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PHYTOPLANKTON ASSEMBLAGES IN BIR M'CHERGA FRESHWATER RESERVOIR (TUNISIA)

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Abstract

Tunisia is a Mediterranean country characterized by aridity on the major part of its territory. To this aridity is added the variability of the Mediterranean climate, with erratic and unpredictable periods of large drought and violent floods, to make of water an often limited resource and distributed unequally in the time and in the space. Facing this shortage of water, Tunisia developed a strategy of surface water resources mobilization. Consequently, the continuous increase of artificial water systems (reservoirs) provided a privileged investigation field for limnological research. Being the first link in the trophic web, phytoplankton plays a basic role in aquatic ecosystems. In fact, the processes, related to it, affect directly or indirectly the environment. Thus, a monitoring of the environmental conditions and phytoplankton assemblages was carried out in Bir M'cherga reservoir, from witch problems of fish mortality was reported in the recent years. In this way, we have identified 50 phytoplankton species spreading across seven classes, namely chlorophyceae, diatoms and cyanobacteria. These latter was the most diversified group since it represented 36% of the species richness, against 34% for chlorophyceae and 14% for diatoms.

In quantitatively terms, the successions of the main phytoplankton species showed large seasonal and inter-annual variations, but with a clear dominance of cyanobacteria. Indeed, it is very important to note that 80% of samples were dominated by filamentous cyanobacteria mainly *Limnothrix sp,* at more than 50% and up to 99%. Thus, the phytoplankton in Bir M'cherga reservoir did not present the classically succession described in the Tunisian reservoirs, where the Chlorophyceae and diatoms are dominant. It seems that the high trophic level of the studied water body contributed largely to the expansion of these micro-organisms. Cyanobacteria pose a potential risk to the environment and public health since they can produce toxic secondary metabolites including hepatotoxins that have carcinogenic potential, neurotoxins and lipopolysaccaride endotoxins. In fact, in Bir M'cherga dam, eight potentially toxic cyanobacteria species, such as *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa* and *Planktothrix agardhi*, have been identified. It is important to note that the hepatotoxicity in Bir M'cherga freshwater, evaluated by PP2A assay, didn't exceed the WHO guideline value for drinking water, but it was positively correlated with temperature, salinity and chlorophyll a.

Keywords: Tunisia, freshwater reservoir, phytoplankton, cyanobacteria, hepatotoxicity.

1 INTRODUCTION

On a planet where more than two thirds is covered by water, the illusion of abundance hid the fact that clean freshwater is increasingly scarce. Indeed, only 2.5% of available water on the globe is a freshwater which 69% is locked in the polar caps and mountain glaciers or stored in the deep aquifers (Gadelle, 1995). Moreover, the quality of surface waters is a major challenge for the future of our planet. In fact, the expansion of urban areas associated with agricultural pressure more and more intensively promote the progressive eutrophication of surface water, which contributes to the deterioration of water quality. This hydrosystems dystrophy leads generally, because of the excessive nutrient enrichment, to symptomatic changes such as increasing production of aquatic macrophytes and phytoplankton species, the modification of transparency and of water coloration, as well as the biodiversity loss and the apparition of algal blooms. These terms refer to any phytoplankton overgrowth when communicating sustained water stain, obvious to the observer (Dauta & Feuillade, 1995).

In freshwater, Cyanobacteria are the most commonly bloom forming taxon. They have many unique features among phytoplankton, such as buoyancy and nitrogen fixation, and the production of a wide variety of bioactive compounds. Several species of cyanobacteria form blooms that are frequently toxic, and these thus pose a health risk for humans and animals (Sivonen & Jones, 1999,). They can produce toxic secondary metabolites including hepatotoxins that have carcinogenic potential, neurotoxins and lipopolysaccaride endotoxins (Carmichael & Falconer, 1993; Carmichael, 2001).

Tunisia, a Mediterranean country located under the influence of the climatic disruptions of the north temperate region and the south Saharan region, is characterized by aridity on the major part of its territory. To this aridity is added the variability of the Mediterranean climate, with erratic and unpredictable periods of

large drought and violent floods, to make of water an often limited resource and distributed unequally in the time and in the space (Benzarti, 2003). Facing this shortage of water, Tunisia developed a strategy of surface water resources mobilization (Ben Mammou & Louati, 2007). Indeed, the works of resources mobilization especially dams play a key role in the economy. They contribute decisively to the water supply in drinking water production, irrigation, industrial and energy production. However, these reservoirs have become progressively more enriched during recent decades. The importance and current extent of eutrophication in Tunisian water bodies has been highlighted in previous studies, showing that these ecosystems present an increasing productivity stimulated continually by fertilizing contributions owing to the important anthropisation and the more dry climate (Mouelhi, 2000; Turki, 2002; Ben Rejab Janhani et al., 2006; Fathalli et al., 2006 and El Herry et al., 2008).

The research conducted in this work has as objective the water characterization of Bir M'cherga reservoir, from witch problems of fish mortality was reported in the recent years, through a monitoring of the environmental conditions and phytoplankton assemblages during a cycle of two years.

2 MATERIALS AND METHODS

2.1 Study area and water sampling

Sampling was carried out bi- monthly from January 2005 to December 2005, and monthly from January 2006 to March 2007. Water samples were collected from the deepest part of the Bir M'Cherga (BM) reservoir, located in the northeast region of Tunisia, 47 km south-western of the capital Tunis (N 36° 30'36'' and E 10° 00'38''). This region is characterized by a semi-arid climate with an annual average temperature of 18.5 °C and an annual rainfall ranging between 350 and 550 mm/year. The Bir M' Cherga water-body is used for irrigation and fishing. It was formed by the damming of the river Oued Miliane in 1971. It has a total capacity of 53 x 10^6 m3, a mean depth of 14.5 m, and a catchment area of 1260 km2. Three sets of samples were collected for phytoplankton assemblage studies, chemical analysis and determination of cyanotoxins.

2.2 Phytoplankton analysis

Phytoplankton samples were preserved with 1% formaldehyde (identification of phytoplankton) and 0.2% Lugol's iodine solution (quantification of phytoplankton). Identification and quantification of the different taxa were carried out using sedimentation chambers and an inverted microscope (Leitz) by the UTERMÖHL technique (Utermöhl, 1958). The cyanobacteria genera were identified using universally accepted taxonomic keys based on cell structure and dimension, and colony morphology, and mucilage characteristics (Komárek and Anagnostidis, 1989, 1999, 2005). The other phytoplankton groups were identified according to morphological features described in Bourrelly (1972, 1981, 1985).

2.3 Physical and chemical water parameters

Water temperature, pH and salinity were measured in situ using a multi-parameter probe (WTW, MultiLine P3, Germany). The dissolved oxygen concentration was dosed with the chemical method of Winkler. Water transparency was measured using a Secchi disk. For nutrient analysis, collected raw water samples were shaken vigorously and aliquots were then used to analysis of nitrate (NO^-_3), nitrite (NO^-_2), ammonium(NH^+_4),orthophosphate (PO_4^{3-}), and total phosphorus (TP) using the methods described in Rodier(1996). Chlorophyll-a concentration was determinate fluorometrically according to the method described by Neuveux (1974).

2.4 Microcystin analysis in environmental samples by PP2A inhibition assay

Microcystin levels in the water samples were measured between January and October 2005 using the colorimetric protein phosphatase 2A (PP2A) inhibition assay. We have performed this assay in the Laboratory "Public Health –Environment", Faculty of Pharmacy, University Paris-Sud 11, France.
This test is based on the dephosphorylation of para-nitro-phenyl phosphate (colorless) to yield para-nitrophenol (yellow) under the action of phosphatase 2A. For each sample, an aliquot (200 ml) was filtered

through a glass microfiber filter (GF/C, Whatman) to separate the toxins dissolved in water (dissolved toxins) from those associated with cyanobacterial cells and adsorbed on particles (particulate toxins). The results were expressed on ng/l Microcystin-LR equivalents (MC-LR equiv ng/l). Relationships between environmental, biological parameters and the concentration of Microcystin were analyzed statistically based the Pearson correlation using the XLSTAT software.

3 RESULTS AND DISCUSSION

3.1 Physicochemical water characterization

The results of the physicochemical parameters for the 24 months of sampling, from January 2005 through March 2007, in the Bir M'cherga reservoir are summarized in Table1 where in the average concentrations of each parameter are indicated. It was characterized by a relatively high water temperature, alkaline water and relatively high salinity. The dam was relatively very well aerated, and transparent with secchi disk extinction depths ranging from 0.51 to 1.5 m. The maximum levels of orthoposphates (0.84 mg / l) and nitrates (16.9 mg / l), the main eutrophic substances (Ryding and Rast, 1994), recorded in the Bir M'cherga reservoir, are among the highest values in Tunisia (Mouelhi, 2000; Turki, 2002; Ben Rejeb Jenhani et al. 2006; Fathalli et al., 2006 and El Herry , 2008). According to the classification proposed by the O.C.D.E (1982) the reservoir can be considered hypertrophic. In fact, during the period of study, the annual average of total phosphorus, total nitrogen and Secchi disk transparency were ranging respectively from 0,23 to 0,55mg/l and from 0,93 to 0.99 m.

Table 1.Extreme and average values for the main physico-chemical water parameters recorded in the Bir M'cherga water body, as well as their correlations during the study period (January 2005 – March 2007).

Parameters	Min – Max	Average
Temperature (°C)	8.9 – 28.4	20.3
salinity	1.2 – 2	1.6
pH	8 – 9.09	8.5
Secchi disk (m)	0.51 – 1.5	0.9
Dissolved oxygen (mg /l)	5.44 – 12.54	9.02
Ammonium (mg /l)	0.06 – 1.94	0.3
Nitrite (mg /l)	0.01 - 1.1	0.08
Nitrate (mg /l)	0.019 – 7.71	1.69
Phosphate (mg /l)	0.04 – 0.41	0.16
Total phosphorus (mg /l)	0.08 – 0.44	0.23

3.2 Phytoplankton composition

Based on microscopic analyses, we have identified 50 phytoplankton species spreading across seven classes, namely chlorophyceae, diatoms and cyanobacteria. These latter was the most diversified group since it represented 36% of the species richness, against 34% for chlorophyceae and 14% for diatoms (figure 1).
In quantitatively terms, the successions of the main phytoplankton species showed large seasonal and inter-annual variations, but with a clear dominance of cyanobacteria. Indeed, it is very important to note that 80% of samples were dominated by filamentous cyanobacteria mainly *Limnothrix sp,* at more than 50% and up to 99%. Thus, the phytoplankton in Bir M'cherga reservoir did not present the classically succession described in the Tunisian reservoirs nor in temperate region, where the Chlorophyceae and diatoms are dominant (Sommer et al., 1986; Padisák, 2004; Ben Rejeb Jenhani et al. 2006; Fathalli et al., 2006; Rolland, 2009) (figure 2). It seems that the high trophic level of the studied water body contributed largely to the expansion of these micro-organisms. Cyanobacteria pose a potential risk to the environment and public health since they can produce toxic secondary metabolites including hepatotoxins that have carcinogenic potential, neurotoxins and lipopolysaccaride endotoxins. In fact, in Bir M'cherga reservoir, eight potentially toxic cyanobacteria species, such as *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa* and *Planktothrix agardhi*, have been identified. The specific composition of planktonic cyanobacteria in this water body may be comparable to those of other Mediterranean and African regions which most described species are cosmopolitan (Chorus & Bartram, 1999). The dominance of cyanobacteria in aquatic ecosystems, in different geographical locations, with considerable variability in size, morphology, salinity and hydrologic conditions,

indicates that climate change may contribute synergistically with anthropogenic nutrient enrichment to the expansion of these micro-organism across the globe (Paerl & Paul, 2012).

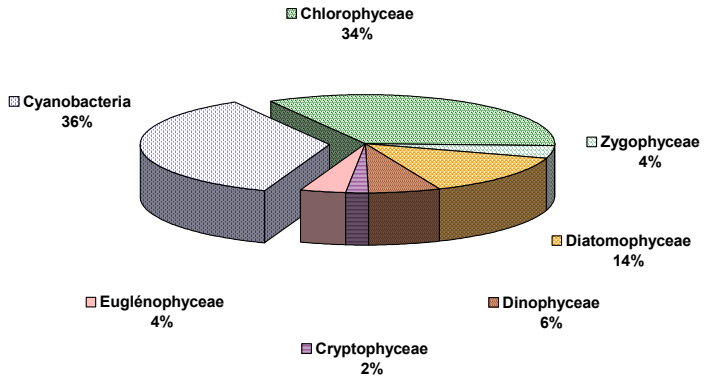

Figure 1. Phytoplankton classes in the Bir M'cherga reservoir

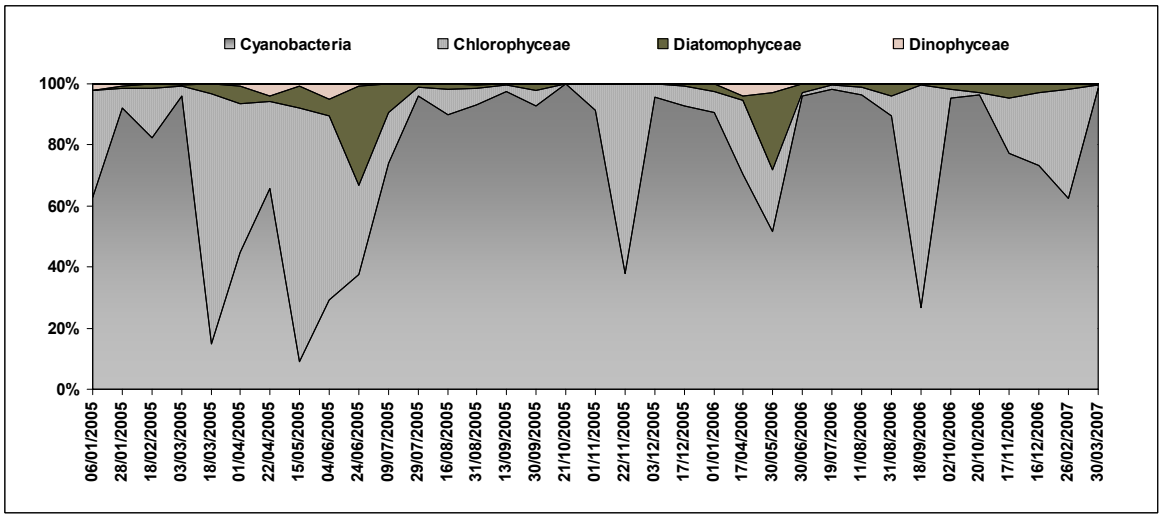

Figure 2. Relative abundance of major phytoplankton groups in Bir M'cherga reservoir during the study period (January 2005 to March 2007).

3.3 Fluctuation of microcystin levels

The total toxicity level in Bir M'cherga reservoir was estimated by the PP2A inhibition assay. This test was performed for the water samples collected during the period from January to October 2005. It showed three peaks recorded at surface water in August, September and October, with the values of 227, 823 and 931 ng/l Microcystin-LR equivalents. Two of these peaks were generated by the dissolved fraction concentration, which greatly exceeded the particulate fraction concentration. This result suggests release of toxin by cell lysis, an interpretation that is consistent with the reduction in the total cyanobacterial density (figure 3). These values were less than those found during a bloom of Microcystis spp. in Algeria (Nasri et al., 2004) and in Morocco (Oudra et al., 2001), where the microcystin concentrations were estimated to be 29,163 and > 500 µg/l, respectively. It is important to note that the hepatotoxicity in Bir M'cherga freshwater, evaluated by PP2A assay, didn't exceed the WHO guideline value for drinking water, but it was positively correlated with temperature, salinity and chlorophyll a.

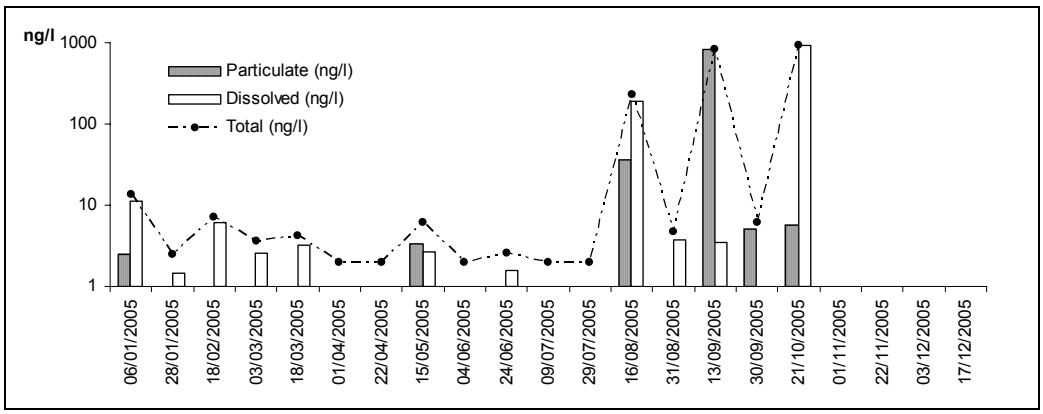

Figure 3. Seasonal fluctuations of dissolved, particulate, and total cyanotoxin expressed as ng/l Microcystin-LR equivalents (MC-LR equiv.ng/l) in surface water samples from the Bir M'cherga reservoir (Tunisia) during the period of January–October 2005.

4 CONCLUSION

The monitoring of the environmental conditions and phytoplankton assemblages carried out in Bir M'cherga reservoir showed 50 phytoplankton species spreading across seven classes, namely chlorophyceae, diatoms and cyanobacteria. These latter was the most diversified taxon. In quantitatively terms, the successions of the main phytoplankton species showed large seasonal and inter-annual variations, but with a clear filamentous dominance of cyanobacteria. Thus, the phytoplankton in Bir M'cherga reservoir did not present the classically succession described in the Tunisian reservoirs nor in temperate region, where the Chlorophyceae and diatoms are dominant. In fact, the high trophic level of the studied water body contributed largely to the expansion of these micro-organisms that pose a potential risk to the environment and public health since they can produce toxic secondary metabolites including hepatotoxins that have carcinogenic potential, neurotoxins and lipopolysaccaride endotoxins. In fact, in Bir M'cherga dam, eight potentially toxic cyanobacteria species, such as *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa* and *Planktothrix agardhi*, have been identified. It is important to note that the hepatotoxicity in Bir M'cherga freshwater, evaluated by PP2A assay, didn't exceed the WHO guideline value for drinking water.

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