Journal of Applied Phycology (2019) 31:3537–3544
https://doi.org/10.1007/s10811-019-01874-x

Toxicity of single steroid hormones and their mixtures toward the cyanobacterium *Microcystis aeruginosa*

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Received: 13 April 2019 / Revised and accepted: 17 July 2019 / Published online: 31 July 2019

Abstract

Several compounds, such as hormones, are released uncontrolled into the aquatic environment, and some of these pollutants have an adverse effect on endocrine systems of humans and other organisms. However, there is insufficient information about the effect of natural and synthetic hormones on non-target organisms, such as cyanobacteria. Therefore, in this study, the adverse effects of hormones, singly and in combination, on *Microcystis aeruginosa* were explored for the first time. Chronic toxicity was evaluated based on biomass and chlorophyll *a* measurements during 14 days of exposure. Growth of cyanobacteria after exposure to 0.1–100 mg L^{-1} of hormones was inhibited in a concentration-dependent manner. In most cases, a low concentration of hormones (0.1–1 mg L^{-1}) did not affect the growth of cyanobacteria, but a higher concentration (> 10 mg L^{-1}) inhibited the growth. The obtained 14-day EC_{50} values were 88.92–355.15 mg L^{-1}. According to these values, the decreasing order of the toxicity of the eight hormones to tested cyanobacteria was 17-α-ethinylestradiol > progesterone > 17ß-estradiol > 5-pregnen-3β-ol-20-one > testosterone> estrone > levonorgestrel > estriol. Moreover, data show that mixed hormones were more toxic than single compounds and more than additive effect was observed. The achieved 14-day EC_{50} values for mixed hormones were 56.66–166.83 mg L^{-1}. Simultaneous presence of several hormones in the aquatic environment may lead to increased toxicity (more than additive effect) and cause serious ecological effects, more harmful than expected.

Keywords *Microcystis aeruginosa* · Cyanobacteria · Hormones · Endocrine-disrupting chemicals · Aquatic pollution · More than additive effect

Introduction

Endocrine-disrupting chemicals (EDCs) are substances destabilizing the endocrine system of organisms (Matsushima 2018; Luo et al. 2019). They are considered extremely dangerous substances because they have a harmful effect on reproductive, nervous, and immune systems (Czarny et al. 2017). Their mechanisms of action rely on their ability to mimic the action of endogenous hormones, antagonism with the synthesis of normal hormones or their metabolism, and modification of the level of hormone receptors (Annamalai and Namasivayam 2015). Due to their prevalence in the environment, as well as their ability to interfere with the endocrine system, these compounds have, in the past decade, gained popularity among scientific communities. The presence of endocrine disruptors in the aquatic environment has become a serious problem, as these compounds may induce negative effects on the hormonal functions of humans and other organisms (Nie et al. 2015; Jia et al. 2019). They lead to reduced fertility as well as problems with development and growth (Aris et al. 2014; Laurenson et al. 2014). EDCs include naturally produced compounds, such as estrogens, progestogens, androgens, and phytoestrogens, as well as a wide range of industrial chemicals and household products (synthetic hormones, polycyclic aromatic hydrocarbons, polychlorinated compounds, alkylphenols, pharmaceuticals, and pesticides). Endocrine disruptors are present in various aquatic matrices (surface waters (Pojana et al. 2007; Gorga et al. 2015), groundwater (Barber et al. 2009; Li et al. 2015), wastewater

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10811-019-01874-x) contains supplementary material, which is available to authorized users.

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(Barber et al. 2006; Chang et al. 2011; Yu et al. 2013), and drinking water (Benotti and Snyder 2009)) at trace concentrations, such as ng L^{-1} or μg L^{-1}.

EDCs have negative effects on aquatic organisms, and therefore, more attention is paid to the occurrence of steroid hormones in the environment. Even low concentrations of hormones (ng L^{-1}) can cause serious disturbances in the functioning of the endocrine system (Khanal et al. 2006; Leonard et al. 2017). It has been proven that their presence in waters can lead to the inhibition of reproductive processes, distortion of gonads, reduction of sperm count, hermaphroditism, and even to the feminization of male fish (Brian et al. 2007; He et al. 2018; Thrupp et al. 2018). So far, most attention has been paid to the study of the environmental impact and fate of estrogens; however, less attention has been paid to other steroids, such as progestogens and androgens. Natural hormones are excreted in urine and feces by humans, farm animals, and aquatic organisms. Their amount is determined by gender, stage of growth, pregnancy, the stage of the menstrual cycle, and the use of contraceptives or hormonal drugs (Leet et al. 2011; Wang et al. 2017). On the other hand, synthetic hormones are widely used in contraceptives, therapies, hormone therapy, livestock and aquaculture production (Laurenson et al. 2014). The mechanisms of bringing these compounds to the environment rely on the discharging of agricultural fertilizers flowing from the fields, which enter the surface waters together with municipal sewage. Many studies show that these types of compounds have a high resistance to the degradation process and a tendency to accumulate in sediments and bioaccumulate in fauna and flora. Therefore, it is important to evaluate the potential risks of hormones for aquatic ecosystems.

There is insufficient information about the effect of hormones on the growth of cyanobacteria. Therefore, the aim of this study was to examine the toxic effect of natural steroid hormones (estrone, 17ß-estradiol, estriol, progesterone, 5-pregnen-3β-ol-20-one, testosterone), which are key compounds in the regulation of female/male reproductive functions, as well as synthetic ones (17-α-ethinylestradiol, levonorgestrel), which are often used as an active ingredient in oral contraceptives. In this study, the toxicity of eight single hormones and their combination on *Microcystis aeruginosa* was investigated for the first time. For this purpose, a toxicity test with single and mixed hormones was performed and the biomass and chlorophyll *a* content of examined cyanobacteria were determined.

Materials and methods

Materials and chemicals

Analytical grade estrone (E1), 17ß-estradiol (E2), estriol (E3), progesterone (PRO), 5-pregnen-3β-ol-20-one (PRE), and testosterone (TST) (purity > 98%) were from Sigma-Aldrich (Germany), and 17-α-ethinylestradiol (EE2) and levonorgestrel (LG) (purity > 97%) were obtained from TCI (Japan). All of the investigated hormones were dissolved in HPLC grade methanol (Chemsolve, Poland) to prepare a 10 mg mL^{-1} stock solution and stored at 4 °C for subsequent experiments.

Microcystis aeruginosa cultures

The freshwater cyanobacterium *Microcystis aeruginosa* PCC 7820 from the Faculty of Biology, University of Lodz, Poland, were used as a test organism. The cultures were grown in 250-mL Erlenmeyer flasks containing 100 mL liquid BG-11 medium (Rippka et al. 1979) and incubated in a phytotron chamber. The Erlenmeyer flasks were maintained at 23/20 °C (light/dark) and a humidity of 30% in a phytotron chamber with alternating periods of light and dark (12 h/12 h). Irradiance was held at 60 μmol photons m^{-2} s^{-1}. The pH (7.64 ± 0.02) of the medium was adjusted using 1 M HCl and/or NaOH solutions. All flasks were shaken at least three times a day to prevent clumping of cells. The initial algal density was 2×10^5 cells mL^{-1}.

Experimental setup

Experiments were performed to study the toxic effect of hormones on *M. aeruginosa*. Hormones were added to each treatment flask separately (E1, E2, E3, EE2, PRO, PRE, LG, and TST) and then mixed (MIX1, MIX2, and MIX3) at different nominal concentration levels: 0.1, 1, 10, 25, 50, 75, and 100 mg L^{-1}. All the investigated hormones were dissolved in methanol and added to the 100 mL of test medium containing *M. aeruginosa* cells to achieve the desired series of concentrations. Treatments with an equivalent amount of methanol, but without the presence of hormones, were included as control samples, which were cultured in the same conditions. MIX1 comprises all the tested compounds (E1, E2, E3, EE2, PRO, PRE, TST, LG), MIX2 one of the most toxic estrogens, progestogen, and androgen (EE2, PRO, TST), and MIX3 one of the most toxic hormones and three with the weakest effect (PRO, E1, E3, LG). MIX1, MIX2, and MIX3 were prepared with 1/8, 1/3, and 1/4 of each compound (mg mL^{-1}), respectively, to achieve the same final concentration to be tested for individual hormones. The concentrations of hormones in the final experiment were established through a preliminary test. All the treatments were measured at 1, 2, 3, 7, 8, 10, 13, and 14 days.

Determination of biomass and chlorophyll *a* content

Biomass and chlorophyll *a* concentrations were measured over 14 days in cultures containing different concentrations of hormones. Biomass was determined according to the modified method of Moheimani et al. (2013). For gravimetric measurement of dry weight, 10 mL of culture was filtered

through pre-weighed and pre-combusted nylon membrane filters (3 μm, diameter 47 mm, Yeti, Germany). The filters were then dried in an oven for 1 h at 105 °C to a constant weight and weighed again. Chlorophyll *a* concentrations were evaluated using an AlgaeChek Ultra fluorometer with fluorescence detection at 685 nm (Modern Water, UK).

Statistical analysis

The experiments were carried out five times for all treatments, and the results are presented as the means of five replicates (mean ± SD, $n = 5$). Experimental data were analyzed with Microsoft Excel software package (Microsoft Corporation, USA) and Statistica (StatSoft) software. Identification and rejection of outliers were tested using the Q-Dixon test (Dixon 1953). Differences between *M. aeruginosa* cultures were considered to be significant at $P < 0.05$.

The EC_{50} values and dose-response equation for 7, 10, 14 days were calculated using linear regression analysis of log-transformed concentration versus % of inhibition using the Microsoft Excel software package.

Results

Chronic toxicity of single hormones

The toxic effect of single and mixed hormones on the growth of *M. aeruginosa* was determined based on biomass and chlorophyll *a* measurements at different concentrations of hormones during 14 days of exposure. The results are shown in Figs. 1 and 2 and Supplementary Material S1. Figure 1 shows the variability of biomass content in cyanobacteria cells during 14 days of exposure to increasing concentrations of hormones. The results obtained from the determination of biomass showed that eight of the investigated hormones were toxic to the studied *M. aeruginosa*. A concentration-dependent decrease in the biomass content was observed, which confirmed the growth-inhibiting effect of single hormones. Compared with the controls, 10–100 mg L^{-1} of 17-α-ethinylestradiol and progesterone significantly inhibited the growth of cyanobacteria ($P < 0.05$), while 0.1 and 1 mg L^{-1} of EE2 and 0.1 mg L^{-1} of PRO resulted in a growth-stimulating effect after 7 days of exposure (Fig. 1d, e). In the case of 17ß-estradiol, 5-pregnen-3β-ol-20-one, and testosterone, the growth inhibition of cyanobacteria cultures was significantly lower than that of 17-α-ethinylestradiol and progesterone. Nevertheless, these hormones inhibited the growth of *M. aeruginosa* at all concentration levels after 7 days of exposure (Fig. 1b, f, and h). In contrast, exposure to estrone, estriol, and levonorgestrel showed a weak effect on the growth of the cyanobacteria (Fig. 1a, c, and g).

Chlorophyll plays an important role in photosynthesis, because it absorbs light energy, which is then converted into the chemical energy associated with biomass. Therefore, chlorophyll content is an important index of algal activity. Similar decreasing trends were observed for the chlorophyll *a* concentration of the exposed cells of *M. aeruginosa*. Within 14 days of incubation of cyanobacteria cultures in the presence of hormones, the content of chlorophyll *a* pigment decreased with increasing exposure for all concentrations of the investigated compounds (Supplementary Material S1).

The EC_{50} values of the eight single hormones are presented in Table 1. In the case of almost all the examined hormones, the toxicity increased with time (E2, EE2, PRO, PRE, TST). The 14-day EC_{50} values of hormones to cyanobacteria ranged from 88.92 to 355.15 mg L^{-1}. Of the various examined hormones, the sensitivity of *M. aeruginosa* to 17-α-ethinylestradiol and progesterone (88.92, 93.63 mg L^{-1}, respectively) was the highest. The cyanobacteria were medium sensitive to 17ß-estradiol, 5-pregnen-3β-ol-20-one, and testosterone (106.04, 141.84 mg L^{-1}, respectively) and the least sensitive to estrone, estriol, and levonorgestrel (276.68, 355.15, 354.75 mg L^{-1}, respectively). As is evident from Table 1 (14 days, EC_{50}), the toxicity of hormones to *Microcystis aeruginosa* decreased in the order: EE2 > PRO > E2 > PRE > TST > E1 > LG > E3.

Chronic toxicity of mixed hormones

The inhibitory effects of mixed hormones on biomass content in *M. aeruginosa* cultures after 14 days of exposure are shown in Fig. 2. The examined cyanobacteria were adversely affected by exposure to mixed hormones. Of all the investigated mixed hormones, the strongest inhibitory effects were observed for all concentrations of MIX1, even after only 3 days of exposure (Fig. 2a). A similar trend was observed in the case of MIX2, which inhibited the growth of *M. aeruginosa* at a concentration of 1–100 mg L^{-1} after the third day (Fig. 2b). Among the mixed hormones, the weakest inhibition was observed after exposition to MIX3. This mixture inhibited the growth at a concentration of 75–100 mg L^{-1} after 7 days. Concentrations below 10 mg L^{-1} stimulated the growth of *M. aeruginosa* after 8 days of exposure (Fig. 2c).

The toxic effect increased with increasing concentrations of the tested mixtures. EC_{50} values of all mixed hormones decreased with time (Table 1). The range of EC_{50} values of mixed hormones varied from 56.66 (14 days) to 261.29 mg L^{-1} (7 days). Fourteen-day EC_{50} values for MIX1 (56.66 mg L^{-1}) and MIX2 (72.71 mg L^{-1}) were lower than those for MIX3 (166.83 mg L^{-1}), indicating the greater toxicity of MIX1 and MIX2. In brief, the following order of toxicity was observed: MIX1 > MIX2 > MIX3.

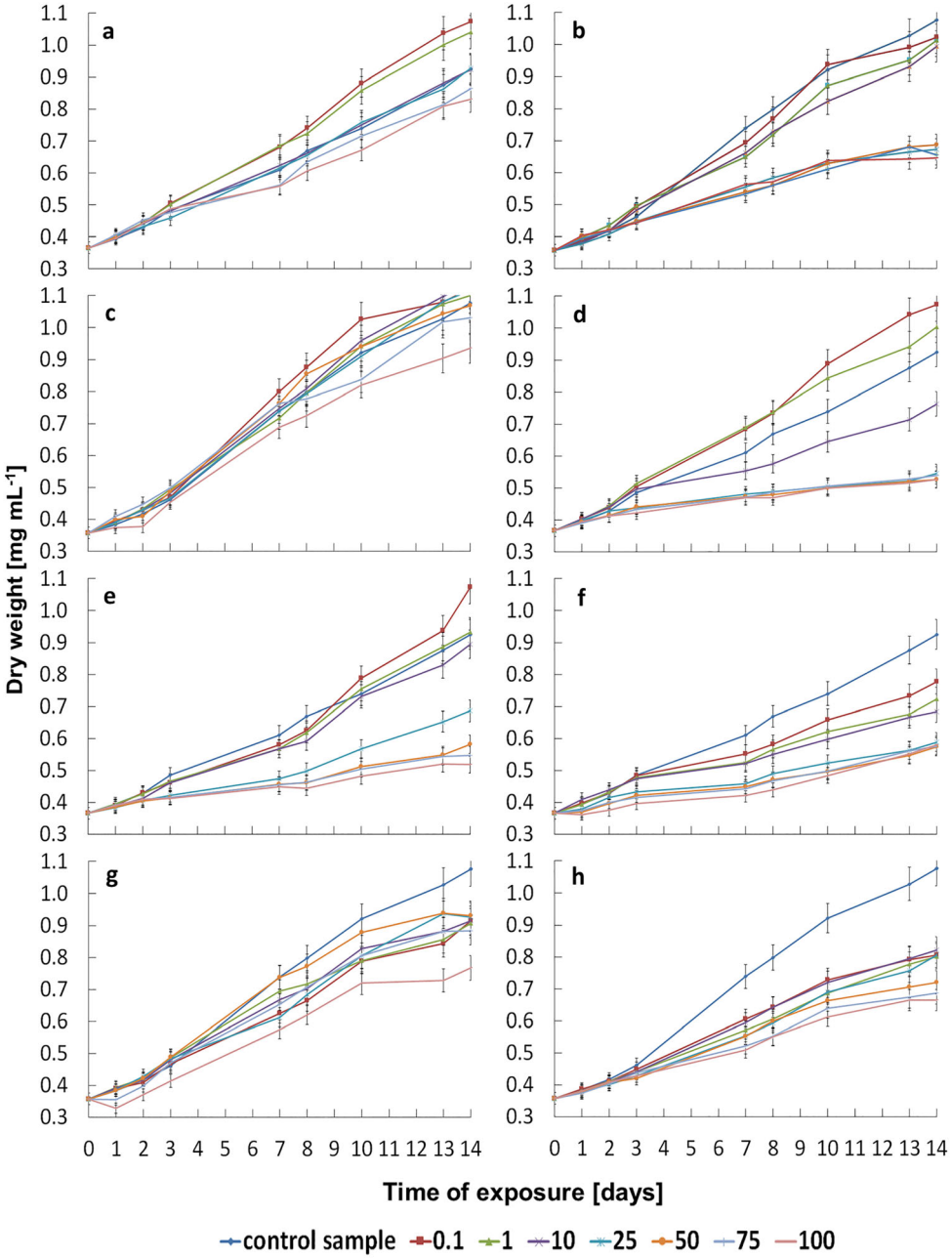

Fig. 1 Changes in biomass content (mg L^{-1}) of *Microcystis aeruginosa* during 14 days of exposure to 0.1, 1, 10, 25, 50, 75, and 100 mg L^{-1} single hormones (a E1, b E2, c E3, d EE2, e PRO, f PRE, g LG, h TST). Error bars represent the standard deviation of five measurements ($P < 0.05$, $n = 5$)

Discussion

In recent years, hormones have attracted considerable attention due to their rapidly rising concentrations in lakes and rivers all over the world (Adeel et al. 2017). It is well known that hormones pose serious threats to soil, plants, the aquatic environment, and humans, and negatively affect many aquatic organism species, such as amphibians and fish (Yuan et al. 2014). Less is known about the impact of hormones on microalgae. The research that can be found in literature focuses on studying the effect of EE2 on algal growth. The effects of single and mixed hormones on the cyanobacterium *M. aeruginosa* were explored for the first time in this study. Balina et al. (2015) found that 100–500 μg L^{-1} of 17-α-ethinylestradiol causes a 100% reduction in the growth rate and induces the initial destruction of algae cells. Similar results were obtained by Pocock and Falk (2014). In their study, the growth of green algae *Chlamydomonas reinhardtii* was negatively affected by EE2 and 7 μM of this hormone reduced the growth rate by 68% compared with the control sample. Liu et al. (2010) reported that the EC_{50} values in a *Navicula incerta* growth inhibition test for EE2 and E2 were 3.21 and > 10 mg L^{-1}, respectively. Salomao et al. (2014) studied the effect of E1, E2, and EE2 on *Desmodesmus subspicatus* and

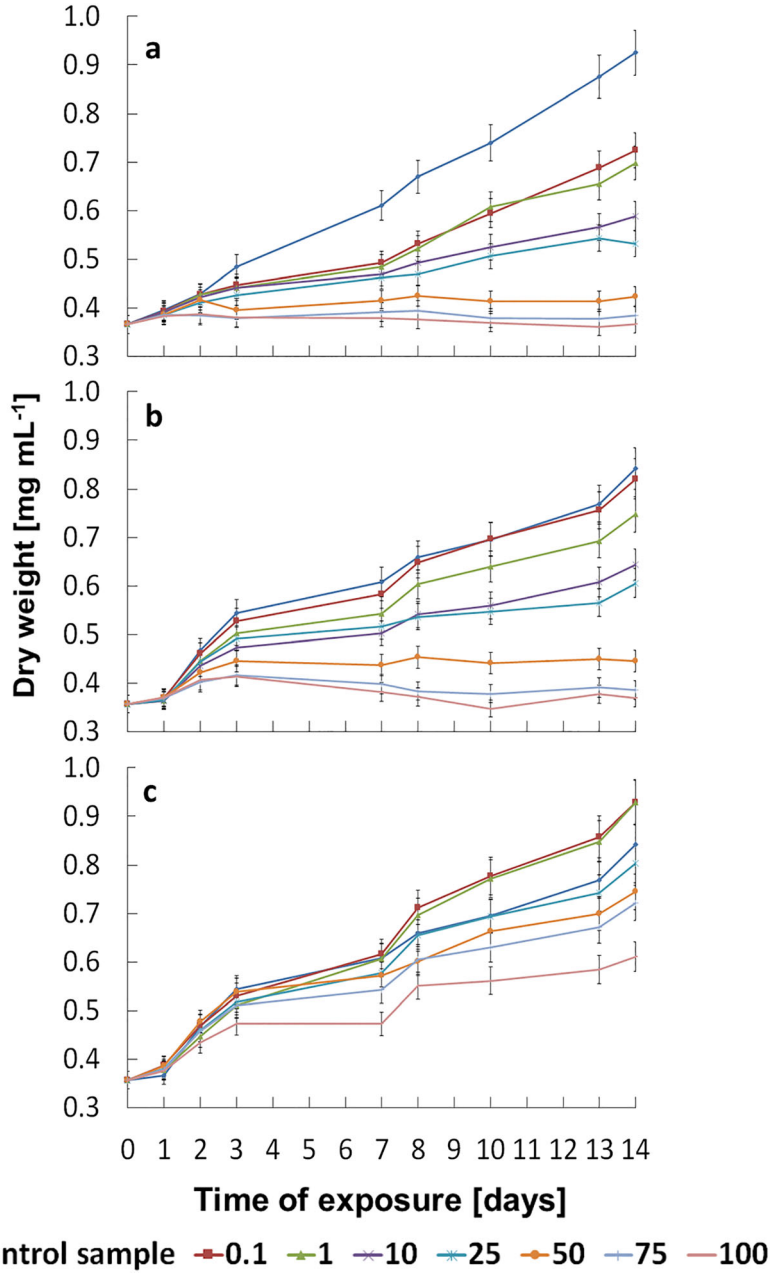

Fig. 2 Changes in biomass content (mg L^{-1}) of *Microcystis aeruginosa* during 14 days of exposure to 0.1, 1, 10, 25, 50, 75, and 100 mg L^{-1} mixed hormones (a MIX1, b MIX2, c MIX3). Error bars represent the standard deviation of five measurements ($P < 0.05$, $n = 5$)

Pseudokirchneriella subcapitata. Their results show that 17-α-ethinylestradiol and 17ß-estradiol were more toxic than estrone to both algae species. The results obtained in the present study confirm that the toxicity of estrogens toward *M. aeruginosa* decreased in the order: EE2 > E2 > E1. However, the achieved EC_{50} values were significantly higher than in the case of studies by other scientists. This difference results from the different culture conditions and different species of test organisms. Peng et al. (2014) indicated that low concentrations, such as 1.6 μM, of progesterone and norgestrel did not significantly affect the growth of *Scenedesmus obliquus* and *Chlorella pyrenoidosa*. Lai et al. (2002), Della Greca et al. (2008), and Maes et al. (2014) studied biotransformation of 17-α-ethinylestradiol by microalgae, and the obtained results showed that a low concentration of pollutants did not affect growth. As in the case of the above-mentioned studies, in the present research, low concentrations (0.1–1 mg L^{-1}) of E2, PRE, LG, and TST did not significantly affect the growth of *M. aeruginosa*. Cheng et al. (2018) reported that 0.01–5 mg L^{-1} of 17-α-ethinylestradiol stimulated the growth of microalgae mutant *Chlorella* PY-ZU1. However, this growth-stimulating effect decreased with increasing concentrations of the pollutant. Low-dose stimulation and high-dose inhibition are associated with the occurrence of the phenomenon of hormesis (Calabrese and Baldwin 2003; Calabrese 2005). Low concentrations of hormones can

Table 1 EC_{50} values (mg L^{-1}) of target single and mixed hormones based on biomass concentration, with 95% confidence intervals ($P < 0.05$)

Hormones	*Microcystis aeruginosa*					
	7 days		10 days		14 days	
	Dose-response equation (R^2)	$EC_{50} \pm SD$ (mg L^{-1})	Dose-response equation (R^2)	$EC_{50} \pm SD$ (mg L^{-1})	Dose-response equation (R^2)	$EC_{50} \pm SD$ (mg L^{-1})
E1	$y = -0.002x + 1.0836$ (0.8309)	288.01 ± 14.40	$y = -0.0023x + 1.1215$ (0.7485)	272.59 ± 13.63	$y = -0.0021x + 1.0941$ (0.7621)	276.68 ± 13.83
E2	$y = -0.0018x + 0.8778$ (0.5993)	211.10 ± 10.56	$y = -0.0031x + 0.9111$ (0.6348)	133.72 ± 6.69	$y = -0.0037x + 0.8928$ (0.7038)	106.04 ± 5.30
E3	$y = -0.0007x + 1.0204$ (0.2406)	766.69 ± 38.33	$y = -0.0018x + 1.0633$ (0.7834)	320.27 ± 16.01	$y = -0.0016x + 1.0543$ (0.8685)	355.15 ± 17.76
EE2	$y = -0.0033x + 1.0175$ (0.6071)	156.84 ± 7.84	$y = -0.0046x + 1.0173$ (0.5826)	113.43 ± 5.67	$y = -0.0052x + 0.9634$ (0.6125)	88.92 ± 4.45
PRO	$y = -0.0022x + 0.9139$ (0.7569)	185.46 ± 9.27	$y = -0.0042x + 0.9938$ (0.8115)	118.81 ± 5.94	$y = -0.0055x + 1.0126$ (0.8162)	93.63 ± 4.68
PRE	$y = -0.0019x + 0.8604$ (0.8217)	187.94 ± 9.40	$y = -0.0021x + 0.8288$ (0.7608)	154.20 ± 7.71	$y = -0.0019x + 0.7649$ (0.6409)	141.84 ± 7.09
LG	$y = -0.0006x + 0.9063$ (0.1116)	648.46 ± 32.42	$y = -0.0005x + 0.8876$ (0.1220)	834.99 ± 41.75	$y = -0.001x + 0.8669$ (0.5727)	354.75 ± 17.74
TST	$y = -0.0011x + 0.7991$ (0.8621)	260.97 ± 13.05	$y = -0.0011x + 0.7751$ (0.8880)	251.11 ± 12.56	$y = -0.0015x + 0.7596$ (0.9212)	173.27 ± 8.66
MIX1	$y = -0.0019x + 0.795$ (0.9687)	155.41 ± 7.77	$y = -0.0033x + 0.7783$ (0.9047)	85.05 ± 4.25	$y = -0.0038x + 0.7156$ (0.8757)	56.66 ± 2.83
MIX2	$y = -0.0031x + 0.9038$ (0.9145)	131.91 ± 6.60	$y = -0.0047x + 0.9148$ (0.9202)	89.12 ± 4.46	$y = -0.0051x + 0.8728$ (0.8910)	72.71 ± 3.64
MIX3	$y = -0.0019x + 1.0061$ (0.9003)	261.29 ± 13.06	$y = -0.0029x + 1.1048$ (0.9769)	206.47 ± 10.32	$y = -0.0035x + 1.0787$ (0.9538)	166.83 ± 8.34

y corresponds to percentage inhibition (%) and *x* stands for log (chemical concentration [mg L^{-1}])

activate the maintenance and repair functions of cyanobacteria cells by increasing the activity of proteins involved in their protection. In contrast, higher concentrations inhibit growth because they damage the functions of maintenance and repair of cells. A similar phenomenon was observed in our study because low concentrations of E1, E3, EE2, and PRO stimulated the growth of *M. aeruginosa*. Previous studies have shown that hormones inhibited the growth of cyanobacteria *Anabaena variabilis* (Czarny et al. 2019a). Similar to the present study, EE2 and PRO were the most toxic of all the tested hormones and the achieved 14-day EC_{50} values were 48.59 and 82.95 mg L^{-1}. PRE, TST, and E2 were moderately toxic to these cyanobacteria (14-day EC_{50}, 283.50, 336.68, and 437.82 mg L^{-1}), while E1, E3, and LG did not affect their growth. The toxicity of hormones to *Anabaena variabilis* seems to be similar as for *M. aeruginosa*. However, *M. aeruginosa* was less sensitive to 17-α-ethinylestradiol and progesterone and more toxic to estrone, 17ß-estadiol, estriol, 5-pregnen-3β-ol-20-one, levonorgestrel, and testosterone.

According to the literature, the toxic effects of single hormones on the growth of cyanobacteria have been tested, and the effects of mixtures have not been evaluated. The only information about the toxic effects of mixtures of hormones on microalgae can be found in the previous research which concerned the estimation of their effect on the cyanobacterium *A. variabilis* and the green algae *C. vulgaris* and *Scenedesmus armatus* (Czarny et al. 2019a, b). The achieved 14-day EC_{50} values for mixed hormones for *A. variabilis*, *C. vulgaris*, and *S. armatus* were 37.00–89.46, 47.89–75.30, and 70.54–697.26 mg L^{-1}, respectively (Czarny et al. 2019a, b). Comparing the results, it can be estimated that *M. aeruginosa* were less sensitive than *A. variabilis* and *C. vulgaris*, and more sensitive than *S. armatus*. The toxicity of the three mixtures of hormones to *M. aeruginosa* was MIX1 > MIX2 > MIX3. The much higher toxicity of MIX1 and MIX2 to cyanobacteria compared with MIX3 might be related to the components of the mixtures. The strongest inhibitory effect on *M. aeruginosa* was observed for EE2 and PRO. It is known that compounds in a mixture can interact with each other, which can result in a stronger (synergistic) or weaker (antagonistic) combined effect than could be expected from the toxicity data of each hormone alone. A more than additive effect arises when the combined effect of pollutants is greater than the sum of the effects of each compound individually (Nielsen et al. 2008). In our study, the sum of the EC_{50} values of single components was greater than the obtained values for mixtures. Therefore, the interactions between hormones result in a more than additive effect.

The toxic effect on *M. aeruginosa* might be related to several factors and mechanisms occurring simultaneously. Even if non-target organisms, such as cyanobacteria, do not have an endocrine system, hormones can affect their growth. It is

known that compounds with an octanol/water partition coefficient (log P) > 3 show high lipophilicity and high bioaccumulation potential (Silva et al. 2012; Blewett et al. 2014; Czarny et al. 2017). The octanol/water partition coefficients of almost all tested hormones were above 3, except for estriol (2.4) (Aris et al. 2014; Ismail et al. 2017). For this reason, hormones can easily cross cell membranes of cyanobacteria. Perron and Juneau (2011) reported that EDCs such as 17ß-estradiol can alter energy flows through photosystem II and affect the photosynthetic activity of *M. aeruginosa* and the green algae *C. reinhardtii* and *P. subcapitata*. Many studies have confirmed that the difference in the toxicity of endocrine-disrupting chemicals varies widely between species (Perron and Juneau 2011; Salomao et al. 2014; Xiang et al. 2018).

Environmental levels of hormones in typical lakes and rivers from different countries fluctuate in the range of ng L^{-1} (Adeel et al. 2017; Ma and Yates 2018). The ecological risk of these compounds can be assessed by comparing the measured environmental concentrations and the EC_{50} values obtained in this study. The results indicate that single and mixed hormones posed no risk to aquatic life such as cyanobacteria. However, taking into account the growing environmental pollution resulting from the increase in the amount of hormones, a much higher risk for aquatic organisms is anticipated.

Conclusions

In this study, the toxicity of eight single and three mixed hormones to *Microcystis aeruginosa* was compared based on biomass, chlorophyll *a* content, and EC_{50} values. Among all the examined single hormones, the sensitivity of the cyanobacterium *M. aeruginosa* to 17-α-ethinylestradiol and progesterone was the highest. In contrast, 17ß-estradiol, 5-pregnen-3β-ol-20-one, and testosterone were moderately toxic and estrone, estriol, and levonorgestrel showed the weakest effect. The achieved 14-day EC_{50} values were from 88.92 to 355.15 mg L^{-1}. Moreover, mixed hormones are more toxic than single compounds. MIX1 and MIX2 were more toxic than MIX3 (56.66, 72.72, 166.83 mg L^{-1}, respectively). According to the obtained EC_{50} values, more than additive effect between hormones may occur, especially when mostly toxic compounds (17-α-ethinylestradiol and progesterone) were the dominant pollutant in the mixture. Therefore, the increased toxicity caused by combined hormones poses a greater risk for the aquatic environment. According to the European Union classification, pollutants with EC_{50} values of 10–100 mg L^{-1} were considered harmful to aquatic organisms, while those with EC_{50} values above 100 mg L^{-1} were not classified. Therefore EE2, PRO, MIX1, and MIX2 may be considered harmful to *M. aeruginosa*. This is the first study to compare the toxic effect of hormones and their mixtures on the growth of *M. aeruginosa*. The measured concentrations of hormones in samples of river and lake water collected in several countries were below the tested concentrations, indicating that these compounds pose a negligible risk to cyanobacteria.

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