SPACE-TIME DYNAMICS AND PARAMETERS OF GROWTH OF TOXIC CYANOBACTERIA IN FRESHWATERS IN CHEFFIA DAM (NORTH-EAST OF ALGERIA)

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ABSTRACT

Cases of morbidity caused by Cyanobacteria toxins have been associated to public water consumption distribution. Blooms of Cyanobacteria induce production of toxins, which constitutes a new topic of sanitary preoccupation, a field where little has been made in Algeria. Indeed, these lower-case small blue algae synthesise cyanotoxines, which are considered as neurotoxins or hepatotoxins for human beings. Cyanobacteria spoil water quality affecting aquatic ecosystem and contribute to accelerated lake eutrophication. In this study, spatial and temporal dynamics and growth parameters of the poisonous Cyanobacteria at the level of the Cheffia dam permitted us:

a) To make an inventory and identify the poisonous Cyanobacteria species which populate the Cheffia dam (December 1999 - November 2000)
b) To determinate the seasonal dynamic of poisonous algae and to follow their evolution according to some physical and chemical parameters of the dam waters,
c) To identify the poisonous Cyanobacteria as eutrophication bio-indicator,
d) To contribute to a better knowledge of these microorganisms which constitute a source of nitrogen and phosphorus imports, to the promising application perspectives in biotechnology.

Finally, it appears through the survey that the level of poisonous Cyanobacteria in the Cheffia dam increases during spring time generating very high concentrations of algae in summer.

Key Words: Cyanobacteria, Eutrophication, Algeria.

INTRODUCTION

The aquatic environment of freshwater is subjected to a series of parameters of which most significant are the physical and chemical composition, the biodiversity, and particularly the temperature and the periodicity of its lighting. With these parameters, the climate and the geological nature of the ground are factors whose influence on the characteristics of the aquatic environment is far from being negligible. According to Wasmund [30], eutrophic lakes are characterized with a progressive disappearance of the biocenosis. Eutrophication is observed when the affluent, the streaming and precipitations join to enrich waters with carbonate, silicate, phosphate and nitrate. It can also support fast multiplication of various microbial floras, which benefit from the noxious change of the composition of water. In such cases, one observes a clear prevalence of Cyanobacteria (blue algae) compared to other planktonic algae. Cyanobacteria are photosynthetic prokaryotes that appeared as insulated cells, in cluster or in filaments. These micro organisms populate a large variety of aquatic environments: freshwaters (rivers, ponds, lakes), domestics wasted water and littoral water. Their growth is supported by particular physicochemical parameters of the aquatic environment. Blooms of Cyanobacteria are by dense formations of cells appearing like a coloured scum or a greenish foamy-layer visible at the surface of water. In fact, the buoyancy of Cyanobacteria is due to the presence of gas vacuoles in the cytoplasm (Klemer [14]).

Massive proliferation of Cyanobacteria is an increasingly frequent phenomenon throughout the world. In Europe, several studies show that 40 to 75 % blooms have toxic properties (neurotoxins, Hepatotoxins, cytotoxins and endotoxins). In Scotland and in Portugal, toxins were detected in 68 % of blooms and in Finland 44% (Codd *et al*. [7]; Sivonen *et al.* [24]). In North America (Wisconsin), a similar study showed that the toxic bloom frequency varies from 20 to 40%, where as in Australia 42% of the blooms are considered as emitting toxins (Repavich *et al.* [21]; Vezie *et al.* [28]). Cyanobacteria deteriorate also the quality of water, which is an important factor in the balance of water ecosystem (Hennion, [10]). Such deterioration has as consequences:

- Considerable production of toxins that generates a great sanitary and medical concern for man, domestic and wild animals.
- Appearance of blooms at the origin of problems of treatment (in cost and effectiveness) and harmful effects at the level of water treatment industry,
- The damage to freshwater ecological balance and the contribution to accelerate the eutrophication of water systems.

In this study, we oriented our investigation in the inventory of toxic genera of Cyanobacteria that populate the Cheffia freshwater dam. We carried out a survey on their seasonal dynamics changes and on the variation of their frequency according to physical and chemical parameters of the water of the dam, which is a source of supply of drinking water to the neighbouring population.

MATERIAL AND METHODS

1. Presentation of the Site of Study

Cheffia dam is on the wadi Bounamoussa located 50 km east of Annaba city of (Northeast of Algeria) and its start-up goes back to 1969. The dam has a capacity of 170 million m^3 and allows to store up to 140 million m^3. The regular volume per year is about 95 million m^3 of which 61 million m^3 are destined to irrigation of agricultural areas and 34 million m^3 intended for industrial and human needs.

2. Methods

2.1. Sampling Procedures

Samplings of water for the identification of Cyanobacteria as well as for measurements and proportioning of physico-chemical parameters of the water are carried out once per month (from December 1999 to November 2000).

Collecting Cyanophycean elements consists in filtering 50 litres of freshwater by means of a filtering phytoplankton net with a diameter of 20 µm. A volume of 100 ml of filtrate is poured into a sterilized dark glass bottle. In order to avoid proliferation of micro-organisms, samplings that are not processed within the following 6 hours, are fixed in 4% formalin solution. Samplings destined for long period of conservation are fixed in 6% formalin solution (Nasri [18]).

2.2. Generic Identification

Generic identification of Cyanobacteria was made regarding to morphological criteria according to keys of identification from Bourrely [2] and Coute [9]. We took into account the following criteria: colour, size, shape of the colonies, shape of the trichome, presence or not of the akinete, presence or not of the gas vacuoles, presence or not of the heterocyst, presence or not of a gelatinous sheath as well as for the trichome and for the colonies, and finally the colour and the aspect of the gelatinous sheath when present.

2.3. Determination of the Density

Countings of Cyanobacteria were carried out under light microscope starting from a drop of 0.1 ml of a homogenized fixed sample. The drop was spread out between a slide and a cover slide. The smear was observed according to horizontal course over the entire length of the cover slide. This operation was repeated 5 times while shifting clearly over the width of the plate, of approximately a field of microscope, so that there is no overlapping in the observation. At the time of these 5 horizontal courses, all Cyanobacteria present, independently of their kinds, are counted under 10 X 40 magnification (Leitao *et al.* [16]; Champiat & Larpent [6]). Calculation of the average monthly and total densities was done according to following formulas:

Average Monthly Density: **A.M.D. = X . 10^3 / 50 (Individuals per Litre).**

With X = Average number of individuals in five (05) slides observed from each sample.

Average Total Density: **A.T.D. = A.M.D. (by Sampling station) x N**

With N = Number of Sampling station.

2.4. Physico-chemical characters of water:

We measured the following physico-chemical parameters: temperature, pH, dissolved oxygen, turbidity and concentration of nitrates and orthophosphates. Water samplings were carried in bottles of glass of 0.5 L. Analysis of these parameters were carried out the same day of sampling in the regional laboratory of Annaba Water Supply Enterprise.

2.5. Statistics

Statistical analysis was performed with the Minitab software for Windows: (Minitab Release Ver. 12.21).

RESULTS AND DISCUSSION

1. Generic Identification of Cyanobacteria in Cheffia Dam

We identified six genera of Cyanobacteria:

- *Microcystis* and *Synechocystis* are circular in shape, generally grouped in colonies. One counts approximately 5000 cells per colony for *Microcystis*, but only 20 cells per colony for *Synechocystis*.
- *Oscillatoria, Pseudanabaena, Lyngbya* and *Anabaena* are filamentous or trichomes in shape.

2. Generic Monthly Mean Densities of Cyanobacteria in Cheffia Dam

Our results (Fig. 1) show that *Microcystis* and *Oscillatoria* are dominant with respectively 68% and 29 % of the whole Cyanobacteria in Cheffia dam whereas the four genera *Synechocystis*, *Pseudanabaena*, *Lyngbya* and *Anabaena* have very low densities representing only 3 % of the identified Cyanobacteria population.

3. Seasonal generic evolution of Cyanobacteria:

Calculation of the global mean seasonal densities of the Cyanobacteria population in the dam (**Fig 2**) shows that the strongest densities are observed in summer (7 997 individuals per litre) then in autumn (31 608 individuals per litre). In winter and spring, the mean seasonal densities are very low (respectively 05 and 08 individuals per litre).

DISCUSSION

Cyanobacteria gather approximately 120 genera and more than 1 500 species. Only 22 genera including 40 species are at the origin of toxic blooms. The most studied cyanotoxins are neurotoxins, which act on the nervous system and paralyse respiratory muscles whereas the hepatic toxins (microcystins and nodularins) damage the liver (Brock [3]; Thebault & Lesne [27]; Carmichael, [5]; Vezie *et al*. [29]). Proliferation of toxic Cyanobacteria is not a new phenomenon. According to Hennion [10], the first reliable case of a proliferation of toxic Cyanobacteria dates from the twelfth century reporting the death of many animals, cattle in particular. However, many researchers (Repavich *et al.*, [21]; Thebault & Lesne [27]; Vezie *et al.* [28]) underline that it is not necessary to be too alarmist, because through the results of their work, they all agree to estimate that 40 % of the bloom proliferations with Cyanobacteria in freshwaters do not present any danger.

During our visits at the dam, we noticed that the surface waves, the wind and the rain disperse the cells in water, decreasing the extent of blooms. Our results in Cheffia dam enabled us to highlight for the first time:

- The presence of toxic blooms of Cyanobacteria,
- The genera *Microcystis* and *Oscillatoria* represent 97 % of identified toxic Cyanobacteria density,
- Toxic blooms (neurotoxic and hepatotoxic) are relatively high in summer and autumn but are not significant in winter and spring.
- The presence of toxic Cyanobacteria in Cheffia dam correlated to seasons of summer and autumn (highest recorded densities), to alkalinity of water (pH > 8), to a moderate temperature (15 - 30°C), to a strong turbidity, to decreased levels of dissolved oxygen and finally to a rise in nitrates and orthophosphates.

Therefore, all these parameters seem to be favourable to the proliferation of Cyanobacteria in general and to blooms of toxic species in particular. Indeed, our results show that toxic Cyanobacteria proliferate better at the end of summer and during autumn particularly if the latter season temperatures do not fall beyond 18°C with a total absence of rain. On the other hand, from December to March, unfavourable environmental conditions for the growth of Cyanobacteria were recorded; significant rain fall, drop of temperature (Mean water Temperature in winter

= 9°C; Mean air temperature in winter = 10°C) and cold windy storms. Carmichael [4] confirmed that in tempered climate countries, Cyanobacteria are totally absent during the cold season (over wintering in the sediments).

At the beginning of the hot periods, explosive development and growth of phytoplankton elements were noticed. In addition, Codd et *al.* [7] and Sivonen et *al.* [24] attest that if Cyanobacteria reach high densities it is because of the mixing of water which makes go up the nutritive substances accumulated at the bottom. Thus, the generic progressions of Cyanobacteria depend on the capacity of adaptation of each genus to the various environmental conditions. Our observations enable us to understand that *Microcystis* and *Oscillatoria* are the most adapted genera in Cheffia dam. Pinckney *et al.* [20] estimate that Cyanobacteria that are most spread in summer months are those that have a great contribution to the enrichment of the phytoplankton biomass.

In Cheffia dam, the growth of Cyanobacteria is supported by a high level of temperature, an important sunning exposure, an alkaline pH, a dissolved oxygen saturation and phosphorus and mineral abundance in the medium. Several studies (Arrignon [1]; Champiat *et al.*[6]; Codd *et al.* [8]; Hennion [10]; Joset [12]; Maggie [17]; Paerl [19]; Sellner [22]; Sivonen *et al*. [25]; Skulberg *et al.* [26]; Thebault & Lesne [27]; Wasmund [30]) indicate that Cyanobacteria are characterized by a massive growth in water at temperature ranging between 15°C and 30°C, with alkaline pH (between 8 and 11), having a shallow depth with significant nitrate concentrations (up to 3.805 mg/L) and orthophosphates (optimal rate of 0.0124 mg/l). It is known that these compounds found in strong concentrations in water systems are always related to urban or agricultural activities.

The threat for a potential intoxication of a whole population is to be feared in the event of a high density of toxic Cyanobacteria. Their toxins can be released following a natural lysis of the cell or following algae treatments such as the use of Copper Sulphate, which makes burst the cells. In this field, Keijola *et al.*[13], Himberg *et al.*[11] and Lambert *et al*.[15] report that Cyanobacteria toxins are soluble in water but resistant to the noxious action of temperature. On the other hand, Lambert *et al*. [15] also pointed out that ionization degrades free toxins by oxidation while cupric treatment kills toxic Cyanobacteria.

Another negative consequence related to the presence of a strong concentration of Cyanobacteria is the formation at the surface of the water of a thick screen preventing the penetration of light into deep water. This is a limiting factor for chlorophyllian organisms living in depth as well as for other species requiring a sufficient quantity of dissolved oxygen. This is why we accept the idea that toxic Cyanobacteria should be considered as bio indicators of a slow death of a water system.

CONCLUSION

The massive development of Cyanobacteria is an increasingly frequent phenomenon throughout the world. In Cheffia hydrologic system, proliferation of these micro algae is correlated positively with water temperature (16°C – 30°C), with pH (8 to 10), with significant phosphorus and nitrogen concentrations, and also with strong sunlight intensity and a low depth.

The discovery of toxic *Microcystis* and *Oscillatoria* in Cheffia dam will lead to great safety and medical concern. Hence, we consider that the best mean to avoid human or animal intoxication is to set up regular surveillance campaigns in the dam particularly during summer and autumn. On the other hand, a severe control in the addition and the amounts of nitrates and phosphates used in agricultural systems beside the wadi Cheffia and Cheffia dam should be applied. Finally, in order to slow or even to put a stop to proliferations of the toxic Cyanobacteria, cupric treatment should be tested under reduced scale before larger application.

REFERENCES

[1] Arrignon, J., Aménagement piscicole des eaux douces. 4ème edition, Technique et documentation LAVOISIER, 631 pp, 1991.

[2] Bourelly, P., les algues d'eau douce. Initiation à la systématique.: Les algues bleues et rouges. Les Eugleniens, Péridiniens et Cryptomonadines, Tome III, 1985.

[3] Brock, T. D., Lower PH limit for the existance of blue-Green algae : Evolutionary and ecologica implications. Science, vol. 179, 1973, pp 480-483.

[4] Carmichael, W.W., Cyanobacteria secondary metabolites – the cyanotoxins. J. Appl. Bacterol., 1992, pp 445-459.

[5] Carmichael, W.W., The cyanotoxins. Advances in Botanical Research, 27, 1997, pp 211-256.

[6] Champiat, D. et Larpent, J. P., Biologie des eaux et techniques, Edition, France, 1998.

[7] Codd, G.A., Bell, S.G. and Brooks, W.P., Cyanobacterial toxins in water. Wat. Sci. Tech., 1989, 21, 3, pp 1-13.

[8] Codd, G.A., Ward, C.J. and Bell, S.G., Cyanobacterial toxins : Occurrence, Modes of Action, Health effects and exposure Routes *in* Applied Toxicology: Approaches Through Basic Science. *Proceedings of the 1996 EUROTOX Meeting,* Alicante, Spain September 22-25, *Archives of Toxicology*, Supplement 19, Ed. J.P. Seiler and E. Vilanova., Springer-Verlag, 1997, pp 399-410.

[9] Coute, A., Diversité chez les micro-algues. Techniques – Sciences – Méthodes, France, N°01, 1995, pp 20-24.

[10] Hennion, M.C., La chimie au service de l'environnement. Pour la Science, 266, décembre 1999, pp 83-84.

[11] Himberg, K. ; Keijola, A. M.; Hiisvirta, H. ; Pysalo, H. & Sivonen, K., The effect of zater treatment processes on the removal hepatotoxins from *Microcystis* and *Oscillatoria* a laboratory study. Wat. Res., 23, 1989, pp 979-984.

[12] Joset, F., Les cyanobactéries ont-elles un avenir en biotechnologie ? Analyse de leurs propriétés Génétiques. Bio sciences-II. N°12, 1983, pp 214-218.

[13] Keijola, A. M.; Himberg, K.; Esala A. L. ; Sivonen, K. et Hiisvirta, L., Removal of cyanobacterial toxins in water treatment processes : laboratory and pilot scale experiments. Tox. Assess., 1988, 3, pp 643-656.

[14] Klemer, A.R., Effects of nutritional status on cyanobacterial buoyancy, blooms and dominance, with special reference to inorganic carbon. Can. J. Bot., 1990, 69, pp 1133-1138

[15] Lambert, T.W.; Holmes, C.F.B, Hrudey, S.E., Absorption of microcystins – LR by activated carbon and removal in full scalewater treatment. Wat. Res., 1996, 30, pp 1411-1422.

[16] Leitao, M.D.A. ; Lassus, P. ; Maggi, P. ; Lebaut, C. ; Chauvin, J. et Truquet, P., Phytoplancton des zones Mytilicoles de la baie de vilaine et intoxication par les coquillages. Institut Scientifique et Technique des pêche Maritimes, Nantes, 1984, 3 pp.

[17] Maggie, P., 1982. Les mortalités de poissons en Baie de Vilaine (juillet 1982). Institut Scientifique et Technique des pêche Maritimes, Nantes. 19 pp.

[18] Nasri, A.B., Etude de la biodiversité des Cyanoprocaryotes et de leur toxines dans un milieu d'eau douce : Lac Oubeira. Thèse de Magister en toxicologie fondamentale et appliquée. Département de biologie, Université *Badji Mokhtar* Annaba, Algérie, 110 pp, 1998.

[19] Paerl, H.W., Nuisance phytoplankton blooms in coastal, estuarine and inland waters. Limnol. Oceanogr., 1988, 33, pp. 823-847.

[20] Pinckney, J. L.; Paerl, H. W.; Harrington, M. B. et Houre, K. E., Annual cycles of phytoplankton community-structure and bloom dyna,ics in the Neuse River Estuary, North carolina. Marine Biology;.131, 1997, pp. 371-381.

[21] Repavich, W.M.; Sonzogni, W.C.; Standridge, J.H.; Wedepohl, R.E. & Meisner, L.F. - Cyanobacteria (blue – green algae) in Wisconsin waters : acute and chronic toxicity. Wat. Res., 1990, 24, pp 225-231.

[22] Sellner, K.G., Physiology, ecology; and toxic properties of marine Cyanobacteria blooms. Limnol. Oceanogr., 42, 1997, pp 1089-1104.

[23] Sivonen, K., Toxic Cyanobacteria in Finnish Freshwaters and the Baltic Sea. Ph.D academic dissertation in Microbiology, University of Helsinki, Finland, 1990.

[24] Sivonen, K.; Niemela, S.I.; Niemi, R.M.; Lepisto, L.; Luoma, T.H. & Rasanen, L.A., Toxic Cyanobacteria (Blue – green algae) in Finnish fresh and coastal waters. Hydrobiologia, 190, 1990, pp 267-275.

[25] Sivonen, K.; Namikoshi, M.; Luukkainen, R.; Fardig, M.; Rouhiainen, L.; Evans, W. R.; Carmichael W. W. ; Rinehart, K. L. et Niemela, S. I. () – Variation of Cyanobacterial hepatotoxins in Finland. Nordic Ecosystem: Dynamics, Processes & Fate. Hydrobiologia, 1995, pp 163-169.

[26] Skulberg, O.M., Codd, G.A., Carmichael, W.W., Toxic blue-green algal blooms in Europe: a growing problem. Ambio, 1984, 13, pp 244-247.

[27] Thebault, L., Lesne, J., Les toxines des Cyanobactéries : quels risque pour la santé ?. Techniques – Sciences – Methodes , France, N° 12, pp 937-940.

[28] Vezie, C. ; Bertru, G. ; Brient, L. ; Lefeuvre, J.C. - Blooms de cyanobacteries hépatotoxiques dans l'ouest de la france", Techniques – Sciences – Methodes , France, N° 10, 1997, pp 39-46.
[29] Vezie, C. ; Brient, L. ; Sivonen K. ; Bertru, G. ; Lefeuvre, J.C. et Salkinoja-Salonen M.– Variation of *Microcystis* content of Cyanobacterial bloomsand isolated strains in Lake Grand – Lieu (France). Microb. Ecol.; 35, 1998, pp 126-135.
[30] Wasmund, N., Phytoplankton periodicity in a eutrophic coastal water of the Baltic Sea. Int. Revue Ges. Hydrobiol., 1997, 79, pp 259-285.

Table 1: Relative correlations between physicochemical parameters and monthly mean densties of Cyanobacteria in the Cheffia dam

	Temperature (°C)		**pH**		**Dissolved Oxygen**		**Nitrates (mg / l)**		**Ortho – Phosphates (mg / l)**		**Turbidity**	
	r	P	r	P	r	P	r	P	r	P	r	P
A.M.D. (Ind./L)	0,485	0,110	0,313	0,323	0,533	0,074	0,775	0,003	0,802	0,002	0,974	0,000

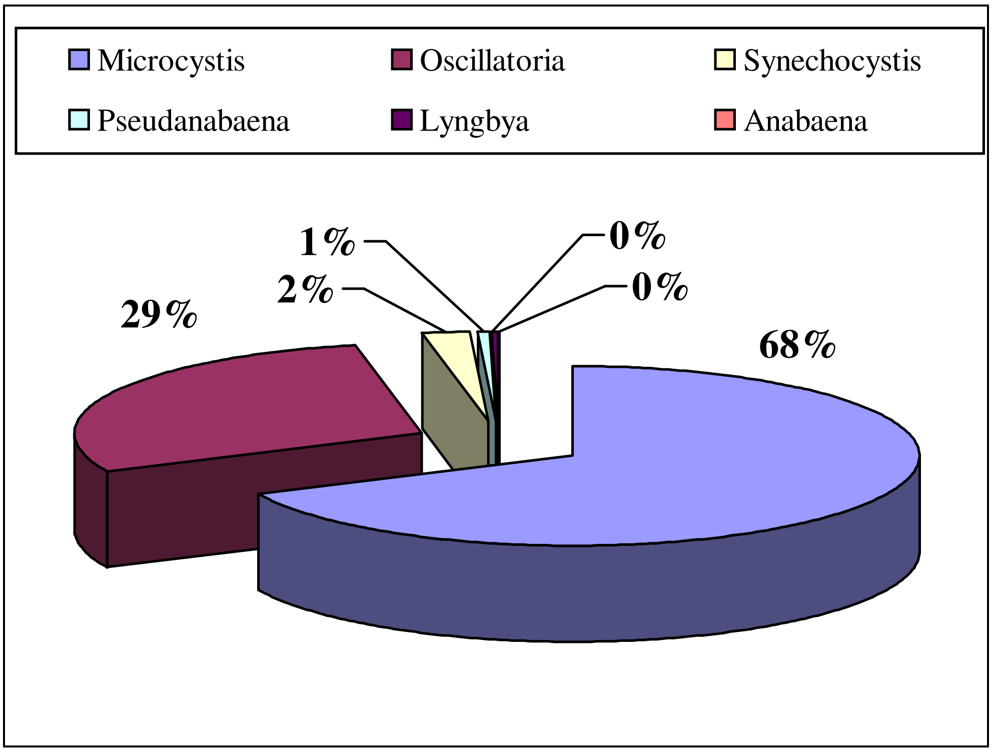

Figure 1: Generic concurrency of toxic Cyanobacteria in Cheffia dam in Algeria (Dec. 1999 – Nov 2000)

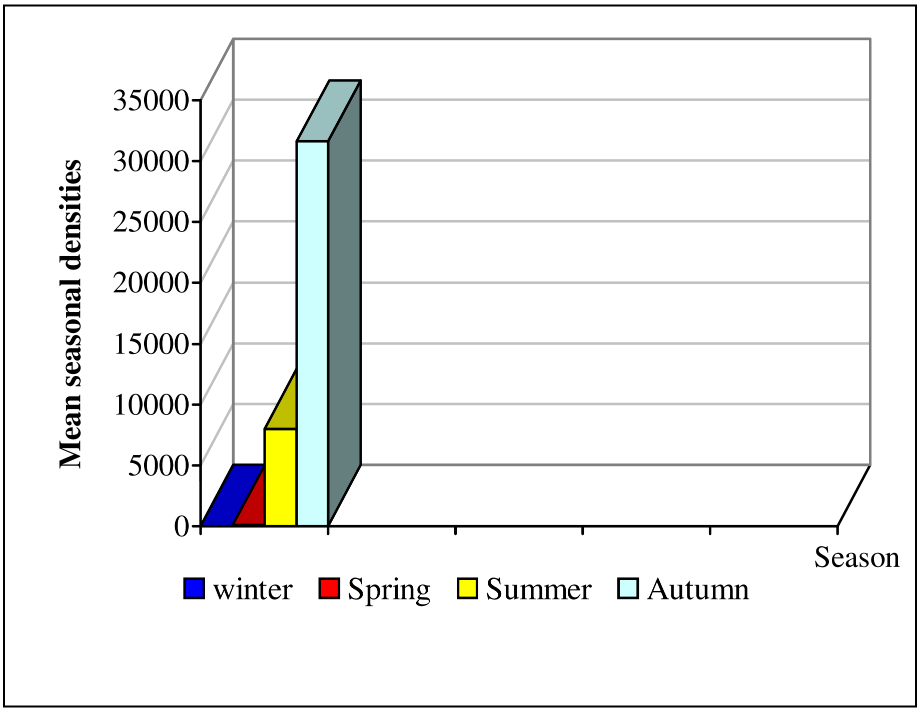

Figure 2: Global mean seasonal densities of toxic Cyanobacteria in Cheffia dam in Algeria (1999-2000)