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Cyanobacterial blooms act as sink and source of endocrine disruptors in the third largest freshwater lake in China☆

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ABSTRACT

Cyanobacterial blooms are of global concern due to the multiple harmful risks they pose towards aquatic ecosystem and human health. However, information on the fate of organic pollutants mediated by cyanobacterial blooms in eutrophic water remains elusive. In the present study, endocrine disruptive potentials of phytoplankton samples were evaluated throughout a year-long surveillance in a large and eutrophic freshwater lake. Severe cyanobacterial blooms persisted during our sampling campaigns. Estrogenic agonistic, anti-estrogenic, anti-androgenic, and anti-glucocorticogenic effects were observed in the phytoplankton samples using in vitro reporter gene bioassays. 27 endocrine disrupting chemicals (EDCs) of different modes of action were detected in the samples via UPLC-MS/MS system. Results from mass balance analysis indicated that the measured estrogenic activities were greater than the predicted estrogenic potencies from chemical analysis, demonstrating that chemical analysis of targeted EDCs is unable to fully explain the compounds responsible for the observed estrogenicities. Results from Spearman's correlation analysis concluded that the concentrations of ten EDCs in phytoplankton samples were negatively correlated with cyanobacterial biomass, suggesting the potential occurrence of biomass bio-dilution effects of EDCs due to the huge biomass of cyanobacteria during bloom seasons. The present study provided complementary information about the potential endocrine disruptive risks of cyanobacterial blooms, which is important for understanding and regulating EDCs in eutrophic lakes.

1. Introduction

Chemical pollutants, such as endocrine disrupting compounds (EDCs), are of great concern due to their industrial and domestic applications and potential adverse effects on metabolism, development, growth and reproduction in exposed freshwater and marine wildlife (Tan et al., 2007). The effects of EDCs include reduced fertility, feminization, reproductive organ anomalies, and changes in sexual behavior of a variety of aquatic organisms (Kidd et al., 2007; Pal et al., 2010). Research has shown the occurrence of EDCs in wastewaters (Yu et al., 2013), natural waters (Yang et al., 2014), and drinking waters (Benotti et al., 2008) all around the world, including synthetic steroid hormones, pharmaceutical drugs, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, alkylphenols, pesticides, but also natural products such as phytoestrogens. Men-made pollutants are released from manufacturing processes and since they are not fully removed by sewage treatment systems, they can enter aquatic systems.

In addition to chemical pollution, aquatic ecosystems are exposed to additional multiple stressors (Ormerod et al., 2010). Eutrophication of freshwaters and coastal marine ecosystems

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resulting from increased anthropogenic nutrient input into receiving waterbodies has become a global problem (Smith, 2003). Harmful algal blooms are frequently concomitant with over-enrichment of nutrients (particularly nitrogen and phosphorus). Eutrophic waterbodies with severe cyanobacterial blooms are observed in Lake Taihu in China (Paerl et al., 2011), Lake Biwa in Japan (Nalewajko & Murphy, 2001), Lake Erie and Michigan in North America (Rinta-Kanto et al., 2005), Lake Winnipeg in Canada (Schindler et al., 2012), Lake Victoria in Africa (Verschuren et al., 2002), the Baltic Sea in Northern Europe (Suikkanen et al., 2007), and other ecologically and economically important lakes, rivers and estuaries globally (Huisman et al., 2006). Dense cyanobacterial blooms increase the turbidity of eutrophic waters, and subsequently suppress the growth of aquatic plants and thereby negatively affect the underwater habitat for many invertebrates and fish species (Scheffer, 2004). In the last few decades, of great concern is the increase in toxin-producing strains of the blue-green algae Microcystis sp. that produce hepatotoxin microcystin (Michalak et al., 2013). More than 100 structural variants of microcystins have been identified and classified as "possibly carcinogenic to humans" (Grosse et al., 2006). A number of studies have been focused on the toxicity of microcystins, such as hepatotoxicity (Takumi et al., 2010), genotoxicity (Žegura et al., 2003), oxidative stress (Amado & Monserrat, 2010), and endocrine-disruptive potentials (Rogers et al., 2011). Besides the estrogenicity from microcystins, more recent studies suggest that cyanobacterium Microcystis might be a natural source of environmental estrogens (Sychrová et al., 2012; Essa & Fathy, 2014; Prochazkova et al., 2018).

Phytoplankton is the first link in the trophic chain, performing an important function in the transfer of organic compounds between biotic and abiotic elements of aquatic ecosystems. It has been indicated that significant accumulation of contaminants in phytoplanktonic phase can occur for example with organic contaminants such as polychlorinated biphenyls (Lynn et al., 2007), polycyclic aromatic hydrocarbons (Wan et al., 2007), dioxins (Wan et al., 2005), polybrominated diphenyl ethers (Frouin et al., 2013), and other EDCs (Staniszewska et al., 2015). Thus, in the context of cyanobacterial blooms, the loading and transformation of EDCs in phytoplankton matrix warrant concern. Particularly, colonial Microcystis, which form by a heterogeneous high-molecular-weight mucilaginous matrix (Forni et al., 1997), might play a considerable role in regulating the biogeochemical cycles of organic pollutants in waterbodies affected by Microcystis blooms.

Lake Taihu, the third largest freshwater lake in China, serves as an important resource for drinking water, aquaculture, irrigation and industrial waters, in addition to being a popular recreational and tourist attraction (Song et al., 2007). Unfortunately, decades of intensive utilization of water resources has transformed this once meso-oligotrophic lake in the 1950s into its present hypertrophic state (Chen et al., 1997). Every spring, large areas of the lake turn green with dense Microcystis blooms that persist well into the autumn (Otten et al., 2012). In addition, the pollution by synthetic organic chemicals has been reported in abiotic and biotic phases from Lake Taihu (Yan et al., 2014; Xie et al., 2015). However, little information is available about the occurrence of endocrine disruptors from cyanobacterial blooms in eutrophic freshwaters.

In the present study, phytoplankton samples were collected throughout a one-year surveillance of Lake Taihu. Firstly, the situations of cyanobacterial blooms and cyanotoxins were evaluated in the north, west, and south of Lake Taihu. Particularly, the phytoplankton compositions and biomass were monitored. Secondly, a bioanalytical approach was employed to evaluate the endocrine disruptive potentials in phytoplankton matrices. Rapid, highly sensitive in vitro reporter gene bioassays were used to measure endocrine disruption potentials, as these have been previously demonstrated to successfully identify endocrine disruption effects in environmental matrices (Kunz et al., 2017; Gehrmann et al., 2018; Kase et al., 2018). For environmental monitoring, one of the advantages of the use of bioassays over chemical analysis is that bioassays can measure both known and unknown EDCs, whereas chemical analysis can only detect known EDCs. Thus, bioassays evaluate the total endocrine disruption potency, offering a more robust and accurate assessment of the risk of EDCs in the environment than chemical analysis (Wernersson et al., 2015; Könemann et al., 2018). However, important quantitative information about concentrations of EDCs in the environment can still be obtained from chemical analysis. Thus, in the third step of the present study, chemical analysis was performed in parallel to the bioassays to gain complementary results about the presence and risk of EDCs in the environment. Mass balance analysis was performed between the two approaches. Finally, multivariate analysis was conducted to gain more insights into the relationship between EDCs contents and phytoplankton compositions.

2. Methods and materials

2.1. Chemicals and materials

All chemicals used in the present study were reagent grade and purchased from Sigma Aldrich (Schnelldorf, Germany). Reference compounds used for bioassays, including estradiol (E2), dihydrotestosterone (DHT), dexamethasone (Dexa), tamoxifen, flutamide, and mifepristone, were prepared in dimethyl sulfoxide (DMSO). Cell culture medium [Dulbecco's Modified Eagle Medium (DMEM)] was obtained from Sigma Aldrich (Schnelldorf, Germany) and Invitrogen (Darmstadt, Germany). Twenty-six natural and synthetic hormonal chemicals and four commonly detected industrial contaminants were selected as the target EDCs based on previous studies in Lake Taihu (Lu et al., 2011; Yan et al., 2012). Descriptions of these substances are provided in Table 1.

2.2. Sample collection and preparation

Samples of phytoplankton were collected using 40 μm phytoplankton net from four sites in each of the north, west, and south regions of Taihu Lake over the period from September 2010 to August 2011 (Fig. 1). The sampling sites were not directly adjacent to the inflows of major effluents. Water samples for phytoplankton species composition were collected at each site from 0 to 0.5 m depth using a vertical sampler, and fixated with Lugol's iodine solution. Fixated phytoplankton samples were identified and enumerated by light microscopy according to the commonly used method by Watanabe and co-workers (Watanabe et al., 1992). In the laboratory, the phytoplankton samples were settled overnight at 4 °C to separate the zooplankton and suspended particles (Watanabe et al., 1992). The phytoplankton samples were lyophilized and stored at −80 °C for further analysis.

2.3. Sample extraction

Due to the limited biomass obtained during the cold season, sites with abundant algal biomass were selected for cyanotoxin and EDCs measurements. Firstly, one portion of the dried phytoplankton samples (50 mg) were weighed for cyanotoxins analysis, and prepared with 5% acetic acid and 80% aqueous methanol used for cyanotoxins extraction (the ratio of solvent volume per dry weight biomass is 20 mL per 50 mg). The obtained extracts were cleaned up using C18 solid phase extraction cartridge (6 cc, 500 mg, Waters, US) and the eluted solutions were stored at −20 °C until high performance liquid chromatography (HPLC) analysis, as

Table 1
The 30 selected EDCs and their measurement parameters for UPLC-MS.

Compounds	Abbreviation	Internal standards	Mode	Parent Ions	Quantification Ions	Confirmation Ions	Retention time (min)
Seven Estrogens							
Estrone	E1	E1-D2	ESI−	269.2	145.1	159	3.58
Estrone-D2	E1-D2	–	ESI−	271.3	185.1	171.1	3.53
17β-Estradiol	E2	E1-D2	ESI−	271	182.9	145	3.17
Estriol	E3	E1-D2	ESI−	287.1	171	158.8	1.87
Diethylstilbestrol	DES	DES-D8	ESI−	267.2	221.9	237	3.69
Diethylstilbestrol-D8	DES-D8	–	ESI−	275	244.8	259	3.65
Dienoestrol	Dieno	DES-D8	ESI−	265	92.8	171	3.78
Hexestrol	Hexe	DES-D8	ESI−	269.1	119	134	3.79
Estradiol benzoate	E2-ben	Proges-D9	ESI+	377.4	105	77	5.8
Eight Androgens							
19-Nortestosterone	Nortes	TES-D3	ESI+	275.3	82.9	109	3.16
Trenbolone	Tren	TES-D3	ESI+	271.2	106.9	91	2.92
Testosterone	TES	TES-D3	ESI+	289.2	97	253.3	3.43
Testosterone-D3	TES-D3	–	ESI+	292.4	97	109	3.35
Methyl testosterone	Me-TES	TES-D3	ESI+	303.4	97	109	3.69
Nandrolone Phenylpropionate	Nan-phen	TES-D3	ESI+	407.1	91	104.9	6.19
Testosterone propionate	TES-pro	TES-D3	ESI+	345.3	109.3	97.2	5.75
Boldenone	Bold	TES-D3	ESI+	287.2	121	135.1	3.05
Epitestosterone	Epite	TES-D3	ESI+	289.2	109.3	97.2	3.81
Six Progesterones							
Norethisterone	Noreth	Proges-D9	ESI+	299.2	109	91	3.44
d(−) Norgestrel	Norges	Nogres-D6	ESI+	313.2	109	245.6	3.99
Norgestrel-D6	Norges-D6	–	ESI+	319.5	251.7	301.5	3.9
Medroxy progesterone	Me-pro	Proges-D9	ESI+	345.3	123	97	4.18
Progesterone	Proges	Proges-D9	ESI+	315.4	97	109	4.7
Progesterone-D9	Proges-D9	–	ESI+	324.4	100.1	113.1	4.67
Megestrol acetate	Me-ace	Proges-D9	ESI+	385.3	224.2	267.2	4.62
Hydroxyprogesterone	Hydrop	Proges-D9	ESI+	429.3	253.5	271.3	5.84
Five Adrenocortical hormones							
Prednisone	Predn	Proges-D9	ESI+	359.5	146.8	313.1	2.17
Cortisone	Corti	Proges-D9	ESI+	361.3	121.3	90.9	2.21
Dexamethasone	Dexa	Proges-D9	ESI+	393.4	373.2	147	2.63
Prednisolone	Prednln	Proges-D9	ESI+	361.4	147.1	307.2	2.21
Methylprednisolone	Me-prednl	Proges-D9	ESI+	375.4	161	120.9	2.52
Four Industrial compounds							
Bisphenol S	BPS	DES-D8	ESI−	248.4	108	91.9	0.76
4-n-Octyl Phenol	OP	DES-D8	ESI−	205.2	189.1	133.3	5.56
Bisphenol A	BPA	DES-D8	ESI−	227.3	212	133.3	3.06
Bisphenol F	BPF	DES-D8	ESI−	199.1	93	197.4	2.46

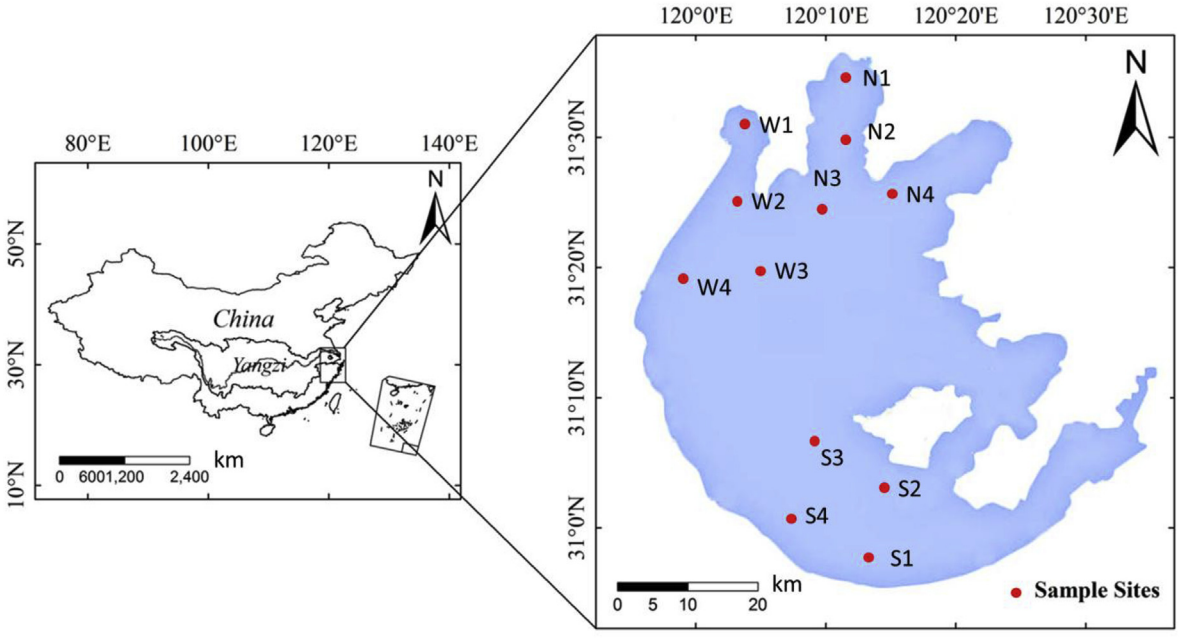

Fig. 1. Map of the sampling sites in Lake Taihu, China.

reported previously (Barco et al., 2005). Secondly, the extraction and purification procedures for the determination of the selected EDCs from phytoplankton samples were performed as previously described for solid samples (Pojana et al., 2007). Briefly, 100 mg freeze-dried phytoplankton samples were sonicated with an extraction solvent mixture (hexane/acetone, 70:30, v/v) for 2 h (the ratio of solvent volume per dry weight biomass is 20 mL per 100 mg). The sonication frequency was 20 kHz. The extraction procedure was repeated three times and the obtained three 20 mL extracts were then combined and filtered through a glass fiber membrane (GF/F, Whatman, Maidstone, UK) and concentrated under gentle nitrogen flow to ca. 500 μL under 20 °C. The concentrated extracts were further purified with a Florisil clean-up column (60–100 mesh, activated at 150 °C overnight) as described elsewhere (Pojana et al., 2007). Meanwhile, in order to evaluate the performance of sample extraction, procedure controls were processed throughout the extraction and clean-up. The final extracts were then dissolved in 300 μL Milli-Q water and stored at 2 °C prior to chemical and bioanalytical measurements.

2.4. U2OS cell culture and reporter gene assay

The assay protocols have been extensively described elsewhere (Van Der Linden et al., 2008; Grund et al., 2011; Maletz et al., 2013). The human U2OS Osteosarcoma 1 cells used in the Estrogen receptor α](ERα), androgen receptor (AR)], and glucocorticoid receptor (GR] reporter gene assays were provides and licenced by BioDetectionSystems (BDS, Amsterdam, The Netherlands). To avoid false negative results, only the extracts tested as non-cytotoxic (cell viability >80%) within the MTT assay were applied in the CALUX assays according to the protocol developed by BDS. Briefly, U2OS-*luc* cells were cultured at 37 °C under 5% CO_2 and high humidity in DF medium (1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's F12) supplemented with 7.5% fetal calf serum (FCS). Cells were seeded into 96 wells plates with DF medium (without phenol red and supplemented with DCC stripped FCS). After 24 h of incubation, the medium was replaced with medium containing stripped FCS and reference compounds with or without extracts for agonistic and antagonistic response testing. After 24 h of exposure, the medium was removed, and the cells were lysed in Triton lysis buffer. The amount of luciferase activity was measured using a luminometer (Berthold, Germany). All measurements were performed in triplicate wells; experiments were conducted at least in triplicate for all samples. For agonistic assays, cells were exposed to reference hormone-receptor agonists [17β-estradiol (E2, 10^{-13}-10^{-10} M), 5α-dihydrotestosterone (DHT, 10^{-12}-10^{-7} M), and dexamethasone (DEX, 3×10^{-11}-10^{-7} M) for ER-, AR-, and GR-CALUX, respectively] or extracts. For antagonistic effects, the assay medium was spiked prior to exposure with the EC50 of agonist, i.e., final concentrations of 3×10^{-11} E2, 4.2×10^{-10} DHT, 1.2×10^{-9} DEX for the anti-ER CALUX, anti-AR CALUX, and anti-GR CALUX, respectively. Tamoxifen (10^{-7}-10^{-3} M), flutamide (10^{-6}-10^{-2} M), and mifepristone (10^{-8}-10^{-3} M) were used as the reference compounds for anti-ER, anti-AR, and anti-GR assays, respectively.

For each bioassays, concentrations of samples were expressed as relative enrichment factors (REF), which can be calculated by enrichment factor of the sample extraction or concentration multiplied by the dilution factors of the extract (Jia et al., 2015).

In the agonistic assays, the response as %effect was obtained by dividing the sample response by the maximum response at the same experiment after subtracting control signal. The control signal refers to solvent control (equation (1)).

$$\text{Agonistic effect\%} = \frac{\text{Signal}_{\text{sample}} - \text{Signal}_{\text{control}}}{\text{Signal}_{\text{max}} - \text{Signal}_{\text{control}}} \quad (1)$$

EC_{10} was defined as the sample REF causing 10% effect and it can be derived from the nonlinear regression fit if a full concentration-effect curve was observed, or, if there is no full curve, the EC_{10} was derived from the slope of the linear range of the sample concentration-effect curve, as presented in equation (2) (Kunz et al., 2017).

$$EC_{10} = \frac{10\%}{\text{slope}} \quad (2)$$

In the antagonistic assays, the effect was expressed as suppression ratio (equation (3)).

$$\text{Antagonistic effect} = 1 - \frac{\text{Signal}_{\text{sample}} - \text{Signal}_{\text{control}}}{\text{Signal}_{\text{agonist}} - \text{Signal}_{\text{control}}} \quad (3)$$

EC_{20} was defined as the sample REF causing 20% of the effect suppression, and similar with EC_{10}, it is calculated directly according to 50% effect concentration (EC_{50}) and the hill slope if a full curve available (equation (4)), or from the slope of the linear regression if no full curve exists, as showed in equation (5) (Kunz et al., 2017).

$$\log EC_{20} = \log EC_{50} + \frac{1}{\text{slope}} \log \frac{1}{4} \quad (4)$$

$$EC_{20} = \frac{0.2}{\text{slope}} \quad (5)$$

2.5. Chemical analysis

The target analytes were analyzed according to a previously described method (Zhang et al., 2011). Briefly, the analysis was performed using Waters ACQUITY UPLC system coupled with Waters Xevo TQ MS (Waters, Milford, MA). The tandem MS system was operated in both positive-ion and negative-ion multiple-reaction-monitoring (MRM) mode. UPLC-MS data were acquired using Masslynx 4.1 software package (Waters, Milford, USA). Recoveries of EDCs in phytoplankton samples were determined by use of external standards. Limit of quantitation and recoveries are shown in Table S1. The detailed detection parameters for UPLC-MS/MS and quality assurance and quality control (QA/QC) are presented in the supplementary file. Estrogenic equivalents predicted from chemical analysis were calculated from concentrations of the targeted estrogenic compounds determined by UPLC-MS/MS analyses and relative potencies (estradiol equivalency factor, EEF) obtained from the in vitro assays of previous studies (Table S4). The compounds that were below the limit of quantification (LOQ) were not included in the calculations of predicted estrogenic equivalents. Calculation of the predicted estrogenic equivalents (EEQ) was according to the following equations:

$$\text{Predicted EEQ (total)} = \sum_{i}^{n} [\textit{compound } i] \times \textit{EEF } i$$

2.6. Data processing

Statistical analyses were performed using R Studio (Version 1.0.143, R Studio, Inc.) and GraphPad Prism 6 (GraphPad Software Inc.) for Windows. Prior to data analysis, data were checked for assumptions of normality and homogeneity of variances using the

Shapiro-Wilk test and Bartlett test. The concentration-response curve was generated using a four-parameter logistic model (drc package, R). The final data are shown as mean ± standard deviation. The non-parametric two variables Spearman's correlation of the phytoplankton and EDCs contents was analyzed using ltm package in "R" (Rizopoulos, 2006). In comparison to Pearson's correlation analysis which assesses linear relationships, Spearman's correlation assesses monotonic relationships. The results of all tests were accepted as significant at $p < 0.05$.

3. Results and discussion

3.1. Status of cyanobacterial blooms and cyanotoxins in Lake Taihu

Eutrophication has long been acknowledged as a driver of the production of phytoplankton biomass in freshwater (Conley et al., 2009). In Lake Taihu, total nitrogen (TN) and total phosphorus (TP), which are largely associated with eutrophication, were present at relatively high concentrations throughout the duration of the sampling period (Fig. 2A and B). The average concentrations of TN and TP were 3.7 mg/L and 0.2 mg/L, respectively. Sampling sites in the western part of the lake contained greater concentrations of both TN and TP loading in comparison with the northern and southern regions ($p < 0.05$). Overall, the concentrations of nutrients from the present sampling campaign are similar to China environmental quality report for Lake Taihu in 2011, indicating the predominant occurrence of eutrophication in the lake.

Given the abundance of nutrients in the lake, severe cyanobacterial blooms are one of the major problems threatening water quality and function in Lake Taihu. As presented in Fig. 2C, high percentages of cyanobacteria in total phytoplankton biomass were observed during the year surveillance. Cyanobacteria were observed to predominate more than 90% of the total phytoplankton cell density in 65%, 44% and 58% of the 48 phytoplankton samples collected in each of the west, north and south areas of the lake, respectively. The phytoplankton cell densities during the different time points of sampling were shown in Figure S1. Cyanobacterial blooms in Taihu Lake exhibited an increase in coverage in all open lake sections over the sampling year, suggesting that situations in these lake regions continue to worsen.

Meanwhile, cyanobacterial blooms are also notorious for the production of cyanotoxins, such as the heptatoxins, microcystins (MCs). We measured three variants of MCs, i.e., MC-RR, MC-YR, and MC-LR in phytoplankton samples from Lake Taihu (Fig. 2D and Figure S2). The three microcystin variants were widely detected in the samples with the highest concentrations of 1128, 527 and 658 mg kg d.w. for MC-RR, MC-YR and MC-LR, respectively. MC contamination has been reported in Lake Taihu by several previous studies (Song et al., 2007; Peng et al., 2010). High intracellular MCs might contribute to a number of adverse outcomes on organisms, such as endocrine disruptive activity (Rogers et al., 2011).

3.2. Bioanalytical evaluation of endocrine-disrupting potentials

The endocrine-disruptive potencies in phytoplankton samples collected during 2010–2011 were examined using in vitro bioassays. All phytoplankton samples exhibited estrogenicity, as presented in Fig. 3A and Figure S3. The estrogenic potencies in the samples ranged from 0.5 to 5.1 ng E2/g d.w., which is greater than previously reported in sediments of Lake Taihu and several other regions of China (Ke et al., 2015; Lei et al., 2015; Lou et al., 2016). Previous studies were mostly focused on either the dissolved EDCs (Prochazkova et al., 2018; Shi et al., 2018) or sediment-bound EDCs (Shi et al., 2017) in eutrophic waterbodies. However, evidence is accumulating with regard to occurrence of estrogenic activity from cyanobacterial blooms (Štěpánková et al., 2011).

In aquatic ecosystems, phytoplankton contribute to both energy transfer and essential biogeochemistry processes in the food web. However, in polluted waters phytoplankton can adsorb, sequester and/or uptake organic pollutants (Baptista et al., 2009; Seto & Handoh, 2009; Maes et al., 2014), which can contribute toward the transfer of multi-classes of contaminants into the food web. Additionally, phytoplankton are responsible for the production of a diverse array of natural products among which are potential endocrine disruptors (Gong et al., 2014). More recently, estrogenic potencies were also found to be associated with cyanobacteria and their blooms in surface waters (Štěpánková et al., 2011; Sychrová et al., 2012; Essa & Fathy, 2014; Procházková et al., 2017a).

The AR CALUX and GR CALUX assays did not result in any effects of the phytoplankton samples (Figure S4). Hu X. et al. (Hu et al., 2013) similarly reported that no androgenic activity was found in raw water samples from Taihu Lake and drinking water samples around the lake. However, a noteworthy finding was the significant anti-estrogenic, anti-androgenic and anti-glucocorticoid activities observed in the present study with concentrations ranging from 1.7 to 6.1 μg tamoxifen/g d.w., 1.1–44.3 μg flutamide/g d.w., and 0.1–0.4 μg mifepristone/g d.w., respectively (Fig. 3B, C, and D). Jianliang Zhao et al., (2011) reported that the levels of anti-estrogenic activity were up to 1296 μg tamoxifen/L in surface water and 89.5 μg tamoxifen/g in sediment of Pearl River in China. In vitro reporter gene assays offer a highly sensitive and cost-effective way to evaluate a series of hormone receptors agonistic effects. However, particularly in environmental monitoring, the co-existing antagonist compounds might suppress the agonists binding to receptors. In this case, the in vitro bioassays represent net endocrine-disruption activity in the balance between agonistic and antagonistic effects (Ihara et al., 2014). A previous study has reported that phthalate esters including diisobutyl phthalate and dibutyl phthalate were identified as major contributors to an AR antagonistic potencies in source waters in China (Hu et al., 2013). Our recent study identified 4-methyl-7-diethylaminocomarin as a highly potent environmental pollutant that acts as AR antagonist (Muschket et al., 2017). Additionally, it has been reported that several commercially available humins elicited significant anti-estrogenic effects, likely through the sorption of E2 on humic substances, changes in membrane permeability for E2, or another specific mechanism (Janošek et al., 2007). However, the number of pollutants that are responsible for antagonistic effects is far from being fully determined.

3.3. Chemical analysis and mass balance

A wide range of EDCs in phytoplankton samples were detected analytically during all sampling periods (Fig. 4). As shown in Fig. 4, 27 of 30 analytes were detected in at least one of the phytoplankton samples. Four estrogens (i.e., estriol, diethylstilbestrol, dienoestrol, and hexestrol), two androgens (i.e., 19-nortestosterone and boldenone), two progesterones (i.e., norethisterone and hydroxyprogesterone), an adrenocortical hormone (i.e., Cortisone), and three industrial pollutants (i.e., bisphenol A, bisphenol S, and bisphenol F) were detected in all samples.

The highest concentrations of estrogens, androgens, progestogens, and adrenocortical hormones in phytoplankton samples were 6.1 (estriol), 54 (boldenone), 22.7 (hydroxyprogesterone), and 151.7 (Cortisone) ng/g d.w., respectively. The concentrations of five adrenocortical hormones were consistently greater than other classes of EDCs, suggesting further studies are required to examine potential effects from GR CALUX assays in comparison to the relatively high content of adrenocortical hormones determined by chemical analysis. Among the four industrial pollutants, bisphenol

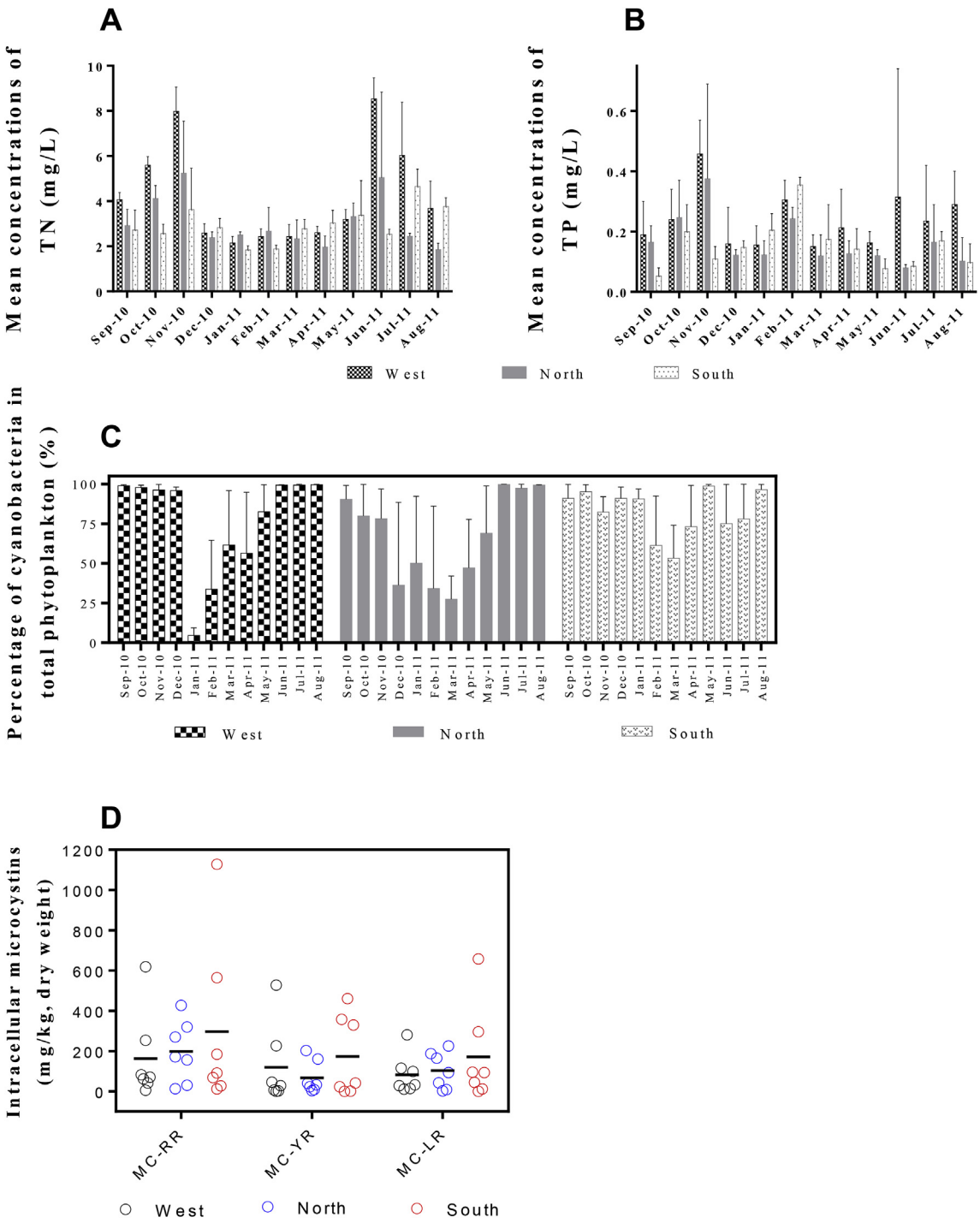

Fig. 2. Nutrient status as a measure of mean (±SD, $n = 3$) concentration of (A) total nitrogen (TN, mg/L) and (B) total phosphorus (TP, mg/L), (C) percent of cyanobacteria in total phytoplankton (%), and (D) cell-bound microcystins (MC-RR, MC-YR, and MC-LR) concentrations (mg/kg, dry weight) were monitored during the one year surveillance in Lake Taihu (2010–2011). The sampling sites are located in the West, North, and South of Lake Taihu.

A was detected with the highest concentration of 3954 ng/g, followed by bisphenol S (547 ng/g), bisphenol F (324 ng/g), and 4-*n*-octyl phenol (58.1 ng/g). The demand and production capacity of bisphenol A in China has grown rapidly due to its importance in the manufacturing of many products such as engineered plastics, food cans, and dental composites/sealants (Huang et al., 2012). This trend will lead to much more bisphenol A contamination in various environmental media. It has been extensively reported that the presence of bisphenol A is ubiquitous in aquatic environments with concentrations of up to 5030 ng/L (Quirós et al., 2005; Voutsa et al., 2006; Huang et al., 2012). More recently, the substitutes of bisphenol A, such as bisphenol S and bisphenol F, are also frequently detected in aquatic environment (Yamazaki et al., 2015). Unfortunately, research has demonstrated that these structural analogues of bisphenol A cause similar effects on ER and AR activities (Rosenmai et al., 2014).

A correlation between the presence of targeted EDCs in cyanobacterial bloom samples and the endocrine effects observed in

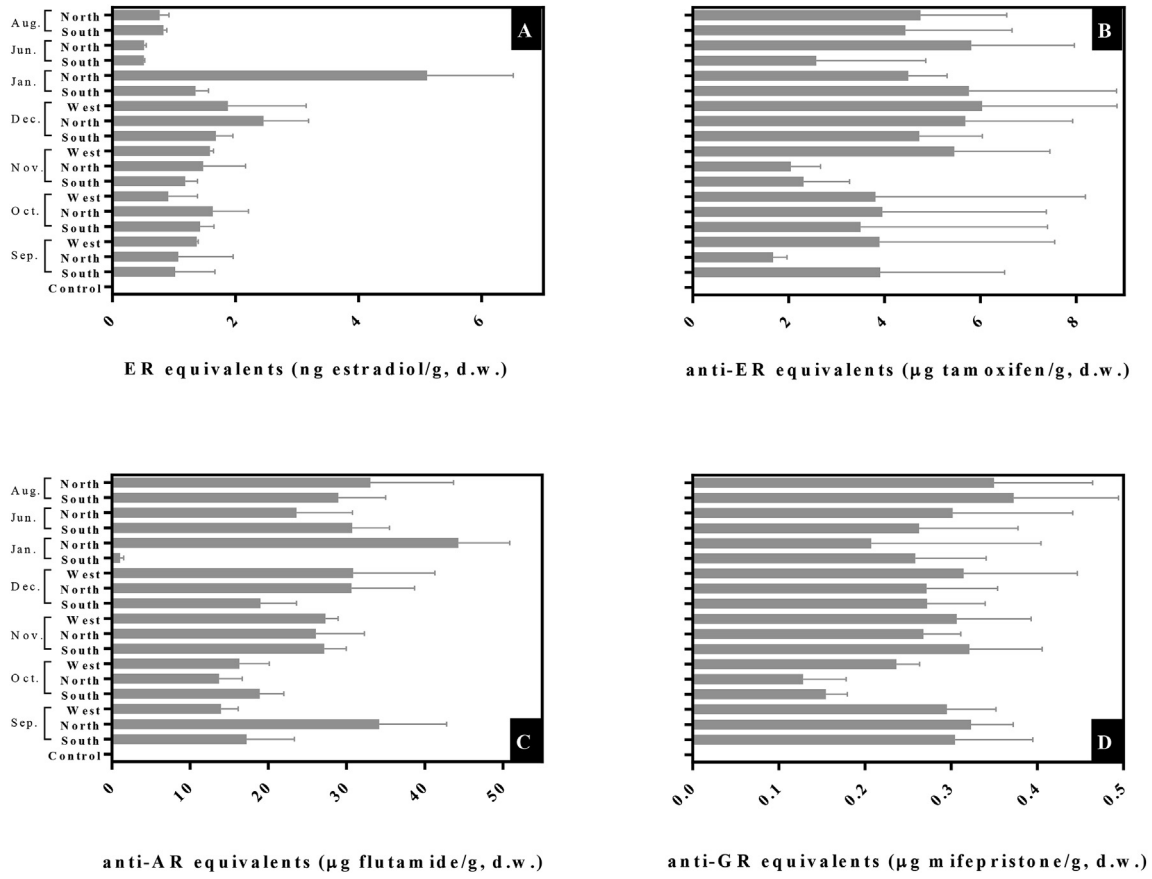

Fig. 3. Estrogen (ER; A), anti-estrogen (anti-ER; B), anti-androgen (anti-AR; C), and anti-glucocorticogen (anti-GR; D) equivalents for phytoplankton samples collected from west, north and south regions of Lake Taihu from Sep. of 2010 to Aug. of 2011 determined by use of U2OS cell reporter gene assays.

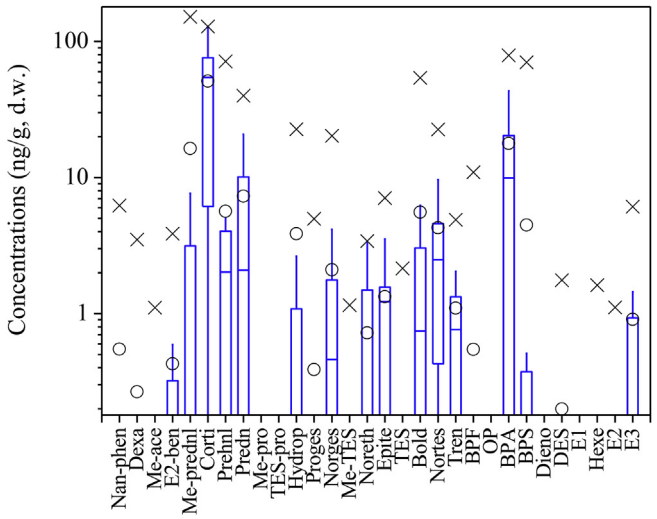

Fig. 4. Box chart of instrumental analysis of 30 endocrine-disruptive compounds (EDCs) in phytoplankton samples. Cross symbol indicates the maximum value; cycle symbol indicates the mean value.

reporter gene assays could not be established in the present study. For example, cyanobacterial bloom samples in this study contained androgenic and glucocorticoid compounds, such as testosterone, testosterone propionate, cortisone, and dexamethasone, yet results from reporter gene assays suggested that these EDCs were either not present in concentrations sufficient to induce androgenic and glucocorticoid effects in the bioassays or were not bioavailable. There are at least three explanations. Firstly, these EDCs were either not present in concentrations sufficient to induce androgenic and glucocorticoid effects in the bioassays or were not bioavailable. Secondly, certain endocrine-disruptive responses may not have been observed due to the coexistence of other compounds with different modes of actions, such that estrogenic effects might be masked due to the presence of testosterone (Sellin Jeffries et al., 2011; Sychrová et al., 2012). Secondly, the occurrence of antagonistic endocrine disrupting effects suppresses the detectability of agonistic endocrine disrupting effects (Ihara et al., 2014) leading to the undetectable of androgenic and glucocorticoid effects in the present in vitro bioassays.

Mass balance analysis of measured and predicted estrogenic activities are shown in Fig. 5. 17 of 20 cyanobacterial samples were calculated with the ratio between measured and predicted estrogenic equivalents greater than one (with a highest value up to 37.62), suggesting that the measured estrogenic potencies could not be explained by the analyzed estrogens in the present study. Both anthropogenic and natural substances contribute to overall estrogenic activity in cyanobacterial blooms samples. The evidence of endocrine-disruptive potentials of naturally produced compounds from cyanobacterial blooms is now growing (Oziol & Bouaïcha, 2010). One recent study suggested that maximum phytoestrogens accounted for only 1.6 pg/L E2 equivalents, responding to maximal 8.5% of the total estrogenicity of the water samples (Procházková et al., 2017b).

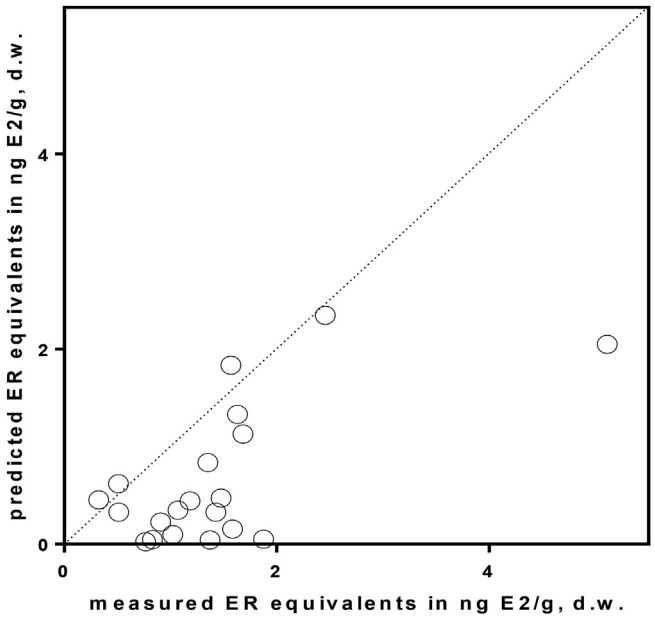

Fig. 5. Correlation between measured estrogenic equivalents based on ER CALUX assays and predicted estrogenic equivalents based on instrumental chemical analysis. The dashed line indicates perfect agreement of the measured estrogenic equivalents and the predicted estrogenic equivalents.

3.4. Multivariate analysis

Statistical analyses were performed to examine possible relationships between the concentrations of EDCs in phytoplankton and phytoplankton biomass. Spearman correlation tests were performed and the results are shown in the datasheet in supplementary file and in Fig. 6. Ten EDCs (i.e., hydroxyprogesterone, methyl testosterone, and dexamethasone, $p < 0.01$; bisphenol S, 19-nortestosterone, testosterone, progesterone, estradiol benzoate, megestrol acetate, and epitestosterone, $p < 0.05$) were negatively correlated with Cyanophyta biomass. Eight EDCs (i.e., 19-nortestosterone and testosterone, $p < 0.01$; bisphenol F, bisphenol S, progesterone, dexamethasone, methyl testosterone, and hexestrol, $p < 0.05$) were positively correlated with Chlorophyta biomass. Hydroxyprogesterone, progesterone, and methyl testosterone were positively correlated with diatom biomass ($p < 0.05$). Dienoestrol, 4-n-octyl phenol, and estradiol benzoate were positively correlated with Chrysophyta biomass ($p < 0.05$). 4-n-octyl phenol, bisphenol A, and estradiol benzoate were positively correlated with Cryptophyta biomass ($p < 0.05$). Similarly, results from the in vitro bioassays were incorporated into Spearman correlation analysis (Fig. 6), demonstrating that the estrogenicities derived from ER-CALUX were negatively correlated with Cyanophyta biomass but positively correlated with the biomass of diatoms and Chrysophyta. The anti-androgenicities derived from anti-AR CALUX were also positively correlated with the biomass of diatoms and chrysophyta.

Physicochemical sorption of organic pollutants to live algae was influenced by several factors including the properties of the sorbing matrix (e.g., surface area, binding sites, and lipids contents) and the hydrophobicity of the agents themselves. Firstly, in comparison to colonial cyanobacteria, unicellular algae (e.g., chlorophyta and diatoms) provide larger surface area for the uptake of organics from water column onto the surface of algal cells (Chen et al., 2017). Secondly, under certain conditions, diatoms and green alga produce more lipids than cyanobacteria (Hu et al., 2008), which can result in a greater tendency to accumulate hydrophobic organic pollutants in algae. Another plausible explanation is that average concentrations of EDCs in phytoplankton declined as the cyanobacteria biomass increased during the bloom events (i.e., bio-dilution).

In this study, cyanobacteria dominated the phytoplankton community during most of the sampling campaigns. Due to the high biomass of cyanobacterial blooms, bio-dilution effects could be used to explain the negative correlation between several EDCs and Cyanophyta biomass. The biomass bio-dilution of the EDCs might subsequently reduce the uptake of these contaminants in freshwater food webs in Taihu Lake. The rapid proliferation of algal biomass usually results in so-called “bio-dilution” of pollutants in the phytoplankton itself (Söderström et al., 2000; Tiano et al., 2014), and might reduce pollution exposure to other organisms in the environment. Pickhardt et al., (2002) reported that bio-dilution of algal blooms may be responsible for lower methylmercury accumulation by zooplankton and fish in alga-rich waters in comparison to alga-deficient waterbodies. Meanwhile, greater occurrence of algal blooms might cause the sedimentation of alga-associated contaminants into the sediment phase after decay of the algal blooms. Concentrations of organochlorine pesticides and PAHs have been observed to be 2.4 to 3.4 times greater in sediments in regions where algal blooms occurred than concentration in regions without the occurrence of algal blooms (Shi et al., 2017). The contaminants thus loaded in sediments might be subsequently redistributed to benthic organisms with events of sediment disturbances and remobilization.

The role of plankton (e.g., as a biological pump) in influencing intercompartment exchange and regional and global distribution of persistent organic pollutants (POPs) has been the focus of recent research (Galbán-Malagón et al., 2012; Nizzetto et al., 2012). In a pelagic lake ecosystem, the primary producers play a key role in alleviating exposure of the trophic web by a) reducing dissolved-phase concentrations of EDCs by uptake or adsorption to phytoplankton; b) enhancing transfer of POPs during the algal bloom and post-bloom seasons; and c) reducing concentrations of EDCs in biota in terms of rapid growth dilution (Nizzetto et al., 2012). Unfortunately, water samples were not collected during our sampling period, thus the determination of bioconcentration factors of EDCs from the water phase to the phytoplanktonic phase were not possible in the present study.

3.5. Future risk assessment of cyanobacterial blooms in eutrophic lakes

Aquatic pollution worldwide is an undesirable by-product of the growing use of industrial chemicals in modern civilization. Sources and fates of these pollutants, such as EDCs deserve increasing attention. While previous studies have shown that sediments act as a source/sink of bioavailable EDCs, few studies provide information regarding the role of cyanobacterial bloom in the biogeochemistry processes of EDCs. Further studies should focus on the bioavailability of algal bloom-associated EDCs to other aquatic organisms.

Lake Taihu is one of most extensively studied freshwater lakes in the world. Hundreds of publications have focused on the hypertrophic situation and frequent occurrence of cyanobacterial blooms in this lake. There is no doubt that the two problematic issues of eutrophication and cyanobacterial blooms contribute to the deterioration of the water quality of Lake Taihu. To this end, monitoring and controlling of cyanobacterial blooms, which are often coupled with the production of cyanotoxins, is of great importance for the environmental and human health risk assessment and water resource utilizations in Lake Taihu. However, the risks of harmful cyanobacterial blooms might be a greater hazard than previously suggested, due to the high amounts of EDCs detected in cyanobacterial bloom samples in the present study. The larger amount of

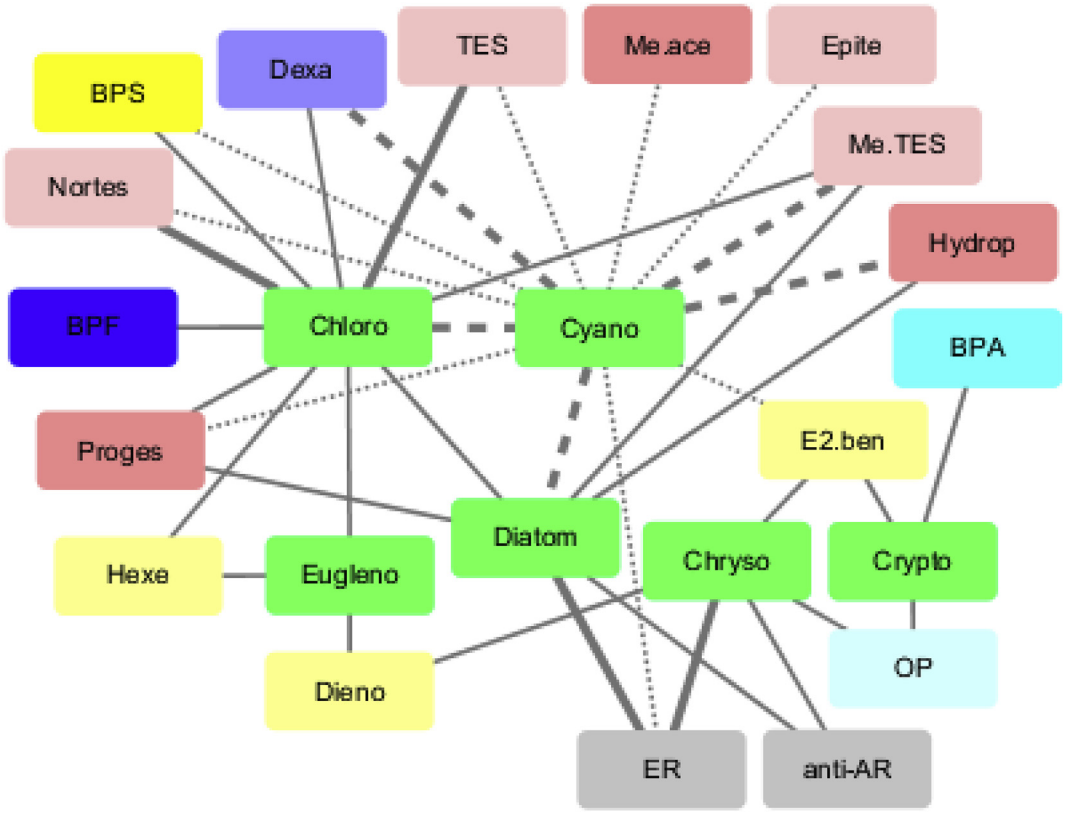

Fig. 6. Spearman correlation network between EDC potentials (instrumental and bioanalytical analysis) in phytoplankton and their biomass (mg/L). Phytoplankton was presented in green nodes. The estrogens, androgens, progestogens, and adrenocortical hormones were presented in light yellow, light red, red, and light blue nodes, respectively. The bioanalytical results were presented in grey nodes. BPA, BPS, BPF, and OP were also presented. Solid lines indicated positive relationship; dotted lines indicated negative relationship. Thin lines indicated the relation with $p < 0.05$ while thick lines indicated strong relation with $p < 0.01$. The abbreviations of different phytoplankton were: Cyano-cyanophyta; Chloro-chlorophyta; Eugleno-euglenophyta; Chryso-chrysophyta; and Crypto-cryptophyta. The abbreviation of EDCs was presented in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cyanobacterial blooms might be a sink or a source of endocrine disruptors, which is a risk of endocrine disruptive effects on aquatic organisms in the water column and humans using cyanobacterial blooms-containing water as drinking source water. The present investigation of endocrine disruptors in cyanobacterial bloom provides insight into the potential risks of cyanobacterial blooms and a comprehensive assessment of cyanobacterial bloom-occurring waterbodies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2018.11.021.

References

Amado, L.L., Monserrat, J.M., 2010. Oxidative stress generation by microcystins in aquatic animals: why and how. Environ. Int. 36, 226–235.
Baptista, M.S., Stoichev, T., Basto, M.C.P., Vasconcelos, V.M., Vasconcelos, M.T.S., 2009. Fate and effects of octylphenol in a Microcystis aeruginosa culture medium. Aquat. Toxicol. 92, 59–64.
Barco, M., Lawton, L.A., Rivera, J., Caixach, J., 2005. Optimization of intracellular microcystin extraction for their subsequent analysis by high-performance liquid chromatography. J. Chromatogr. A 1074, 23–30.
Benotti, M.J., Trenholm, R.A., Vanderford, B.J., Holady, J.C., Stanford, B.D., Snyder, S.A., 2008. Pharmaceuticals and endocrine disrupting compounds in US drinking water. Environ. Sci. Technol. 43, 597–603.
Chen, W., Chen, Y., Gao, X., Yoshida, I., 1997. Eutrophication of Taihu and its control. Agric. Eng. J 6, 109–120.
Chen, W., Jia, Y., Liu, A., Zhou, Q., Song, L., 2017. Simultaneous elimination of cyanotoxins and PCBs via mechanical collection of cyanobacterial blooms: an application of "green-bioadsorption concept". J. Environ. Sci. 57, 118–126.
Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., et al., 2009. Controlling eutrophication: nitrogen and phosphorus. Science 323, 1014–1015.
Essa, A., Fathy, S., 2014. Sex hormonal disruption by cyanobacterial bioactive compounds. J. Appl. Microbiol. 116, 700–709.
Forni, C., Telo, F.R., Caiola, M.G., 1997. Comparative analysis of the polysaccharides produced by different species of Microcystis (Chroococcales, Cyanophyta). Phycologia 36, 181–185.
Frouin, H., Dangerfield, N., Macdonald, R.W., Galbraith, M., Crewe, N., Shaw, P., et al., 2013. Partitioning and bioaccumulation of PCBs and PBDEs in marine plankton from the strait of Georgia, British Columbia, Canada. Prog. Oceanogr. 115, 65–75.
Galbán-Malagón, C., Berrojalbiz, N., Ojeda, M.-J., Dachs, J., 2012. The oceanic biological pump modulates the atmospheric transport of persistent organic pollutants to the Arctic. Nat. Commun. 3, 862.
Gehrmann, L., Bielak, H., Behr, M., Itzel, F., Lyko, S., Simon, A., et al., 2018. (Anti-) estrogenic and (anti-) androgenic effects in wastewater during advanced treatment: comparison of three in vitro bioassays. Environ. Sci. Pollut. Control Ser. 25, 4094–4104.
Gong, Y., Wang, X., Indran, I.R., Zhang, S.-J., Lv, Z., Li, J., et al., 2014. Phytoplankton blooms: an overlooked marine source of natural endocrine disrupting chemicals. Ecotoxicol. Environ. Saf. 107, 126–132.
Grosse, Y., Baan, R., Straif, K., Secretan, B., El Ghissassi, F., Cogliano, V., et al., 2006. Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins. Lancet

Oncol. 7, 628–629.
Grund, S., Higley, E., Schönenberger, R., Suter, M.J., Giesy, J.P., Braunbeck, T., et al., 2011. The endocrine disrupting potential of sediments from the Upper Danube River (Germany) as revealed by in vitro bioassays and chemical analysis. Environ. Sci. Pollut. Control Ser. 18, 446–460.
Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., et al., 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J. 54, 621–639.
Hu, X., Shi, W., Wei, S., Zhang, X., Feng, J., Hu, G., et al., 2013. Occurrence and potential causes of androgenic activities in source and drinking water in China. Environ. Sci. Technol. 47, 10591–10600.
Huang, Y., Wong, C., Zheng, J., Bouwman, H., Barra, R., Wahlström, B., et al., 2012. Bisphenol A (BPA) in China: a review of sources, environmental levels, and potential human health impacts. Environ. Int. 42, 91–99.
Huisman, J., Matthijs, H.C., Visser, P.M., 2006. Harmful Cyanobacteria. Springer Science & Business Media.
Ihara, M., Ihara, M.O., Kumar, V., Narumiya, M., Hanamoto, S., Nakada, N., et al., 2014. Co-occurrence of estrogenic and antiestrogenic activities in wastewater: quantitative evaluation of balance by in vitro ERα reporter gene assay and chemical analysis. Environ. Sci. Technol. 48, 6366–6373.
Janošek, J., Bittner, M., Hilscherová, K., Bláha, L., Giesy, J., Holoubek, I., 2007. AhR-mediated and antiestrogenic activity of humic substances. Chemosphere 67, 1096–1101.
Jia, A., Escher, B.I., Leusch, F.D., Tang, J.Y., Prochazka, E., Dong, B., et al., 2015. In vitro bioassays to evaluate complex chemical mixtures in recycled water. Water Res. 80, 1–11.
Könemann, S., Kase, R., Simon, E., Swart, K., Buchinger, S., Schlüsener, M., et al., 2018. Effect-based and chemical analytical methods to monitor estrogens under the European Water Framework Directive. Trac. Trends Anal. Chem. 102, 225–235.
Kase, R., Javurkova, B., Simon, E., Swart, K., Buchinger, S., Könemann, S., et al., 2018. Screening and risk management solutions for steroidal estrogens in surface and wastewater. Trac. Trends Anal. Chem. 102, 343–358.
Ke, X., Wang, C., Zhang, H., Zhang, Y., Gui, S., 2015. Characterization of estrogenic receptor agonists and evaluation of estrogenic activity in the sediments of Liaohe River protected areas. Mar. Pollut. Bull. 100, 176–181.
Kidd, K.A., Blanchfield, P.J., Mills, K.H., Palace, V.P., Evans, R.E., Lazorchak, J.M., et al., 2007. Collapse of a fish population after exposure to a synthetic estrogen. Proc. Natl. Acad. Sci. Unit. States Am. 104, 8897–8901.
Kunz, P.Y., Simon, E., Creusot, N., Jayasinghe, B.S., Kienle, C., Maletz, S., et al., 2017. Effect-based tools for monitoring estrogenic mixtures: evaluation of five in vitro bioassays. Water Res. 110, 378–388.
Lei, B., Kang, J., Wang, X., Liu, Q., Yu, Z., Zeng, X., et al., 2015. The toxicity of sediments from Taihu Lake evaluated by several in vitro bioassays. Environ. Sci. Pollut. Control Ser. 22, 3419–3430.
Lou, S., Lei, B., Feng, C., Xu, J., Peng, W., Wang, Y., 2016. In vitro toxicity assessment of sediment samples from Huangpu River and Suzhou River, Shanghai, China. Environ. Sci. Pollut. Control Ser. 23, 15183–15192.
Lu, G., Yan, Z., Wang, Y., Chen, W., 2011. Assessment of estrogenic contamination and biological effects in Lake Taihu. Ecotoxicology 20, 974.
Lynn, S.G., Price, D.J., Birge, W.J., Kilham, S.S., 2007. Effect of nutrient availability on the uptake of PCB congener 2, 2′, 6, 6′-tetrachlorobiphenyl by a diatom (Stephanodiscus minutulus) and transfer to a zooplankton (Daphnia pulicaria). Aquat. Toxicol. 83, 24–32.
Maes, H.M., Maletz, S.X., Ratte, H.T., Hollender, J., Schaeffer, A., 2014. Uptake, elimination, and biotransformation of 17α-ethinylestradiol by the freshwater alga Desmodesmus subspicatus. Environ. Sci. Technol. 48, 12354–12361.
Maletz, S., Floehr, T., Beier, S., Klümper, C., Brouwer, A., Behnisch, P., et al., 2013. In vitro characterization of the effectiveness of enhanced sewage treatment processes to eliminate endocrine activity of hospital effluents. Water Res. 47, 1545–1557.
Michalak, A.M., Anderson, E.J., Beletsky, D., Boland, S., Bosch, N.S., Bridgeman, T.B., et al., 2013. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. Proc. Natl. Acad. Sci. Unit. States Am. 110, 6448–6452.
Muschket, M., Di Paolo, C., Tindall, A.J., Touak, G.r., Phan, A., Krauss, M., et al., 2017. Identification of unknown antiandrogenic compounds in surface waters by effect-directed analysis (EDA) using a parallel fractionation approach. Environ. Sci. Technol. 52, 288–297.
Nalewajko, C., Murphy, T.P., 2001. Effects of temperature, and availability of nitrogen and phosphorus on the abundance of Anabaena and Microcystis in Lake Biwa, Japan: an experimental approach. Limnology 2, 45–48.
Nizzetto, L., Gioia, R., Li, J., Borga, K., Pomati, F., Bettinetti, R., et al., 2012. Biological pump control of the fate and distribution of hydrophobic organic pollutants in water and plankton. Environ. Sci. Technol. 46, 3204–3211.
Ormerod, S., Dobson, M., Hildrew, A., Townsend, C., 2010. Multiple stressors in freshwater ecosystems. Freshw. Biol. 55, 1–4.
Otten, T., Xu, H., Qin, B., Zhu, G., Paerl, H.W., 2012. Spatiotemporal patterns and ecophysiology of toxigenic Microcystis blooms in Lake Taihu, China: implications for water quality management. Environ. Sci. Technol. 46, 3480–3488.
Oziol, L., Bouaïcha, N., 2010. First evidence of estrogenic potential of the cyanobacterial heptotoxins the nodularin-R and the microcystin-LR in cultured mammalian cells. J. Hazard Mater. 174, 610–615.
Paerl, H.W., Xu, H., McCarthy, M.J., Zhu, G., Qin, B., Li, Y., et al., 2011. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): the need for a dual nutrient (N & P) management strategy. Water Res. 45, 1973–1983.
Pal, A., Gin, K.Y.-H., Lin, A.Y.-C., Reinhard, M., 2010. Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects. Sci. Total Environ. 408, 6062–6069.
Peng, L., Liu, Y., Chen, W., Liu, L., Kent, M., Song, L., 2010. Health risks associated with consumption of microcystin-contaminated fish and shellfish in three Chinese lakes: significance for freshwater aquacultures. Ecotoxicol. Environ. Saf. 73, 1804–1811.
Pickhardt, P.C., Folt, C.L., Chen, C.Y., Klaue, B., Blum, J.D., 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. Proc. Natl. Acad. Sci. Unit. States Am. 99, 4419–4423.
Pojana, G., Gomiero, A., Jonkers, N., Marcomini, A., 2007. Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon. Environ. Int. 33, 929–936.
Procházková, T., Sychrová, E., Javůrková, B., Večerková, J., Kohoutek, J., Lepšová-Skácelová, O., et al., 2017a. Phytoestrogens and sterols in waters with cyanobacterial blooms-Analytical methods and estrogenic potencies. Chemosphere 170, 104–112.
Procházková, T., Sychrová, E., Javůrková, B., Večerková, J., Kohoutek, J., Lepšová-Skácelová, O., et al., 2017b. Phytoestrogens and sterols in waters with cyanobacterial blooms - analytical methods and estrogenic potencies. Chemosphere 170, 104–112.
Prochazkova, T., Sychrova, E., Vecerkova, J., Javurkova, B., Otoupalikova, A., Pernica, M., et al., 2018. Estrogenic activity and contributing compounds in stagnant water bodies with massive occurrence of phytoplankton. Water Res. 136, 12–21.
Quirós, L., Céspedes, R., Lacorte, S., Viana, P., Raldúa, D., Barcelò, D., et al., 2005. Detection and evaluation of endocrine-disruption activity in water samples from Portuguese rivers. Environ. Toxicol. Chem. 24, 389–395.
Rinta-Kanto, J., Ouellette, A., Boyer, G., Twiss, M., Bridgeman, T., Wilhelm, S., 2005. Quantification of toxic Microcystis spp. during the 2003 and 2004 blooms in western Lake Erie using quantitative real-time PCR. Environ. Sci. Technol. 39, 4198–4205.
Rizopoulos, D., 2006. ltm: an R package for latent variable modeling and item response theory analyses. J. Stat. Software 17, 1–25.
Rogers, E.D., Henry, T.B., Twiner, M.J., Gouffon, J.S., McPherson, J.T., Boyer, G.L., et al., 2011. Global gene expression profiling in larval zebrafish exposed to microcystin-LR and Microcystis reveals endocrine disrupting effects of cyanobacteria. Environ. Sci. Technol. 45, 1962–1969.
Rosenmai, A.K., Dybdahl, M., Pedersen, M., et al., 2014. Are structural analogues to bisphenol a safe alternatives? Toxicol. Sci. 139 (1), 35–47.
Söderström, M., Nylund, K., Järnberg, U., Lithner, G., Rosén, G., Kylin, H., 2000. Seasonal variations of DDT compounds and PCB in a eutrophic and an oligotrophic lake in relation to algal biomass. AMBIO A J. Hum. Environ. 29, 230–237.
Scheffer, M., 2004. Ecology of Shallow Lakes. Springer Science & Business Media.
Schindler, D.W., Hecky, R.E., McCullough, G.K., 2012. The rapid eutrophication of Lake Winnipeg: greening under global change. J. Great Lake. Res. 38, 6–13.
Sellin Jeffries, M.K., Conoan, N.H., Cox, M.B., Sangster, J.L., Balsiger, H.A., Bridges, A.A., et al., 2011. The anti-estrogenic activity of sediments from agriculturally intense watersheds: assessment using in vivo and in vitro assays. Aquat. Toxicol. 105, 189–198.
Seto, M., Handoh, I.C., 2009. Mathematical explanation for the non-linear hydrophobicity-dependent bioconcentration processes of persistent organic pollutants in phytoplankton. Chemosphere 77, 679–686.
Shi, P., Zhou, S., Xiao, H., Qiu, J., Li, A., Zhou, Q., et al., 2018. Toxicological and chemical insights into representative source and drinking water in eastern China. Environ. Pollut. 233, 35–44.
Shi, W., Yu, N., Jiang, X., Han, Z., Wang, S., Zhang, X., et al., 2017. Influence of blooms of phytoplankton on concentrations of hydrophobic organic chemicals in sediments and snails in a hyper-eutrophic, freshwater lake. Water Res. 113, 22–31.
Smith, V.H., 2003. Eutrophication of freshwater and coastal marine ecosystems a global problem. Environ. Sci. Pollut. Control Ser. 10, 126–139.
Song, L., Chen, W., Peng, L., Wan, N., Gan, N., Zhang, X., 2007. Distribution and bioaccumulation of microcystins in water columns: a systematic investigation into the environmental fate and the risks associated with microcystins in Meiliang Bay, Lake Taihu. Water Res. 41, 2853–2864.
Staniszewska, M., Nehring, I., Zgrundo, A., 2015. The role of phytoplankton composition, biomass and cell volume in accumulation and transfer of endocrine disrupting compounds in the Southern Baltic Sea (The Gulf of Gdansk). Environ. Pollut. 207, 319–328.
Štěpánková, T., Ambrožová, L., Bláha, L., Giesy, J.P., Hilscherová, K., 2011. In vitro modulation of intracellular receptor signaling and cytotoxicity induced by extracts of cyanobacteria, complex water blooms and their fractions. Aquat. Toxicol. 105, 497–507.
Suikkanen, S., Laamanen, M., Huttunen, M., 2007. Long-term changes in summer phytoplankton communities of the open northern Baltic Sea. Estuar. Coast Shelf Sci. 71, 580–592.
Sychrová, E., Štěpánková, T., Nováková, K., Bláha, L., Giesy, J.P., Hilscherová, K., 2012. Estrogenic activity in extracts and exudates of cyanobacteria and green algae. Environ. Int. 39, 134–140.
Takumi, S., Komatsu, M., Furukawa, T., Ikeda, R., Sumizawa, T., Akenaga, H., et al., 2010. p53 Plays an important role in cell fate determination after exposure to microcystin-LR. Environ. Health Perspect. 118, 1292.
Tan, B.L., Hawker, D.W., Müller, J.F., Leusch, F.D., Tremblay, L.A., Chapman, H.F., 2007. Comprehensive study of endocrine disrupting compounds using grab and

passive sampling at selected wastewater treatment plants in South East Queensland, Australia. Environ. Int. 33, 654–669.
Tiano, M., Tronczyński, J., Harmelin-Vivien, M., Tixier, C., Carlotti, F., 2014. PCB concentrations in plankton size classes, a temporal study in Marseille Bay, Western Mediterranean Sea. Mar. Pollut. Bull. 89, 331–339.
Van Der Linden, S.C., Heringa, M.B., Man, H.-Y., Sonneveld, E., Puijker, L.M., Brouwer, A., et al., 2008. Detection of multiple hormonal activities in wastewater effluents and surface water, using a panel of steroid receptor CALUX bioassays. Environ. Sci. Technol. 42, 5814–5820.
Verschuren, D., Johnson, T.C., Kling, H.J., Edgington, D.N., Leavitt, P.R., Brown, E.T., et al., 2002. History and timing of human impact on Lake Victoria, East Africa. Proc. R. Soc. Lond. B Biol. Sci. 269, 289–294.
Voutsa, D., Hartmann, P., Schaffner, C., Giger, W., 2006. Benzotriazoles, alkylphenols and bisphenol A in municipal wastewaters and in the Glatt River, Switzerland. Environ. Sci. Pollut. Control Ser. 13, 333–341.
Wan, Y., Hu, J., Yang, M., An, L., An, W., Jin, X., et al., 2005. Characterization of trophic transfer for polychlorinated dibenzo-p-dioxins, dibenzofurans, non-and mono-ortho polychlorinated biphenyls in the marine food web of Bohai Bay, North China. Environ. Sci. Technol. 39, 2417–2425.
Wan, Y., Jin, X., Hu, J., Jin, F., 2007. Trophic dilution of polycyclic aromatic hydrocarbons (PAHs) in a marine food web from Bohai Bay, North China. Environ. Sci. Technol. 41, 3109–3114.
Watanabe, M.M., Kaya, K., Takamura, N., 1992. Fate of the toxic cyclic heptapeptides, the microcystins, from blooms of microcystis (cyanobacteria) in a hypertrophic lake1. J. Phycol. 28, 761–767.
Wernersson, A.-S., Carere, M., Maggi, C., Tusil, P., Soldan, P., James, A., et al., 2015. The European technical report on aquatic effect-based monitoring tools under the water framework directive. Environ. Sci. Eur. 27, 7.
Xie, Z., Lu, G., Liu, J., Yan, Z., Ma, B., Zhang, Z., et al., 2015. Occurrence, bioaccumulation, and trophic magnification of pharmaceutically active compounds in Taihu Lake, China. Chemosphere 138, 140–147.
Yamazaki, E., Yamashita, N., Taniyasu, S., Lam, J., Lam, P.K., Moon, H.-B., et al., 2015. Bisphenol A and other bisphenol analogues including BPS and BPF in surface water samples from Japan, China, Korea and India. Ecotoxicol. Environ. Saf. 122, 565–572.
Yan, Z., Lu, G., Liu, J., Jin, S., 2012. An integrated assessment of estrogenic contamination and feminization risk in fish in Taihu Lake, China. Ecotoxicol. Environ. Saf. 84, 334–340.
Yan, Z., Yang, X., Lu, G., Liu, J., Xie, Z., Wu, D., 2014. Potential environmental implications of emerging organic contaminants in Taihu Lake, China: comparison of two ecotoxicological assessment approaches. Sci. Total Environ. 470, 171–179.
Yang, J., Li, H., Ran, Y., Chan, K., 2014. Distribution and bioconcentration of endocrine disrupting chemicals in surface water and fish bile of the Pearl River Delta, South China. Chemosphere 107, 439–446.
Yu, Y., Wu, L., Chang, A.C., 2013. Seasonal variation of endocrine disrupting compounds, pharmaceuticals and personal care products in wastewater treatment plants. Sci. Total Environ. 442, 310–316.
Žegura, B., Sedmak, B., Filipič, M., 2003. Microcystin-LR induces oxidative DNA damage in human hepatoma cell line HepG2. Toxicon 41, 41–48.
Zhang, H.-C., Yu, X.-j., Yang, W.-c., Peng, J.-f., Xu, T., Yin, D.-Q., et al., 2011. MCX based solid phase extraction combined with liquid chromatography tandem mass spectrometry for the simultaneous determination of 31 endocrine-disrupting compounds in surface water of Shanghai. J. Chromatogr. B 879, 2998–3004.
Zhao, J.L., Ying, G.G., Yang, B., Liu, S., Zhou, L.J., Chen, Z.F., et al., 2011. Screening of multiple hormonal activities in surface water and sediment from the Pearl River system, South China, using effect-directed in vitro bioassays. Environ. Toxicol. Chem. 30, 2208–2215.