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Article abstract

In situ fluorescence probes have attracted growing interest for the on-line monitoring of cyanobacteria in drinking water treatment plants. The probes rely on the fluorescence of pigments such as phycocyanin and chlorophyll-a to detect respectively cyanobacteria and green algae. They offer direct and simultaneous multiparameter measurements and opportunity for online monitoring which can enable water operators to improve cyanobacteria management during the drinking water process. However, fluorescence probes can be influenced by interference sources which may results in biased measurements. The impact of these factors on probe readings can make the calibration and validation process difficult for operators. Hence, the aim of the study was to calibrate and validate fluorescence probe performance (here YSI EXO2 probe) for varying laboratory grown phytoplankton species. Although good linear correlation between raw probe fluorescence readings and cyanobacteria cell concentrations was found, measurement bias was observed using this probe in water samples with high turbidity (62 NFU) or Dissolved Organic Carbon concentration (10 mg•L^{-1}). These data showed the potential of fluorescence probes deployment in cyanobacteria monitoring with a deeper understanding of the potential interference sources that is required to interpret data correctly.

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CONTINUOUS MONITORING OF CYANOBACTERIAL BLOOMS: BENEFITS AND CONDITIONS FOR USING FLUORESCENCE PROBES

Surveillance en continu des proliférations en cyanobactéries : avantages et conditions d'utilisation des sondes par fluorescence

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ABSTRACT

In situ fluorescence probes have attracted growing interest for the on-line monitoring of cyanobacteria in drinking water treatment plants. The probes rely on the fluorescence of pigments such as phycocyanin and chlorophyll-a to detect respectively cyanobacteria and green algae. They offer direct and simultaneous multiparameter measurements and opportunity for online monitoring which can enable water operators to improve cyanobacteria management during the drinking water process. However, fluorescence probes can be influenced by interference sources which may results in biased measurements. The impact of these factors on probe readings can make the calibration and validation process difficult for operators. Hence, the aim of the study was to calibrate and validate fluorescence probe performance (here YSI EXO2 probe) for varying laboratory grown phytoplankton species. Although good linear correlation between raw probe fluorescence readings and cyanobacteria cell concentrations was found, measurement bias was observed using this probe in water samples with high turbidity (62 NFU) or Dissolved Organic Carbon concentration (10 $mg{\cdot}L^{-1}$). These data showed the potential of fluorescence probes deployment in cyanobacteria monitoring with a deeper understanding of the potential interference sources that is required to interpret data correctly.

Key Words: Cyanobacteria, fluorescence probe, monitoring, phycocyanin.

RÉSUMÉ

La mesure en continu de pigments spécifiques aux algues et cyanobactéries (chlorophylle-a et phycocyanine) par des sondes de fluorescence *in situ* peut être une réponse au besoin exprimé par les gestionnaires de plans d'eau et opérateurs de filières de traitement d'eau potable pour aider à la gestion des proliférations de microalgues. Ces sondes permettent de quantifier le nombre total de cellules d'algues vertes et de cyanobactéries grâce à la mesure de fluorescence émise par leurs pigments respectifs : chlorophylle-a et phycocyanine.

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L'objectif principal de cette étude était de valider l'utilisation d'une sonde de fluorescence *in situ* (EXO2, YSI) afin de vérifier l'impact potentiel des principales interférences de fluorescence, notamment la présence de biomasse algale (Chlorophycées), la turbidité et la matière organique. Les données produites en laboratoire ont confirmé que les sondes permettent une bonne corrélation entre la concentration cellulaire et les lectures de fluorescence. Certains paramètres de qualité d'eau tels que la turbidité (à 62 NFU) ou la teneur en carbone organique dissous (10 mg·L^{-1}) peuvent néanmoins influencer la prédiction de la teneur en cellules de cyanobactéries. Ces données montrent le potentiel d'utilisation des sondes de fluorescence *in situ* pour la surveillance des cyanobactéries, grâce au développement de recommandations qui permettront d'aider à interpréter et utiliser correctement les données issues de la surveillance en ligne.

Mots-clés : Cyanobactéries, sonde par fluorescence, surveillance en ligne, phycocyanine.

1. INTRODUCTION

Climate change and increased human activity has contributed to the challenging issue of cyanobacteria and associated metabolite management. The increasing frequency and intensity of cyanobacterial blooms including: 1) repeated widespread poisoning of animals, fish, other aquatic living beings and humans by cyanotoxins (ROSSOM *et al.*, 1994); 2) toxic cell accumulation in water treatment processes and toxin breakthrough into drinking water that has led to instances of advisories against drinking the tap water in some communities (ZAMYADI *et al.*, 2012a); 3) breakthrough of unpleasant cyanobacterial taste & odor compounds to finished water leading to customer dissatisfaction (IZAGUIRRE *et al.*, 1982); 4) human health effects after recreational exposure to nontoxic cyanobacterial cells (PILOTTO *et al.*, 1997) and 5) human exposure to cyanotoxins by consumption of plants irrigated with cyanotoxin contaminated surface water or recycled water from sewage (SAQRANE and OUDRA, 2009). Due to the harmful effects of toxic cyanobacteria, water authorities across the globe have adopted management strategies to improve the handling of bloom events (WORLD HEALTH ORGANIZATION, 1999; US EPA, 2015). In order to support such management plans, identification and enumeration of cyanobacteria are performed on a regular basis. However these relatively time-consuming methods performed on grab samples do not allow monitoring of cyanobacteria with sufficient spatio-temporal resolution. In this context, field probes measuring the fluorescence of photosynthetic pigments of cyanobacteria may represent an interesting approach for monitoring cyanobacteria and providing an *in situ* estimation of cyanobacteria cell density. Cyanobacteria possess chlorophyll-a (chl-a), as well as phycocyanin (PC) and phycoerythrin (PE) which are photochemically active pigments. PC is the phycobilisome pigment of blue-green cyanobacteria while PE is specific to red cyanobacteria which are mainly found in marine environment. The photoemission of light energy by pigments is the process used to develop *in situ* fluorescence monitoring equipment (MCQUAID *et al.*, 2011). PC absorbs red and orange light at wavelengths between 610 and 630 (absorption peak at 620 nm) and emits fluorescence in the bands of wavelengths between 600 and 700 (emission peaks at 647 nm). Moreover, fluorescence technology has become far more advanced in recent years with specific light-emitting diodes (LED) and optical filters, offering the opportunity to significantly improve upon existing fluorescent probe technology (HENDERSON *et al.*, 2009). Field probes measuring PC fluorescence can then potentially used to perform spatio-temporal monitoring of cyanobacterial blooms and offer a cost effective opportunity for on-line monitoring which can enable water operators to improve cyanobacteria management during the drinking water process (BRIENT *et al.*, 2008; ZAMYADI *et al.*, 2012a; MACÁRIO *et al.*, 2015). However, recent widespread development and application of *in situ* fluorometric probes by both scientists and water utilities have also led to recognition of major issues associated with the undertaking of these measurements, particularly around interferences (ZAMYADI *et al.*, 2012b). Bias in the probe technical function, turbidity, phytoplankton cell characteristics can influence *in situ* fluorescence measurements (CATHERINE *et al.*, 2012). There is a need to quantify these interferences and identify suitable correction technologies (ZAMYADI *et al.*, 2016).

The impact of these factors on probe readings make the calibration and validation process difficult for operators. Hence the aim of this study was to validate fluorescence probe performance (here YSI EXO2 multiparameter probe) for varying laboratory grown phytoplankton species and environmental conditions.

2. MATERIAL AND METHODS

2.1 *In situ fluorescence probe*

YSI (Xylem Analytics, France) EXO2 mulitparameter probe which contained sensors for the following water quality parameters: temperature, specific conductivity, dissolved oxygen, pH, turbidity, fluorescence Dissolved Organic Matter (fDOM), chlorophyll-a and phycocyanin and equipped with anti-fouling wiper, was used in this study. The EXO total algae sensor contains two excitation beams: a blue excitation

beam that directly excites chlorophyll-a molecule, and an orange excitation beam that excites phycocyanin pigment, with a narrow excitation and emission wavelengths bandpass (±5 nm). All experiments were made using the EXO calibration cup filled with around 400 mL of water to be analyzed. Data communication was ensured using USB signal output adapter and the KOR software. The direct readings of the probe were recorded using the manufacturer original calibrations. During all the assays, mixing was manually achieved by shaking.

2.2 Cyanobacteria and algae cultures

The following cultures were grown separately in their corresponding media: *Microcystis aeruginosa* (CCAP 1450/1) in Cyanobacteria BG-11 media (Sigma Aldrich), *Chlorella vulgaris* (CCAP 211/11B) *Desmodesmus subspicatus* in Algae Culture Broth (Fluka Analytical). *C. vulgaris* and *D. subspicatus* are two freshwater green algae, selected for their known capacity of chlorophyll-a. *M. aeruginosa* is the most common toxic cyanobacterial species responsible for bloom in eutrophic fresh water (PAERL and OTTEN, 2013). After inoculation with 50 mL of a previous grown algae culture in a stationary phase, cultures were incubated in batch of 500 ml culture media in 1 L Erlen flasks. Incubation was performed at room temperature (20-25 °C) under a 6h rotating light-darkness flux (10 000 lux·m^{-2}) with agitation by rotatory movement (100 rpm). After around 2 weeks of incubation, cell counts were conducted by light microscopy using a counting chamber at 40x.

2.3 Laboratory validation assays

Water samples were sourced either as tap water from Le Pecq drinking water distribution system (France) either as river raw water (Seine River collected at le Pecq, France). Tap water residual chlorine was neutralized by addition of sodium thiosulfate (20 mg·L^{-1}) for 30 min. Serial dilutions in 500 mL of *M. aeruginosa*, *C. vulgaris*, and *D. subspicatus* cultures were performed. In a second set of assays, two concentrations of green algae (*Chlorella* or *Desmodesmus*, at 10 000 and 100 000 cells·mL^{-1}) were added in sequence to a range of concentration of *M. aeruginosa* (5.10E+3 to 10E+6 cells·mL^{-1}). The effect of natural turbidity and fluorescent/colored dissolved organic matter was respectively studied by using raw Seine River water (turbidity of 62 NFU, Total Organic Carbon of 4.2 mg·L^{-1} and Dissolved Organic Carbon [DOC] of 2.8 mg·L^{-1}) and commercial humic acid solution (Sigma Aldrich reference 53 680). A stock solution of the acid humic solution at 1 g·L^{-1} was made by dissolving 1 g in 1 L of deionized water followed by a filtration through a glass fiber filter (GF/F Whatman, nominal pore size 0.7 µm).

3. RESULTS AND DISCUSSION

3.1 Linearity of PC quantification using Microcystis aeruginosa culture

In the first culture assays in mono-suspensions, five concentrations of *M. aeruginosa* between 5 000 and 300 000 cells·mL^{-1} from three independent culture batches were measured by the EXO2 probe. This range of concentration includes the alert levels as recommended respectively by the Australian Drinking Water Guidelines (2011) equivalent to 65 000 cells·mL^{-1} of *M. aeruginosa* and WHO guidelines for safe recreational water environments with Level 1 (20 000 cells·mL^{-1}) and Level 2 (100 000 cells·mL^{-1}) thresholds. As observed by ZAMYADI *et al.* (2012a), the repeatability of the probe measurements in tap water were good over the full range of quantification with standard deviation from 1.1% to 7.7%. Figure 1 shows also the good linearity of the relationship between BGA-PC and *M. aeruginosa* cell concentrations even when all data from the three different batches were pooled with a global linear coefficient about 0.99. Similarly good linearity was recorded when *M. aeruginosa* cells were spiked into river water (turbidity of 61.7 NFU; DOC of 2.8 mg·L^{-1}). Although no interference for quantifying cyanobacteria cells below 5.10E+5 cells·mL^{-1} was shown in turbid surface water, somewhat a difference in BGA-PC reads can be observed at concentration above 10E+6 cells·mL^{-1}. At this concentration, quantification of *Microcystis* may be underestimated of about 25%. Using bentonite for simulating turbidity, ZAMYADI *et al.* (2012b) showed that this bias could reach up to 60%.

Considering the limit of detection (LoD) calculated as the mean plus 3SD of the blank and the limit of quantification (LoQ) as the mean plus 10SD of the background noise (mean = -1.36 RFU, SD = 0.13), the LoD and LoQ of the method are respectively 670 and 2 450 cells·mL^{-1} in tap water. This is consistent with the observation of BASTIEN *et al.* (2011) using the YSI 6600 probe. However using the manufacturer's calibration negative values are observed for BGA-PC readings (expressed in RFU or µg·L^{-1}) for *M. aeruginosa* concentration below 10 000 cells·mL^{-1}, leading difficult the interpretation of low cyanobacteria range. Using EXO multiparameter probes outputs, linear relationships were also achieved using the chlorophyll-a output and in much lower extent with turbidity (Figure 2). Interestingly, a linear relationship was also observed between fDOM outputs and *M. aeruginosa* cell concentration. Some authors (FUKUZAKI *et al.*, 2014) had recently proposed that fDOM could be used for the monitoring of marine phytoplankton by the direct production of fluorescent characteristics of algae exudates.

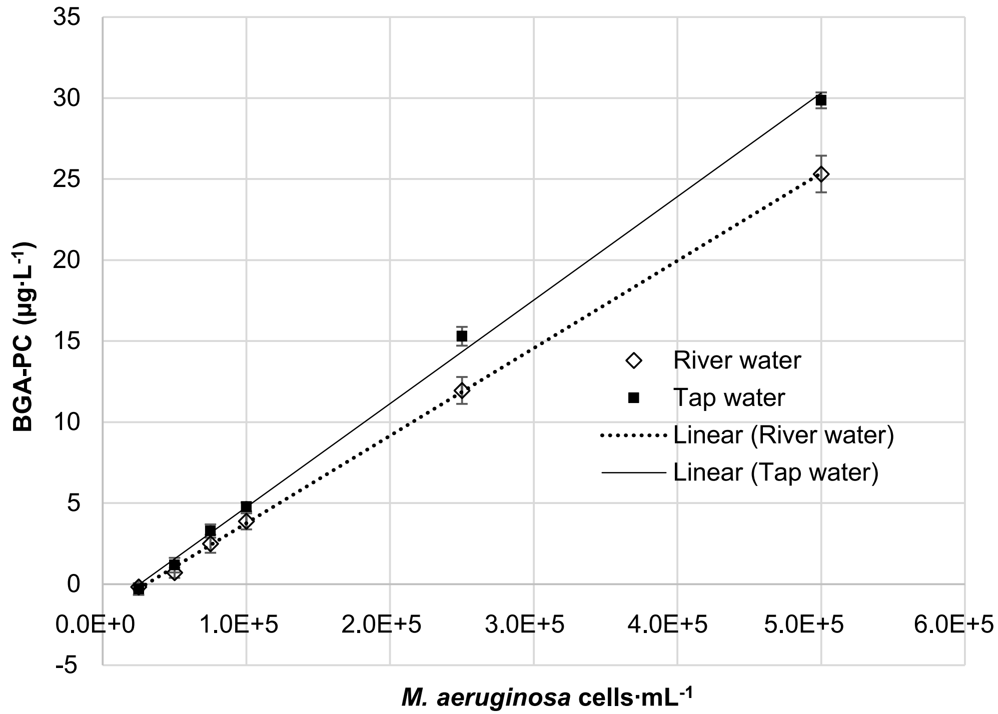

Figure 1. Relationships between the EXO2 outputs (BGA-PC in Relative Fluorescence Units or µg·L^{-1}) and *Microcystis aeruginosa* cell concentration in tap water or river water.
Relation linéaire entre les mesures de phycocyanine et la concentration de* Microcystis aeruginosa *(cellules·mL^{-1}) dans l'eau de rivière et dans l'eau potable..

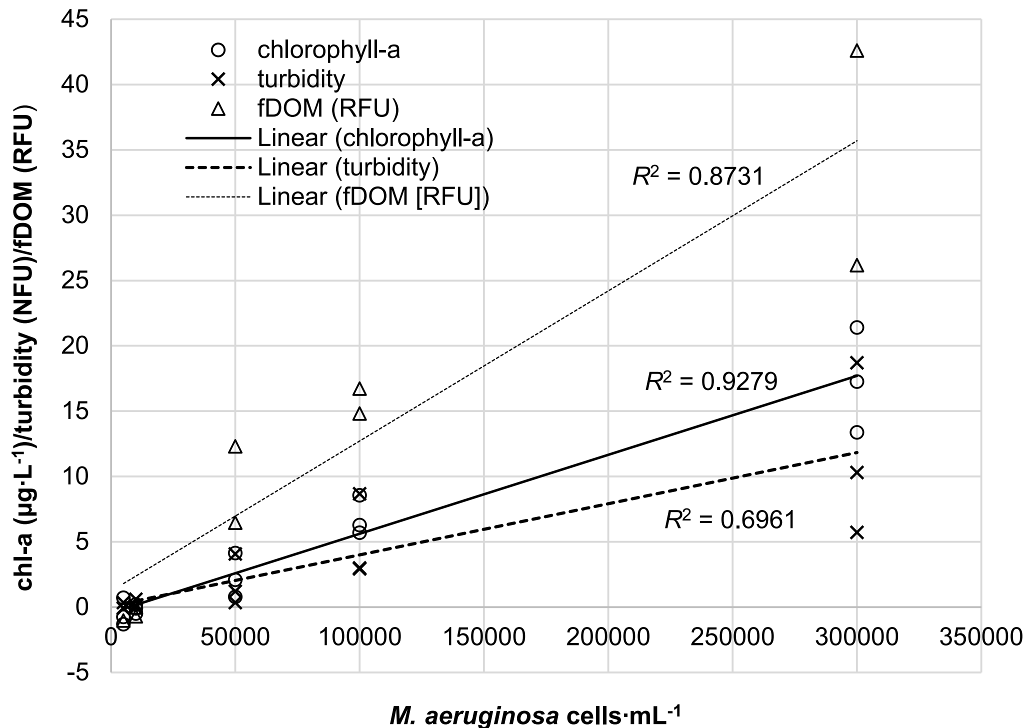

Figure 2. Relationships between the EXO2 outputs (chlorophyll-a in µg·L^{-1}, turbidity in NFU and fDOM in Relative Fluorescence Units) and *Microcystis aeruginosa* cell concentration.
Relations linéaires entre les mesures de chlorophylle-a (µg·L^{-1}), de turbidité (NFU) et de matière organique dissoute fluorescente (RFU) et la concentration de* Microcystis aeruginosa *(cellules·mL^{-1}).

3.2 Phycocyanin quantification in presence of chl-a and/or turbidity

First, two dilution ranges from two Chlorophyceae cultures (with concentration from 5.10E+3 to 10E+6 cells·mL^{-1}) were made in tap water and measured by EXO2 probe. Results showed that *Chlorella vulgaris* and *Desmodesmus subspicatus* cultures exhibit high chl-a content as expected, with good linearity coefficients (R^2 of 0.992 and 0.988 respectively) but can also present some fluorescence detected by the BGA-PC sensor, especially for high green algae concentrations. At concentration level of 5.10E+5 cells·mL^{-1} of *C. vulgaris* and *D. subspicatus* respectively (corresponding to high level of chlorophyll-a above 350 µg·L^{-1}), around 7 and 15 µg·L^{-1} of BGA-PC can be measured in absence of cyanobacteria (Figure 3). Secondly, a dilution range of cyanobacteria *Microcystis aeruginosa* concentrations were measured in presence (or not) of different amounts of *Chlorella* or *Desmodesmus* cells. As shown in figure 4, when moderate to high (up to 60 µg·L^{-1}) background of chlorophyll-a brought by green algae, the BGA-PC measurement of *M. aeruginosa* is only significantly impacted ($p < 0.05$) for high cyanobacteria cell concentration in presence of *C.vulgaris*. This observation is in contradiction with previous observation made by ZAMYADI *et al*. (2012) using YSI 6600 phycocyanin sensor. This suggest that the more precise excitation and emission wavelength bandpass (±5 nm) used in the new version of EXO2 probe limits successfully the impact of chlorophyll-a from eukaryotic algae fluorescence. The combined effect of turbidity and chlorophyll-a was also studied by adding different quantities of *C. vulgaris* cells (corresponding to respectively 16 and 30 µg·L^{-1} of chl-a) in the Seine River sample (62 NFU turbidity). No additional effect of chlorophyll-a was observed for the PC quantification from a range of *M. aeruginosa* cells concentration.

3.3 Phycocyanin quantification in presence of dissolved organic matter

Fluorescent Dissolved Organic Matter, also known as yellow substances, is the optically active portion of dissolved organic matter (DOM), and is mainly sourced from terrestrial humic and fulvic substances. This form of colored dissolved organic matter have the potential to influence fluorescence readings due to their ability to alter light transmission, as well as their natural fluorescence capabilities. Some manufacturers like BBE have integrated a yellow substance correction factor in their fluorescence sensor instrument (as for the Algae Online Analyser). Assays were performed using a solution made from a commercial humic acid extract providing a

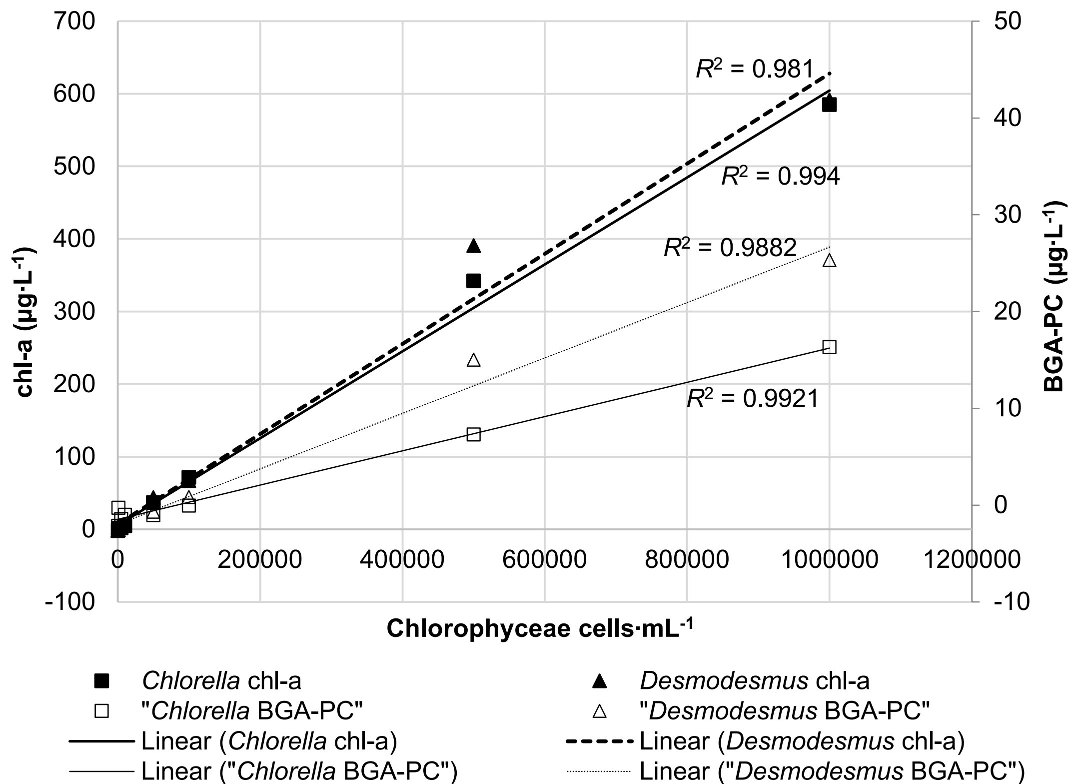

Figure 3. Relationships between the EXO2 outputs (chlorophyll-a and BGA-PC both in µg·L^{-1}) and *Chlorella vulgaris* or *Desmodesmus subspicatus* cell concentration.
Relations linéaires entre les mesures de chlorophylle-a et phycocyanine (en µg·L^{-1}), et la concentration de Chlorella vulgaris ou Desmodesmus subspicatus (cellules·mL^{-1}).

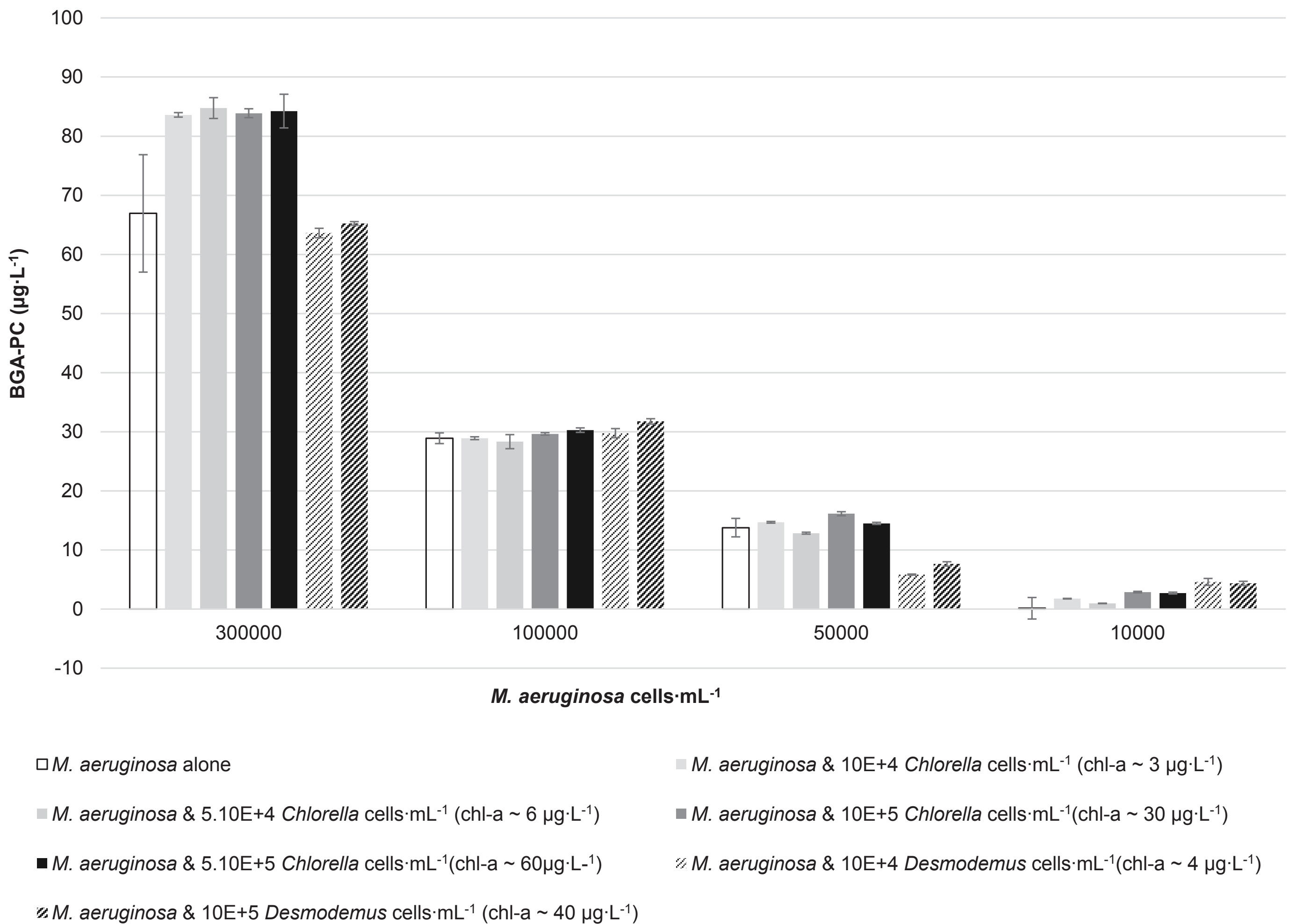

Figure 4. Impact of chlorophyll-a (*Chlorella vulgaris* or *Desmodesmus subspicatus*) at 10E+4 and 10E+5 (cells·mL-1) on BGA-PC measures from *Microcystis aeruginosa*.
Influence de la chlorophylle-a sur la quantification de la phycocyanine pour différentes concentrations en Microcystis aeruginosa..

constant level of 10 mg·L^{-1} of DOC. Such DOC concentration corresponds to the concentration of dissolved organic matter present in some surface waters and reservoirs (AWAD *et al.*, 2017) and attributed to flushing of the upper organic soil horizons. For this solution, the following optical and fluorescence backgrounds were observed: fDOM background of 166 (±1) ppb, turbidity of 7.4 (± 0.1) NFU, chlorophyll-a of 25.3 (±0.1) µg·L^{-1}. In comparison with BGA-PC readings made in tap water for the quantification of a range of *M. aeruginosa* cells concentrations, the difference of 0.8 BGA RFU at the y axis crossing correspond to the BGA-PC background from the humic acid solution. This difference could however lead to an overestimation that can be slight (around 10-15%) for high level of BGA concentration (>10E+5 cells·mL^{-1}) but can reach 100% for lower concentration (5.10E+3 to 10E+5 cells·mL^{-1}). These results show that the content of high fDOM (proxy of high DOC) can cause interference with BGA quantification using PC sensor, especially for a range of low cyanobacteria cell concentration.

4. CONCLUSION

In situ fluorescence probes have attracted growing interest as an on-line monitor of cyanobacteria in drinking water treatment plants (BASTIEN *et al.*, 2011). Probes like YSI EXO2 offer direct and simultaneous multiparameter measurements, cost-effective and long-term operation, which should enable water operators to improve cyanobacteria management during the drinking water process. However, fluorescence probes can be influenced by interference sources which may results in biased measurements. Here the results showed that technical progress in sensor technology can be proposed by improving the accuracy of the excitation and emission wavelength bandpass for limiting the impact of high chlo-a background from eukaryote green algae. However other parameters such as turbidity and fDOM/DOC should be monitored, especially when significant fluctuation of these water quality parameters are occurring in the aquatic system to be monitored.

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