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First report of *Planktothrix rubescens* bloom from the Algerian freshwater reservoir Hammam Debagh

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Abstract. Massive cyanobacteria blooms have become a worldwide concern problem due to the multiple nuisances they can cause. The impacts of climate change are distinct from other environmental constraints controlling its population dynamics. The current study was accomplished in the monomictic reservoir Hammam Debagh (North-Eastern Algeria). A sampling campaign was conducted during May and June 2012 in four surface sampling stations and the water column in the center of this reservoir. This study aims to investigate the surface bloom of *Planktothrix rubescens* (De Candolle ex Gomont 1892) Anagnostidis and Komarek, 1988; observed for the first time in an Algerian reservoir with a focus on the environmental variable changes in 2012.

During this thick epilimnetic bloom, *P. rubescens* spread in all water column layers, reaching 6.4×10^6 cells mL^{-1} at 2m from the surface recorded on 11.05.2012. Meanwhile, at the surface *P. rubescens* abundances ranged from 3×10^5 to 3.6×10^6 cells mL^{-1} . The biomass was so high that the signal from the phycocyanin probe became saturated attending a value of $200 \mu\text{g L}^{-1}$ PC in the top 1 m. In addition, the biogenic compounds especially P- PO_4 and NO_3 show a high concentration of 0.48 mg L^{-1} and 5 mg L^{-1} respectively. The red pigmented cyanobacterium bloom altered environmental conditions in

the reservoir Hammam Debagh, since primarily oxygen concentration shows a minimum of 2.63 mg L⁻¹ and water transparency did not exceed 1m (min= 40cm). The vertical profile of temperature performed on 29.05.2012 over 33m; shows a stratified water column ranging from 22.6°C in the upper subsurface to 9.8°C in the bottom hypolimnion. Finally, suitable meteorological conditions were observed during 2012.

Keywords: cyanobacteria, *Planktothrix rubescens*, phycocyanin, microcystin, Reservoir Hammam Debagh

Introduction

In recent years, lakes hydrodynamics and thermal stratification period have been significantly altered due to the rise in water temperature and the extension of the period with suitable temperature for potential harmful algae growth (Komatsua *et al.*, 2007; De Stasio *et al.*, 1996). Cyanobacteria cells are equipped with the necessary physiological tools to thrive in changing environments (Neilan *et al.*, 2013). Thus, a global intensification of cyanobacteria bloom was expected (Chirico *et al.*, 2020; Paerl 2014; Wood *et al.*, 2011). During cyanobacteria bloom episodes, their concentration can maintain cells densities of more than 500 000 cells L⁻¹ over several months, thus creating a significant restraint on the use of the water resource (Sulis *et al.*, 2014; Ernst *et al.*, 2007). Hence, this increasing problem impacts the ecosystem integrity as well as human and animal health by drinking unfiltered water, fish consumption or swimming in it (Downing *et al.*, 2001; Carmichael *et al.*, 2001). Cyanotoxin production is one of the most dangerous consequences of cyanobacterial bloom, with microcystins, primarily associated with liver injuries, being the most widespread and likely to occur when certain cyanobacterial taxa are present (Bartram *et al.*, 1999). Other toxins, such as neurotoxins, are less common.

Planktothrix rubescens (basionym *Oscillatoria rubescens*, De Candolle ex Gomont 1892) Anagnostidis and Komarek, 1988 is a filamentous bloom-forming cyanobacteria. This red pigmented species can tolerate low temperatures and prefers low light. Moreover, it exhibits high plasticity in the water column due to the presence of gas vesicles. Hence, it can form blooms in deeper, thermally stratified lakes with moderate nutrient pollution (Janse *et al.*, 2005). In fact, Reynolds *et al.* (2002) describe it as an R-strategist. When compared with *Planktothrix agardhii*; *P. rubescens* exerts a significant allelopathic effect on the diversity and biomass of phytoplankton (Lenard *et al.*, 2022).

Freshwater cyanobacteria bloom became a common problem in Algeria. During the last decade, several species have been reported including *Microcystis sp.* (Bouhaddada *et al.*, 2016; El-Herry *et al.*, 2009; Nasri *et al.*, 2008), *Planktothrix agardhii* (Benayache *et al.*, 2022; Saoudi *et al.*, 2017), *Cylindrospermopsis raciborskii* (Charifi *et al.*, 2019; Bouaicha and Nasri, 2004) and *Aphanizomenon issatschenkoi* (Boussadia *et al.*, 2015). Blooms attributed to the species mentioned above occur commonly in the Mediterranean freshwater ecosystems. However, *P. rubescens* is a common bloom forming cyanobacterium in deep Northern and prealpine European oligotrophic to mesotrophic lakes (Vareli *et al.*, 2009). The current study aims to describe a *P. rubescens* bloom observed in the reservoir Hammam Debagh (North of Algeria) in 2012, being the first record of this species blooming in Algeria. The focus was on environmental variations to better understand this “burgundy-blood phenomenon” observed in the surface of the reservoir.

Materials and methods

Study area

Hammam Debagh (36.4713889 N, 7.214166 E) is a warm monomictic reservoir in the north east of Algeria (Fig. 1), situated at 800m above sea level and the depth can vary from 20m to 60m. The total volume of about 185 million cubic meters occupies a surface area of 6.5km². The main water source for the lake is the river Bouhamdene, formed by the confluence of the rivers Zenati and Sabath. This is a source of drinking water for 180000 inhabitants, irrigation (13.000 ha), and small fish farming activities (e.g. *Carassius carassius*, *Cyprinus carpio*). Meteorological data such as air temperature, precipitation, and water evaporation were provided by the weather station situated near to the Hammam Debagh reservoir.

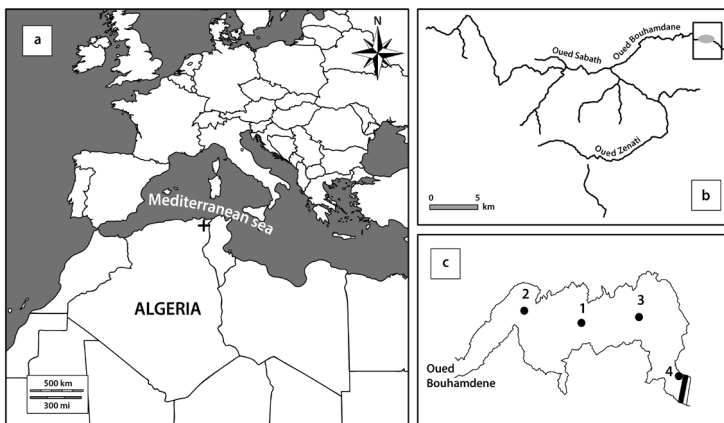


Figure 1. Geographical positions. a) Localization of the reservoir in Algeria; b) The Bouhamdene watershed; c) location of the four sampling stations in the reservoir

Sampling strategies

Surface water sampling was carried out along a west east transect, from May to July 2012, from four sampling stations named S1, S2, S3 and S4 for *P. rubescens* abundances and Microcystins analysis. Samples were collected at various depths in the water column using an integrated Ruttner sampler (Hydro-Bios, Germany). Samples were collected at 20cm interval in the first 1m and then at 2, 3, 5, 10, 18, 23, 33m in order to determine the abundances of *P. rubescens*.

Limnological parameters and phycocyanin measurements

Secchi depth (Zs) was used to estimate water transparency with a 25cm diameter disc. Zs is used for calculating the euphotic zone (i.e. the layer receiving sufficient light for photosynthesis occurring) as $Z_{eu} = 2.5 \times Z_s$. Water temperature, pH, conductivity, and dissolved oxygen concentration were measured *in situ* using a 3420 IDS multi-parameter (WTW, Germany). In addition, phycocyanin (PC) concentration was measured with a TriOS microFlu-blue fluorescent prob with an accuracy of 0.02 $\mu\text{g/LPC}$. The sensor is equipped with ultra-bright red LEDs, of an excitation wavelength of 620 nm, detection wavelength of 655 nm, and band-width 10 nm. Finally, the nutrient (PO_4 , NO_3) analysis was performed by the National Agency of Hydraulic Resources (ANRH).

P. rubescens identification and biomass estimation

Cyanobacteria taxonomic determination took place according to Komárek and Anagnostidis (1999, 2005) by microscopic observations of morphological characteristics. *P. rubescens* abundance was determined in a Nageotte chamber using a light Axiostar Plus microscope (Carl Zeiss, Germany) equipped with a UI-1240SE camera (IDS, Germany) as described in Guellati *et al.*, 2017. Cells quantification was estimated by dividing the length of the filament by the mean lengths of the cells. The number of cells per filament is the mean of 30 filaments.

Microcystins concentration

Samples for Microcystin analyses were filtrated through a GF/C microfiber glass filter (Whatman, Germany), and then frozen. Microcystin was extracted from a 1000 mL filtered water sample in 10mL of 75% methanol for 1h. Filters were crushed then the suspension was centrifuged (10 min at 4000 rpm) and the resulting supernatant kept at room temperature for the analysis. Enzyme-linked immunosorbent Assay technique (ELISA) with a 96-well Microcystin ADDA ELISA Kit (Abraxis LLC, Warminster PA) utilized for the microcystin analyses. The ELISA quantifiable toxin range was 0.15–5 mg/L. Finally, absorbance was read at a single wave length of 450nm using a microplate photometer (MindrayMR-96A). All samples were treated using the same approach.

Results

Task force and strategies to cope with the emergency

The observation of this cyanobacterium was first reported in March 2012 by a local inhabitant of the Hammam Debagh municipality. The spectacular bloom of *P. rubescens* was observed in June 2012, since which a task force was created to cope with the emergency and tackle jointly the contamination of this reservoir, which supplies drinking water to the Guelma willaya, and irrigates the Guelma-Boucheougouf valley. This group includes the scientific team of the EMMAL laboratory, the National Agency of Dams and transfers (ANBT), the Hammam Debagh reservoir water treatment agency (ADE), the National agency of Hydraulic Resources (ANRH), and the Environment Department of Guelma. Several actions conducted by the force task including i) Several actions to reduce human exposure and food consumption (banning fishing, alternative drinking, and irrigation sources. ii) A monitoring campaign started immediately to characterize the hazard within the reservoir and in the treated water: cyanobacterium counting and toxin analysis. iii) A bank filtration was conducted to reduce *P. rubescens* biomass (Fig. 2). This can also be highly effective in removing both cyanobacterial cells and dissolved toxins (Chorus and Bartram, 2021). iv) Additional water treatment using activated carbon for example.

Change in limnological parameters and nutrient

Physico-chemical variables recorded in the subsurface water during the peak of growth period of *P. rubescens* bloom summarized in table 1. As a consequence of the bloom special conditions were observed as attested by the low water transparency not exceeding 1m combined with low dissolved oxygen concentration (min=2.63 mg L⁻¹) during the sampling period. It is interesting to show that the highest values of P-PO₄ in 2012 noticed on May with 0.48±0.16 mg L⁻¹. However the highest values of NO₃ recorded in February and March with 5 mg. L⁻¹ (Fig. 3). A vertical profile of temperature was performed on 29.05.2012 over 33m; it shows a stratified water column ranging from 22.6°C in the upper subsurface to 9.8°C in the bottom hypolimnion. The metalimnion defined as the layer with a temperature gradient ≥0.5°C extended from 5m to 18m (Fig. 1S).

a.



b.



c.



Figure 2. *Planktothrix rubescens* surface bloom in the reservoir Hammam Debagh. a) global view of the bloom. b), c) bank filtration to reduce the cyanobacteria biomass

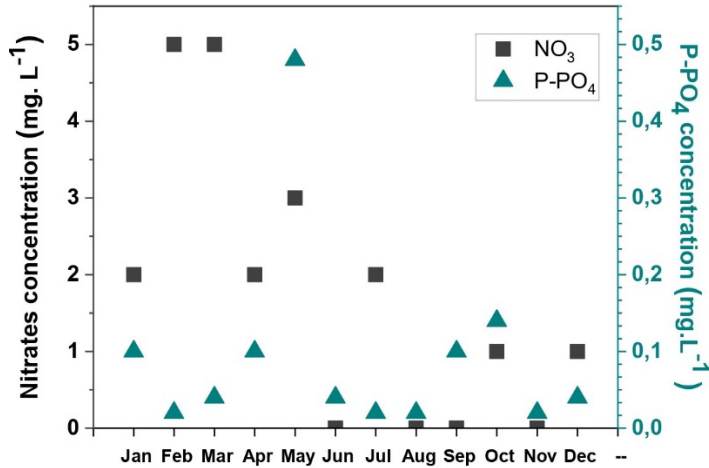


Figure 3. Concentration of biogenic compounds (NO₃, P-PO₄) in the water surface of the reservoir Hammam Debagh during 2012

Microscopic identification and population dynamics of *P. rubescens*

During the spectacular *P. rubescens* bloom a drastic reduction of green algae and diatoms diversity occurred. Maximum abundances reached 6.4×10^6 cells mL⁻¹ in the water column as observed at 2m from the surface of the reservoir Hammam Debagh. The distribution pattern of *P. rubescens* included the entire water column being significantly lower in the hypolimnion layer with 5000-7500 cells mL⁻¹ (Fig. 4 b). On the surface high abundances ranging from 3×10^5 to 3.6×10^6 cells mL⁻¹ estimated respectively on the 06 June at St1 and on the 29 May in St4 (Fig. 4a). It is important to note that the senescence state of *P. rubescens* observed on 29 May and 06 June.

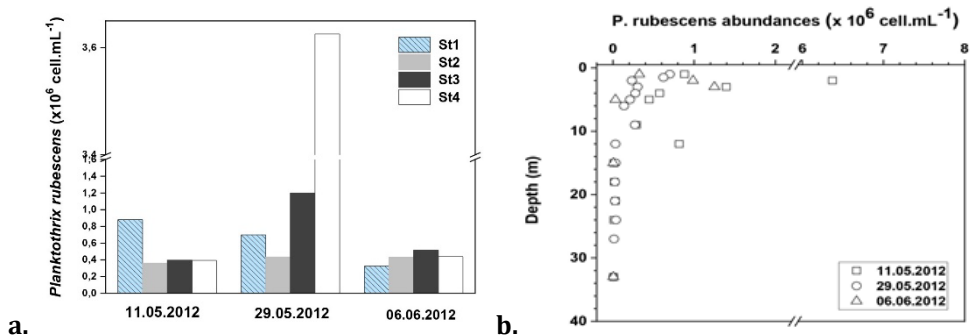


Figure 4. Variation of *P. rubescens* abundances in Hammam Debagh reservoir. a) Surface water in the four sampling stations, b) Water column in the center (St1)

Change in cyanobacterial related phycocyanin pigment

Fig. 5 demonstrates clearly the *P. rubescens* bloom dynamics in the four sampling stations over the euphotic zone. Globally, fluorescens measurements on 29.05.2012 show the highest concentration of the phycocyanin pigment. Moreover, the biomass was so high in St3 and St4 that the signal from the PC probe had become saturated in the top 1 m at a value of 200 $\mu\text{g L}^{-1}$ PC.

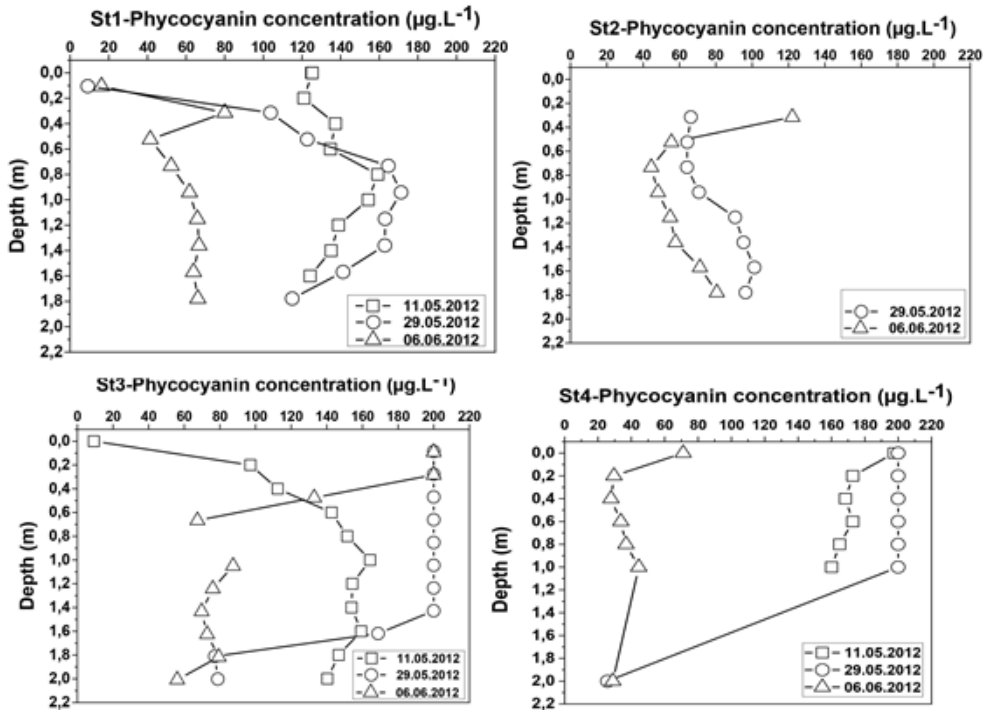


Figure 5. Phycocyanin concentration measured in the four sampling stations with fluorescent probe TRIOS Micro-Flue blue

Microcystin analysis

Intracellular Eq. MC LR concentrations detected at the water surface were below the WHO guidelines for drinking water ($1 \mu\text{g L}^{-1}$). They were only detected on 29 May with $0.45 \mu\text{g L}^{-1}$ Eq. MC-LR in St4 (data not shown). On the same date, samples taken at the water treatment agency (ADE) showed concentrations of 0.23 and $0.1 \mu\text{g L}^{-1}$ Eq. MC-LR before and after the treatment of water used for human consumption.

Discussion

The surface bloom of red colored cyanobacterium observed in the Hammam Debagh reservoir was an unexpected observation in our region. Generally, *P. rubescens* particularized as a cold water stenotherm species (Legnani *et al.*, 2005). It is known to form a metalimnetic blooms in deep-lakes located in Central and Northern Europe (Garneau *et al.*, 2013; Jann-Para *et al.*, 2004; Jacquet *et al.*, 2005; Ernst *et al.*, 2009; Fastner *et al.*, 1998; Micheletti *et al.*, 1998), and in shallow lakes in Scandinavia (Halstvedt *et al.*, 2007) and in Canada (Nürnberg *et al.*, 2003). Despite the importance of the reservoir Hammam Debagh, and in both terms of size and utilization (drinking water supply, irrigation and fisheries), there have been only a few studies of the phytoplankton. The lack of a long series of data, does not allow the identification of the process that causes the *P. rubescens* bloom. However, results from this study show strong variability in P-PO₄ on the water surface during 2012 with a maximum concentration of 0.48mg L⁻¹ recorded in May. Similar concentrations observed in Vico Lake located in central Italy during a bloom of *P. rubescens* (Manganelli *et al.*, 2016). Hence, neither phosphorus nor nitrate (max= 5mg L⁻¹ in February and March) were limited during the growth of *P. rubescens* in the reservoir Hammam Debagh. These findings are in accordance with previous studies which conclude that increased nutrient levels lead to *P. rubescens* surface blooms (Almodóvar *et al.* 2004; Sulis *et al.*, 2014; Trbojević *et al.*, 2019).

A study conducted from 2013 to 2015 in this monomictic reservoir showed that thermal stratification occurs from April to September. However, the overturn began in autumn (October-November) (Guellati *et al.*, 2017). It is interesting that 2012 characterized by a lowest rainfall and a snowywinter (Fig. 2S), enabling an increase of nutrients reaching the upper parts of the water column by the overturn. Our observation suggests that this cold stenotherm population resisted successfully to these conditions making an inoculum population for the following spring. Moreover, *P. rubescens* hardly grazed by herbivorous consumers, partly because of its filamentous morphology, formed by hundreds of single cells in up to 5 mm long fibers (Knapp *et al.*, 2021). In addition to good nutrient conditions and resistance to low water temperature in winter, spring 2012 was warmer; not to mention the role of “warm” conditions in spring and summer likely to enhance cell metabolism and lead to cyanobacterium bloom expansion (Moiron *et al.*, 2021; Posch *et al.*, 2012; Gallina *et al.*, 2011). Akçalan *et al.* (2014) have demonstrated that air temperature indirectly affects cyanobacteria via the stabilization of the water column, favoring the buoyant cyanobacteria to produce more gas vesicles to float up to the surface leading to the Burgundy-blood phenomenon.

It is evident that the *P. rubescens* bloom altered environmental conditions in the reservoir Hammam Debagh, since the primary oxygen concentration shows a minimum of 2.63 mg L⁻¹. Several studies attested that during Cyanobacteria bloom, oxygen levels are low mainly due to the increased microbial decomposition of organic material (Grossart and Simon, 2007; Hoikkala *et al.*, 2016). Moreover, the surface bloom clearly affected concomitantly alkalinity and transparency (40cm in May 29) in the water column. However, despite the high abundance of *P. rubescens* attending several millions of cells per milliliter, very weak concentrations of Eq. MC-LR detected during the pic of the “Burgundy-blood phenomenon” observed in June. A study carried out in 2012 in Lake Alto Flumendosa Sardinia by Stefanelli *et al.* (2017) reported a concentration of 9.74 µg L⁻¹ of total microcystins when *P. rubescens* was dominant on May 2012 with an abundance on the order of 1.3 x10⁶ cells ml⁻¹. Most of the toxicity studies on this red filamentous cyanobacterium reported a high concentration of hepatotoxic microcystins (Humbert *et al.*, 2001; Jacquet *et al.*, 2005; Halstvedt *et al.*, 2007) with two to four variants especially MC-LR and MC-RR (Briand *et al.*, 2005; Blom *et al.*, 2001, Cerasino *et al.*, 2016; Moiron *et al.*, 2021). The absolute identification of microcystin variants among *P. rubescens* population in the reservoir Hammam Debagh would require further studies.

In addition, studies on the microcystin activated and inactivated gene (*mcy*) filament quotas could explain the low concentration of microcystin in this reservoir. A study performed by Kurmayer *et al.* (2004) reported that all the studied *P. rubescens* filaments have the MC synthetase genes, and that the co-occurrence of MC producers and non-MC producers were only due to the inactivation of the *mcy* genes. The same study reported a particular high percentage of inactive *mcy* genotypes found in a lake with a higher density of *P. rubescens*. Hence, the *mcy* genes might down regulated in these particular strains by environmental factors. Thus, the question of how environmental factors influence the toxicity of cyanobacteria remains an important challenge. In fact, several studies reported the role of nitrogen, phosphorous, trace metals, growth temperature, light and pH likely to influence the metabolic pathway of hepatotoxins production (Sivonen 1990; Neilan *et al.*, 2013). Nutrients indirectly influence the toxin production since they affect the growth rate of cyanobacteria as attested by a positive linear relationship between the microcystin content of cells and their specific growth rate. Moreover, low iron concentrations correlated with increased toxin production (Long *et al.*, 2001).

Conclusion

In this study we suggest that the low light and winter low temperature conditions favor the emergence of *Planktothrix rubescens* to constitute the inoculum population when environmental condition were better in spring (nutrient, temperature, stratified and stable water column). However, the questions on the low toxicity of *P. rubescens* despite the high abundances recorded in this reservoir need further studies particularly molecular analysis of the involvement of *mcy* gene in microcystin production. Finally, sampling for longer period is needed to build predictive models of water quality of the reservoir Hammam Debagh.

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