluation of antimicrobial agents for veterinary use in the ecotoxicity test using microalgae. Chemosphere 57, 1733—1738, https://doi.org/10.1016/j.chemosphere.2004.07.017.

Fu, L., Huang, T., Wang, S., Wang, X., Su, L., Li, C., Zhao, Y., 2017. Toxicity of 13 different antibiotics towards freshwater green algae Pseudokirchneriella subcapitata and their modes of action. Chemosphere 168, 217—222, https://doi.org/10.1016/j.chemosphere.2016.10.043.

Gao, Q.T., Tam, N.F.Y., 2011. Growth, photosynthesis and antioxidant responses of two microalgal species, Chlorella vulgaris and Selenastrum capricornutum, to nonylphenol stress. Chemosphere 82 (3), 346—354, https://doi.org/10.1016/j.chemosphere.2010.10.010.

González-Pleiter, M., Gonzalo, S., Rodea-Palomares, I., Legane, F., Rosal, R., Boltes, K., Marco, E., Fernandez-Piñas, F., 2013. Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: implications for environmental risk assessment. Water Res. 47, 2050—2064, https://doi.org/10.1016/j.watres.2013.01.020.

Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), Culture of Marine Invertebrate Animals. Plenum Press, New York, 26—60.

Gulkowska, A., He, Y., So, M.K., Yeung, L., Giesy, J.P., Lam, P.K., Martin, M., Richardson, B.J., 2007. The occurrence of selected antibiotics in Hong Kong coastal waters. Mar. Poll. Bull. 54, 1287—1293, https://doi.org/10.1016/j.marpolbul.2007.04.008.

Guo, J., Selby, K., Boxall, A., 2016. Comparing the sensitivity of chlorophytes, cyanobacteria and diatoms to major-use antibiotics. Environ. Toxicol. Chem. 35, 2587—2596, https://doi.org/10.1002/etc.3430.

Halling-Sørensen, B., 2000. Algal toxicity of antibacterial agents used in intensive farming. Chemosphere 40 (7), 731—739, https://doi.org/10.1016/S0045-6535(99)00445-2.

Hernando, M.D., Mezcua, M., Fernandez-Alba, A.R., Barcelo, D., 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta 69, 334—342, https://doi.org/10.1016/j.talanta.2005.09.037.

Holten Lüzhøft, H.C., Halling-Sørensen, B., Jørgensen, S.E., 1999. Algal toxicity of antibacterial agents applied in Danish fish farming. Arch. Environ. Contam. Toxicol. 36, 1—6, https://doi.org/10.1007/s002449900435.

Ji, K., Kim, S., Han, S., Seo, J., Lee, S., Park, Y., Choi, K., Kho, Y.-L., Kim, P.-G., Park, J., Choi, K., 2012. Risk assessment of chlortetracycline, oxytetracycline, sulfamethazine, sulfathiazole, and erythromycin in aquatic environment: Are the current environmental concentrations safe? Ecotoxicology 21, 2031—2050, https://doi.org/10.1007/s10646-012-0956-6.

Jiao, S., Zheng, S., Yin, D., Wang, L., Chen, L., 2008. Aqueous oxytetracycline degradation and the toxicity change of degradation compounds in photoirradiation process. J. Environ. Sci. 20 (7), 806—813, https://doi.org/10.1016/S1001-0742(08)62130-0.

Karło, A., Wilk, A., Ziembińska-Buczyńska, A., Surmacz-Górska, J., 2015. Cultivation parameters adjustment for Effective algal biomass production. Rocz. Ochr. Śr. 17, 275—288.

Kolar, B., Arnuš, L., Jeretin, B., Gutmaher, A., Drobne, D., Durjava, M.K., 2014. The toxic effect of oxytetracycline and trimethoprim in the aquatic environment. Chemosphere 115, 75—80, https://doi.org/10.1016/j.chemosphere.2014.02.049.

Kołodziejska, M., Maszkowska, J., Białk-Bielińska, A., Steudte, S., Kumirska, J., Stepnowski, P., Stolte, S., 2013. Aquatic toxicity of four veterinary drugs commonly applied in fish farming and animal husbandry. Chemosphere 92, 1253—1259, https://doi.org/10.1016/j.chemosphere.2013.04.057.

Krzemińska, I., Pawlik-Skowrońska, B., Trzcińska, M., Tys, J., 2014. Influence of photoperiods on the growth rate and biomass productivity of green microalgae. Bioprocess Biosyst. Eng. 37, 735—741, https://doi.org/10.1007/s00449-013-1044-x.

Kümmerer, K., 2009. Antibiotics in the environment. In: Kümmerer, K. (Ed.), Pharmaceuticals in the Environment. Springer, Berlin, 75—88.

Kümmerer, K., 2010. Pharmaceuticals in the Environment. Annual Review of Environment and Resources 35 (1), 57—75, http://dx.doi.org/10.1146/annurev-environ-052809-161223.

Lalumera, G.M., Calamari, D., Galli, P., Castiglioni, S., Crosa, G., Fanelli, R., 2004. Preliminary investigation on the environmental occurrence and effects of antibiotics used in aquaculture in Italy. Chemosphere 54 (5), 661—668, https://doi.org/10.1016/j.chemosphere.2003.08.001.

Leal, J.F., Esteves, V.I., Santos, E.B.H., 2016. Use of sunlight to degrade oxytetracycline in marine aquaculture's waters. Environ. Pollut. 213, 932—939, http://dx.doi.org/10.1016/j.envpol.2016.03.040.

Lorenzen, C.J., 1967. Determination of chlorophyll and pheopigments: spectrophotometric equations. Limnol. Oceanogr. 12, 343—346, https://doi.org/10.4319/lo.1967.12.2.0343.

Lyman, J., Fleming, R.H., 1940. Composition of sea water. J. Mar. Res. 3, 134—146.

McArdell, C.S., Molnar, E., Suter, M.J.F., Giger, M., 2003. Occurrence and Fate of Macrolide Antibiotics in Wastewater Treatment Plants and in the Glatt Valley Watershed, Switzerland. Environ. Sci. Technol. 37 (24), 5479—5486, https://doi.org/10.1021/es034368i.

Nödler, K., Voutsa, D., Licha, T., 2014. Polar organic micropollutants in the coastal environment of different marine systems. Mar Pollut. Bull. 85, 50—59, https://doi.org/10.1016/j.marpolbul.2014.06.024.

Oh, M-H., Kang, Y., Lee, C., Chung, I., 2005. Effects of Six Antibiotics on the Activity of the Photosynthetic Apparatus and Ammonium Uptake of Thallus of Porphyra yezoensis. Algae 20 (2), 121—125, https://doi.org/10.4490/algae.2005.20.2.121.

Pan, X., Deng, C., Zhang, D., Wang, J., Mu, G., Chen, Y., 2008. Toxic effects of amoxicillin on the photosystem II of Synechocystis sp. characterized by a variety of in vivo chlorophyll fluorescence tests. Aquat Toxicol. 89 (4), 207—213, https://doi.org/10.1016/j.aquatox.2008.06.018.

Pazdro, K., Borecka, M., Siedlewicz, G., Białk-Bielińska, A., Stepnowski, P., 2016. Analysis of the Residues of Pharmaceuticals in Marine Environment: State-of-the-art, Analytical Problems and Challenges. Curr. Anal. Chem. 12 (3), 202—226, http://dx.doi.org/10.2174/1573411012666151009193536.

Pereira, J.H.O.S., Vilar, V.J.P., Borges, M.T., González, O., Esplugas, S., Boaventura, R.A.R., 2011. Photocatalytic degradation of oxytetracycline using TiO2 under natural and simulated solar radiation. Sol. Energy. 85 (11), 2732—2740, https://doi.org/10.1016/j.solener.2011.08.012.

Rachmilevitch, M., DaCosta, Huang, B., 2006. Physiological and biochemical indicators for stress tolerance. In: Huang, B. (Ed.), Plant—Environment Interactions. Taylor & Francis, Boca Raton, FL, 321—355.

Santos, L.H.M.L.M., Araújo, A.N., Fachini, A., Pena, A., Delerue-Matos, C., Montenegro, M.C.B.S.M., 2010. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J. Haz. Mat. 175, 45—95, https://doi.org/10.1016/j.jhazmat.2009.10.100.

Schmidt, A.S., Bruun, M.S., Dalsgaard, I., Pedersen, K., Larsen, J.L., 2000. Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four Danish rainbow trout farms. Appl. Environ. Microbiol. 66 (11), 4908—4915, https://doi.org/10.1128/AEM.66.11.4908-4915.2000.

Seoane, M., Rioboo, C., Herrero, C., Cid, Á., 2014. Toxicity induced by three antibiotics commonly used in aquaculture on the marine microalga Tetraselmis suecica (Kylin) Butch. Mar. Environ. Res. 101, 1—7, https://doi.org/10.1016/j.marenvres.2014.07.011.

Siedlewicz, G., Pazdro, K., Borecka, M., Kornowska, K., Białk—Bielińska, A., Stepnowski, P., 2014. Determination of Tetracyclines Residues in the Gulf of Gdansk (Southern Baltic Sea) Sediments Using a Tandem Solid-Phase Extraction with Liquid Chromatography Coupled with Tandem Mass Spektrometry. In: Zieliński, T., Pazdro, K., Dragan-Górska, A., Weydmann, A. (Eds.), Insights on Environmental Changes. GeoPlanet: Earth and Planetary Sciences. Springer, Switzerland, 33—45.

Siedlewicz, G., Borecka, M., Białk-Bielińska, A., Sikora, K., Stepnowski, P., Pazdro, K., 2016. Determination of antibiotic residues in southern Baltic Sea sediments using a tandem solid-phase extraction and liquid chromatography coupled with tandem mass spectrometry. Oceanologia 58 (3), 221—234, https://doi.org/10.1016/j.oceano.2016.04.005.

Siedlewicz, G., Borecka, M., Białk-Bielińska, A., Winogradow, K., Stepnowski, P., Pazdro, K., 2018. Presence, concentrations and risk assessment of selected antibiotic residues in sediments and near-bottom waters collected from the Polish coastal zone in the southern Baltic Sea — Summary of 3 years of studies. Mar. Pollut. Bull. 129 (2), 787—801, https://doi.org/10.1016/j.marpolbul.2017.10.075.

Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971. Purification and properties of unicellular blue-green algae (Order Chroococcales). Bacteriol. Rev. 35, 171—205.

Strasser, R.J., Srivastava, A., Tsimilli-Michael, M., 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus, M., Pathre, U., Mohanty, P. (Eds.), Probing Photosynthesis: Mechanism, Regulation and Adaptation. Taylor and Francis, UK, 445—483.

Strasser, R.J., Tsimilli-Michael, M., Srivastava, A., 2004. Analysis of the chlorophyll a fluorescence transient. In: Papadogeorgiou, G.C., Govindjee (Eds.), Chlorophyll a Fluorescence: A Signature of Photosynthesis. Springer, Netherlands, 321—362.

Strickland, J.D.H., Parsons, T.R., 1968. A practical handbook of seawater analysis. Fish Res. Board Can. Bull. 169. Fish Res. Board of Canada, Ottawa, 293 pp.

Strickland, J.D.H., Parsons, T.R., 1972. A practical handbook of seawater analysis. Fish Res. Board Can. Bull. 167, 2nd edn., Fish Res. Board of Canada, Ottawa, 310 pp.

Tsiaka, P., Tsarpali, V., Ntaikou, I., Kostopoulou, M.N., Lyberatos, G., Dailianis, S., 2013. Carbamazepine-mediated prooxidant effects on the unicelular marine algal species *Dunaliella tertiolecta* and the hemocytes of mussel *Mytilus galloprovincialis*. Ecotoxicology 22, 1208—1220, https://doi.org/10.1007/s10646-013-1108-3.

Van Boeckel, T.P., van Gandra, S., Ashok, A., Caudron, Q., Grenfell, B.T., Levin, S.A., Laxminarayan, R., 2014. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. The Lancet Infectious Diseases 14, 742—750, https://doi.org/10.1016/S1473-3099(14)70780-7.

Van der Grinten, E., Pikkemaat, M.G., Van den Brandhof, E.J., Stroomberg, G.J., Kraak, M.H.S., 2010. Comparing the sensitivity of algal, cyanobacterial and bacterial bioassays to different groups of antibiotics. Chemosphere 80, 1—6, https://doi.org/10.1016/j.chemosphere.2010.04.011.

Wang, J., Hu, J., Zhang, S., 2010. Studies on the sorption of tetracycline onto clays and marine sediment from seawater. J. Colloid Interface Sci. 349, 578—582, https://doi.org/10.1016/j.jcis.2010.04.081.

Wille, K., Noppe, H., Verheyden, K., Vanden Bussche, J., De Wulf, E., Van Caeter, P., Janssen, C.R., De Brabander, H.F., Vanhaecke, L., 2010. Validation and application of an LC-MS/MS method for the simultaneous quantification of 13 pharmaceuticals in seawater. Anal. Bioanal. Chem. 397, 1797—1808, https://doi.org/10.1007/s00216-010-3702-z.

Xu, D., Xiao, Y., Pan, H., Me, i Y., 2019. Toxic effects of tetracycline and its degradation products on freshwater green algae. Ecotoxicol. Environ. Safe. 174, 43—47, https://doi.org/10.1016/j.ecoenv.2019.02.063.

Xu, J., Zhang, Y., Zhou, C, Guo, C., Wang, D., Du, P., Luo, Y., Wan, J., Meng, W., 2014. Distribution, sources and composition of antibiotics in sediment, overlying water and pore water from Taihu Lake, China. Sci. Total Environ. 497—498, 267—273, https://doi.org/10.1016/j.scitotenv.2014.07.114.

Yang, Y., Fu, J., Peng, H., Hou, L., Liu, M., Zhou, J.L., 2011. Occurrence and phase distribution of selected pharmaceuticals in the Yangtze Estuary and its coastal zone. J. Hazard. Mater. 190, 588—596, https://doi.org/10.1016/j.jhazmat.2011.03.092.

Zhang, W., Zhang, M., Lin, K., Sun, W., Xiong, B., Guo, M., Cui, X., Fu, R., 2012. Ecotoxicological effect of carbamazepine on *Scenedesmus obliquus* and *Chlorella pyrenoidosa*. Environ. Toxicol. Pharmacol. 33 (2), 344—352, https://doi.org/10.1016/j.etap.2011.12.024.

Zushi, K., Kajiwara, S., Matsuzoe, N., 2012. Chlorophyll a fluorescence OJIP transient as a tool to characterize and evaluate response to heat and chilling stress in tomato leaf and fruit. Scientia Horticulturae 148, 39—46, https://doi.org/10.1016/j.scienta.2012.09.022.

Żak, A., Musiewicz, K., Kosakowska, A., 2012. Allelopathic activity of the Baltic cyanobacteria against microalgae, Estuar. Coast. Shelf Sci. 112, 4—10, https://doi.org/10.1016/j.ecss.2011.10.007.