Harmful cyanobacterial toxic blooms in waste stabilization ponds

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Abstract A coccoid picocyanobacterium *Synechocystis* sp. (0.6-2 µm of cell diameter) was found to be dominant during summer period in the experimental wastewater stabilisation pond of Marrakesh. The taxonomy of this isolated strain was confirmed by electron microscope study. The general patterns of ultrastructure and the mode of cell division resemble *Chroococcales*. The cyanobacterium strain was axenic and cultured on both inorganic Z8 and BG13 media. Mammalian toxicity was confirmed by mice bioassay. The major sympton of poisoning was severe diarrhoea. Histopathological study shows a slight hepatotoxicosis associated with a pronounced change in the intestinal mucosa which shows swelling and destruction of villi epithelium and shedding of enterocytes into the lumen. Although slow, these kinds of poisoning are comparable to those induced by okadiac acid intraperitoneal mice injection (diarrhetic shellfish poisoning "DSP" toxins). By using the enzyme-linked immunosorbent assay (ELISA), the amount of hepatotoxins "microcystins" was determined. The result shows that Synechocystis can produce a small amount of total microcystine [an average of 15 μg^{-1} dry weight corresponding to 20 ng(10^9cell)$^{-1}$]. These findings lead us to consider *Synechocystis* as both a potent neurotoxin and hepatotoxin producer. Because of the confirmed cyanobacterium toxicity, an eventual ecological implication should be considered. However, a toxic chronic test experiment on Daphnia was simultaneously carried out. Juvenile *D. magna* (less than 24 hours old), were fed three concentrations (10^4, 10^6, 10^8 cells / ml) of *Synechocystis*. A group of organisms fed with *Chlorella vulgaris* (3. 10^5 cells/ml) and another group without food, were studied as control treatments. Only animals cultured with 10^4 cells/ml of cyanobacterium survived at 80% until the end of the test (21 days). Reproduction and normal growth occurred in control treatments fed with *Chlorella vulgaris* and the group fed with the lowest concentration of *Synechocystis*. One-way ANOVA statistical analyses show significant differences in *Daphnia* survival and growth, between treatments with and without *Synechocystis* and between treatments with and without food. In terms of this study, there is evidence that toxic picocyanobacteria blooms occurring in wastewater stabilization ponds of Marrakesh, could have harmful repercussions on zooplanktonic, bacteria and other algae communities. Consequently, this will constitute a possible hindrance for sewage self-purification process and system treatment performance. In addition, the reuse of such treated wastewater effluent for irrigation will constitute an additional, potent, health hazard for animals and human's.
Keywords Chlorella; daphnia; microcystins; picocyanobacteria; synechocystis; wastewater

Introduction

The assimilative capacity of stabilization ponds systems depends on the interaction between the ecosystem's various biotic and abiotic factors. Among the biotic factors, algae play an essential role in the self-purification process. During biological sewage purification using stabilization ponds or other biological treatment systems, microalgae can operate and affect the treatment process in many ways (Wong and Tam, 1998). Although the quality of wastewater effluent and climatic conditions may vary, some algal genera are common to

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wastewater stabilization ponds. In Marrakesh sewage ponds, the picocyanobacterium *Synechocystis* sp. is of particular interest (Mezrioui and Oudra, 1998). The picocyanobacteria flora, essentially *Synechoccocus* and *Synechocystis* genera, can account for a significant fraction of phytoplankton productivity and biomass in various aquatic systems (Lincoln and Hill, 1980; Maeda *et al*., 1992; Mezrioui and Oudra, 1998). There are advantages to using eukaryotic microalgae rather than prokaryotes (cyanobacteria) for biological treatment purposes. Toxic cyanobacteria are now recognised as a hazard to human and animal health. The bloom-forming cyanobacterium *Microcystis* seems to be the most isolated toxic strain studied and the most widely distributed among toxigenic blue-green algae (Watanabe *et al*., 1996). The taxonomic connection between *Synechocystis* and *Microcystis* (Chroococcales) leads one to think about their potential toxicity and toxin production. In addition, the toxicity of some of *Synechocystis* and *Synechococcus* isolated strains has been confirmed (Lincoln and Carmichael, 1981; Mitsui *et al*., 1987; Oudra et al. 1998a). So far, toxic picocyanobacteria blooms have not been seriously taken into consideration. Therefore, much interest should be given to these kinds of blue-green algae, which could develop nuisance blooms. Current literature dealing with cyanobacteria-zooplankton interaction reports that there is evidence of a negative effect of toxic cyanobacteria on aquatic herbivorous invertebrate zooplankton survival, growth and reproduction, as Daphnids (Reinikainen *et al*.,1995; Sbiyyaa *et al*., 1998) and rotifers (Gilbert, 1996) and fish (Bury et al., 1996). Some observations concerning the possible effect of *Synechocystis* blooms on the disappearance of cladocerean populations (e.g. *Miona micrura*) from the experimental wastewater stabilisation pond of Marrakesh, have been reported by Tifnouti and Pourriot (1989). According to these findings, it is evident that toxic picocyanobacteria blooms occurring in sewage treatment ponds, could present a possible hindrance to the self-purification process and will constitute an extra sanitary hazard for wastewater reuse. The aim of this work was to investigate the toxicological aspects of this isolated picocyanobacterium *Synechocystis* and its eventual ecological implications (whether the toxins present an acute and chronic hazard to Cladocera, *Daphnia magna*).

Materials and methods

Cultivation of cyanobacterium and toxicological tests

For mammalian toxicity (mice bioassay), the isolation and purification of the cyanobacterium strains were performed using 2.5% agar BG13 solid medium (Ferris and Hirsch, 1991). The strain was axenic, according to the method previously described (Oufdou et al., 1998). The cultures were routinely maintained in 1000 ml of BG13 medium in 2 litre conical flasks, and were constantly illuminated by cool white fluorescent light, which give a measured irradiance of 62 $\mu E\ m^{-2}\ s^{-1}$ (Quantameter Li-cor 185A). The room temperature was kept at 26°C. The culture flasks were constantly agitated by magnetic stirring. The cells were harvested by centrifugation at the end of the logarithmic phase of growth. The lyophilized cells in 0.9% NaCl, were then assayed to determine the intraperitoneal (i.p) lethal dose for 50% of the animals tested (LD_{50}) using male Swiss mice 18-22g. After injection, the animals were observed continuously for four hours. Symptoms and survival times were recorded. For confirmation of poisoning signs, histopathological examination of target organs (liver and intestine) was performed. By application of the highly sensitive enzyme-linked immunosorbent assay, the content of microcystins toxins produced by *Synechocystis* strain, was determined (An and Carmichael, 1994) using the Millipore plates kits " EnviroGrad ^{TM} Microcystins Plate Kit SDP222S4".

For the Daphnid toxicity test, the cyanobacterium non-axenic strain was cultured under 24 $\mu E\ m^{-2}\ s^{-1}$ fluorescent lights cool-white continuous illumination, at 20°C room temperature in 5-l Erlenmeyer flasks containing 3l of the inorganic medium Z8 (Kotai, 1972). The

cells were harvested during the exponential growth phase and concentrated by centrifugation to obtain the highest cells concentration (10^{12} cell)/ml, algal suspension, from which culture dilutions (10^{8}, 10^{6}, 10^{4} cells/ml) were prepared. The algal food suspensions were stored in the dark at 4°C, all the *Synechocystis* aliquot suspensions were changed each week. The direct cells count was determined by the Haemacytometer-Mallassez, 0.2 mm deep.

Cultivation of Cladoceran (Daphnia magna)

For the experiment, female *Daphnia magna* were obtained by keeping mature individuals (picked from culture maintained as a clone at Department of Zoology, Faculty of Science, Porto). The Daphnids were cultured in standard medium for toxicity testing M4 medium (Elendt and Bias, 1990). They grew under favourable conditions (20°C temperature and 14/10-light/dark cycle) and were fed with 3.10^{5} cells/ml of *Chlorella vulgaris* (green algae growing on Z8 medium). After some days, juvenile animals (0-24 hours old) were picked for the experiment. An appropriate number of juveniles were randomly separated into similar conditions. Five replicates were used for each treatment concentration (one animal in 50 ml of M4 medium). The daphnids used for the chronic test were fed daily with cyanobacteria cells suspensions and the culture medium was renewed daily. Control experiment series were kept without food or fed with *Chlorella vulgaris*. During the experiment time (21 days) and for each treatment, the *D. magna* size was estimated by measuring via animal exuvia, the first exopodite of the second antenna which was significantly correlated to body length (r^2 =0.99). This method was previously described and statistically discussed by Barros (1994). The experiments reported above were run with a new-born *Daphnia* with around 1.5 mm body length. The experiments include also the estimation of survival time of *Daphnia* exposed to various concentrations of cyanobacteria strain and reproduction behaviour in each experimental series.

Results and discussion

Morphology and taxonomy of theisolated cyanobacterium strain

Among the smallest known planktonic cyanobacteria are the unicellular coccoid, ovoid and rod-shaped picoplanktonic species (cell ranging in linear dimension from 0.2 to 3 μm) that compare with the Eubacteria in both shape and size (Pearl, 1988). It appears that the picocyanobacteria bloom occurring periodically during the hot period, was one of the peculiarities of the Marrakesh sewage pond operating under an arid climate. The picocyanobacteria bloom was in major part constituted by the chroococcoid *Synechocystis* genus, the dominance of which induce is an apparent change of the color of the pond effluent which turns a brilliant green (Mezrioui and Oudra, 1998). A similar phenomenon was observed and described in large outdoor algal cultures used for protein production (Lincoln and Hill, 1980). According to *in situ* growing wild strain size (0.6-1.2 μm of cell diameter), the species identified most closely with, although *S. parvula* Perfiliew as described by Stramach (1966). The cultured laboratory strain had a larger cell diameter, which ranged from 1.4 μm to 2.6 μm. This last change leads us to refer to the genus *Synechocystis*, without species designation. This genus occurs widely in both marine and freshwater habitats. The electron micrograph shown in Figure 1 pictures the major fine structural components of the prokaryote chroococcaean cells with polyphosphate and cyanophycin granules forming a circular cluster in the centre of the cell and with the photosynthetic thylakoides entirely peripheral.

Toxicological study on the cyanobacterium

An axenic cyanobacterial culture was harvested at the end of the logarithmic growth phase.

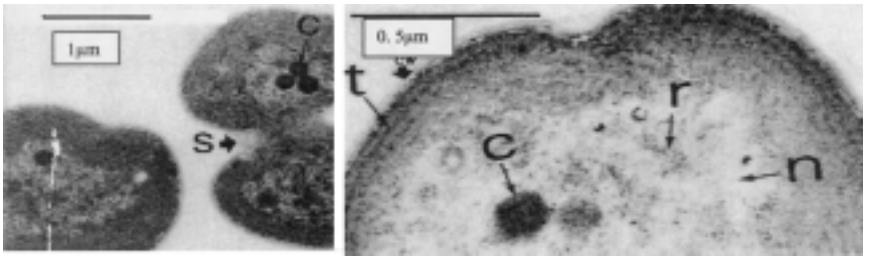

Figure 1 Electron micrograph of Synechocystis cells magnified x11000 and x19000 showing ultrastructure of picocyanobacterium (S: septum formation, C: cyanophycin granule, n: nucleoplasm, r: ribosomes, t: thylakoïds (photosynthetic lamellae), cw: cell wall).

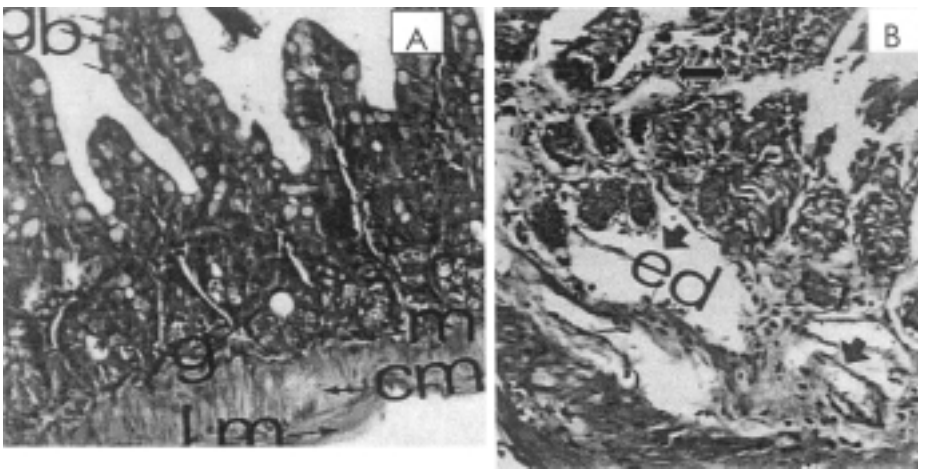

Figure 2 Histological examination of the small intestine of mice (E&H stain). A: normal structure of the villi of control intestine with normal goblet cells (gb), intestinal gland in crypt region (g), muscularis mucosae (m) and circular and longitudinal muscle (cm, lm) (x200). B: mice small intestine acutely poisened by i.p injection of *Synechocystis* aqueous extract, shoeing intestinal damage with shedding of epithelium of the villi (↔) and destruction of the crypt region by oedema (edm) (x400).

Concerning the mammalian toxicity assessment, the results show that an intraperitoneal mice injection (i.p) of lyophilised material at 650 mg/ kg was lethal in all cases. The calculated 24-h LD_{50} ranged from 150 to 350 mg/kg. The survival time at the lethal dose was about 7-12 hours. The major poisoning symptoms manifested were ataxia, paleness, piloerection, "salivation" and severe diarrhoea, which appears 15-30 min after injection. Histopathological examination showed slight liver damage, with some extensive haemorrhaging and sinusoid capillary destruction. Nevertheless, this observed liver injury was less drastic than that induced by (i.p) injection of *Microcystis* cells (Oudra *et al*. 1998b). In order to investigate the poisoning symptoms, histopathological examination on the small intestine was also performed. The results showed a pronounced change in the intestinal mucosa with swelling and destruction of epithelium of the villi and shedding of enterocytes into the lumen, resulting in a complete collapse of the villi architecture. There was also visible damage of the crypt region (Lieberküln glands) and an inflammatory reaction was also observed (Figure 2).

The results showed that *Synechocystis* could induce two kinds of poisoning with hepatotoxic and gastrointestinal disturbance symptoms. The content of hepatoxins "microcystins" was determined by application of ELISA technique. The result shows that *Synechocystis* can produce a small amount of microcystine (cyclic heptapetide), On average 15 µg/g dry weight. This estimated content of toxins seems to be very slight in

comparison with *Microcystis* Moroccan isolated strain, which contains 600 µg/g dry weight (Oudra et al., submitted). It is recognized that "microcystins" were considered the principal hepatotoxic toxins produced by *Microcystis* strains (Watanabe *et al*., 1996). The hepatotoxic effect of a *Synechocystis* sauvageau sp. isolated from a large outdoor algal culture used for protein production has been also confirmed by Lincoln and Carmichael (1981). Nevertheless, these authors reported that mice poisoning symptoms were essentially hepatotoxic without any signs of diarrhoea. The reported that the general effects of *Synechocystis* sauvageau toxin are indicative of a peptide of the anatoxin-c type, although the survival time was longer than the 1 to 3 hours typical of anatoxin-c. In our case, we could not suggest the existence of anatoxin-c type with a survival time longer (7 to 12 hours). Working with *Synechocystis* strain, which induce diarrheal effects as a major mice poisoning symptoms, led us to compare the intestine damage with that induced by okadaic acid (OA) and dinophytoxins (DTX) in suckling mice and rats, respectively, described by Tero et al. (1986) and Lange *et al*. (1990). The enterotoxic activity of OA and DTX was also observed by Hamano et al. (1986) by means of suckling mice bioassay. They confirmed the destructive effect on epithelium of small intestine with oedema of *lamina propria* of villi. In this present investigation, the poisoning effect caused by *Synechocystis* on the small mice intestine appears to be as drastic as that described by Tero *et al*. (1986) and Hamano *et al*. (1986). According to these findings, we suspected the existence of a mixture of microcystins and other diarrheagenic toxins (like DSP toxin compounds).

Toxic chronic effect of *Synechocystis* on *Daphnia* (ecological implication)

The first results obtained from this experiment to test the chronic toxicity effect of *Synechocystis* on *Daphnia magna*, show that without an alternative adequate food source, the ingested cyanobacterium cells are very toxic to juvenile *D. magna* at a high concentration level (10^8cells/ml). In comparison with control treatments (Daphnids without food and fed with *Chlorella vulgaris* (3×10^5 cells/ml) the survival time of animals feeding with 10^8 cells/ml and 10^6 cells/ml of *Synechocystis* did not exceed 7 days. Only animals cultured with 10^4 cells/ml of the cyanobacterium survived at 80% until the end of the experiment (21 days) (Figure 3A). The result of one-way ANOVA analysis (Table 1) showed a highly significant effect of food nature levels on *Daphnia* survival ($P<0.05$). The Newmann-Keuls test, confirmed that concentrations of cyanobacteria influence strongly the animal's survival time. Concerning the toxic effect of ingesting cyanobacterial cells on Daphnia reproduction, it appears that normal reproduction only occurred in the control treatment with *Chlorella vulgaris* (Table 2). However, *D. magna* cultured with the lowest and sub-lethal concentration of *Synechocystis* (10^4cells/ml), showed a delay in its reproduction cycle. The results confirm that toxic ingested cells of *Synechocystis* could inhibit or delayed the *Daphnia* reproduction.

The experiments show a significant effect of cyanobacterial ingested cells on development and growth of these animals. There was a significant difference in *Daphnia* size between treatment with and without *Synechocystis* and between treatment with and without food (Figure 3B). According to each experiment conditions, the estimated *D. magna* growth rates were remarkably different (Table 2). It is evident that the growth rate of *D. magna*, feeding on sub-lethal *Synechocystis* dilution was more important than that recorded in the control experiment without food. The toxic effect of *Synechocystis* reduced *D. magna* growth rate by 29% - 75% of controls fed with *Chlorella vulgaris*. Daphnia starvation reduces the growth at 45%. The results of a one-way ANOVA analysis, show a significant effect of higher cyanobacteria concentration on daphnid growth ($P<0.05$). The Newman-Keuls test, showed that each food quality has a specific action on daphnia growth (Table 3). Toxins produced by several species of cyanobacteria are a potentially serious

environmental problem. These substances can be acutely toxic as evidenced by the death of livestock and other animals that have been exposed to them. Although, the mammalian toxic effects of cyanobacterium *Synechocystis* genus have been confirmed its effect on Cladocera was scarcely reported (Arnold, 1971). Our results showed that *Synechocystis* ingested cells are toxic to the Cladocera, *D. magna*. The same results were found by many authors working with *Microcystis*, *Oscillatoria* strains (Nizan *et al*., 1986; Demott *et al*. 1991; Sbiyyaa *et al*., 1998). The exposure of *D. magna* to high concentrations of *Synechocystis* without an alternative food, induced acute toxicity which caused total mortality within 120 hours of culture time.

Daphnids fed with sub-lethal amount of *Synechocystis* (10^4cells/ml), had a long survival time, but the reproduction and growth were slightly affected. We have recent evidence that *D. magna* is influenced by nutritional quality of food and cyanobacterial toxins can directly impact Cladoceran production, growth and survival. Consequently, *D. magna* cannot survive on a *Synechocystis* diet. This toxicity may have resulted essentially from cyclic heptapeptide " microcystins " which was determined among other unidentified compounds in this cyanobacteria strain culture (around 15 μg/g dry weight). These products

Table 1 Chronic effect of *Synechocystis* ingested cells on survival of *Daphnia*. Results of one way ANOVA analyse (5%) and Newmann–Keuls test.

Food	%survival	Homogenous groups	
Chl	100	A	
No	100	A	
Syn1	88	A	
Syn2	38	B	
Syn3	19	C	
	Df	**F**	**P**
Total variation	9.9		
Food	4	53.48	0.00
Residual variation	95		

Table 2 Computation of *Daphnia* global growth rate and total number of viable new-born Daphnids, according to food level and quality. (*Growth rate calculated from only 5 days of survival time).

Nature of food	No	Chl	Syn1	Syn2	Syn3
Growth rate (day-1)	0.11	0.24	0.18	0.07	0.11*
Cumulus of new-born	0	133	5	0	0

Table 3 Chronic effect of *Synechocystis* ingested cells on Growth of *Daphnia*. Results of one way ANOVA (5%) analyse and Newmann–Keuls test .

Food	Mean growth	Homogeneous groups	
Chl.	3.73		A
Syn1	3.03		B
No	2.47		B C
Syn2	2.15		C
	Df	**F**	**P**
Total variation	79		
Food	3	11.64	0.00
Residual variation	76		

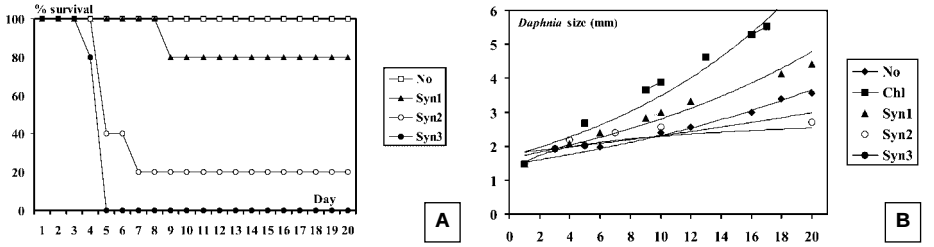

Figure 3 A: Effect of *Synechocystis* ingested cells on *Daphnia magna* survival. B: Variation of *Daphnia magna* growth size according to food quality. (Syn1: 10^4cell /ml, Syn 2: 10^6cell /ml and Syn 3: 10^8cell /ml of *Synechocystis*. Chl: Concentration of *Chlorella vulgaris* cells (3.10^5cell /ml). No: Without food.)

realised essentially by *Microcystis* strain have been reported as toxicant to zooplankton in particular Cladoceran species (Watanabe *et al*., 1996).

Conclusions

The first part of results obtained in this study confirms the toxicity of one strain of *Synechocystis* genus isolated from wastewater oxidation ponds. Hepatotoxic effect's were induced by "microcystins" toxins produced by *Synechocystis* strain. Diarrheal effects associated with hepatotoxicosis confirm that *Synechocystis* could produce a mixture of various toxins. Results of the chronic toxicity on *Daphnia* (Cladocera) test show the eventual ecological implication of this picocyanobacteria bloom in reducing *Daphnia* population. This toxic picocyanobacterium was found dominant during the summer period in the experimental wastewater stabilization ponds of Marrakech. This strain has been also recognised as a biotic selective agent against bacterial populations, in particular pathogenic bacteria growing in wastewater effluent (Mezrioui et al., 1994). In addition, harmful cyanobacterial toxins could affect all aquatic organisms in the food chain (Kotak *et al*., 1996), particularly rotifers (Gilbert, 1996), and fish (Bury *et al*., 1996), and even the photosynthetic activity of plants (Abe *et al*., 1996). In stabilisation ponds, self-purification process could not be complete without a positive microalgae, bacteria and zooplankton interaction. Generally, green algae present many advantages, whereas toxic cyanobacteria pose technical problems to this biological treatment system.

References

Abe, T., Lawson, T. Weyers, J.D.B. And Codd, G.A. (1996). Microcystsin-LR inhibit photosynthetic of *Phaseolus vulgaris* primary leaves-implication for current spray irrigaution pratice. *New Phyytologist*, **133**, (4), 651-658.

An, J., and Carmichael, W.W. (1994) . Use of a colorimtric protein phosphatase inhibition assay and linked immunosorbent assay for the study of microcystins and nodularins. *Toxicon.*, **32**, 1495-1507.

Arnold, D.E., (1971). Ingestion, assimilation, survival and reproduction by *Daphnia pulex* fed seven species of blue green-algae. *Limnol. Oceangr.*, **16**, 906-920.

Barros, P., (1994). Implicaçoes ecotoxicologicas de cianobactérias em cladocéros. *Trabalho de Master*. Faculdade de ciências e tecnologia da universidade de Coimbra-Portugal. 84p.

Bury, N.R., Eddy, F.B. and Codd, G.A. (1996). Stress reponses of brown trout, *salmo truta* L. to the cyanobacterium, Microcystsis aeruginosa. *Envir. Toxicol. Water Quality*, **11**(3), 187-193.

Chu, F.S., Huang, X. and Wei, R.D. (1990) . Enzyme-linked immunosorbent assay for microcystins in blue green algal blooms. *J. Assoc. analyt. Chem.*, **73 ,** 451-456.

Demott, W.R., Zhang, Q. and Carmichael, W.W. (1991). Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of daphnia. *Limnol. Oceanogr.*, **36**, 1346-1357.

Elendt, B., and Bias, W.R. (1990). Trace nutrient deficiency in *Daphnia magna* cultured in standard medium for toxicity testing. Effects of the optimisation of culture conditions on life history parameters of *Daphnia magna*. *Wat. Res.***, 24**, 1157-1167.

Ferris, M.J. and Hirsch, C.F. (1991). Methods for isolation and cultivation of cyanobacteria. *Appl. Environ. Microbiol.*, **57**, 1448-1452.

Gilbert, J.J., (1996). Effect of temperature on the reponse of plankton rotifers to a toxic cyanobacterium. *Ecology*, **77**(4), 1174-1180.

Hamano, Y., Kinoschita, Y. and Yasumoto, T. (1986). Enteropathogenicity of diarrhetic shellfish toxins in intestinal models. *J. Food Hyg. Soc. Jpn.*, **27**, 375-379.

Komarek, J., (1976). Taxonomic review of the genera *Synechocystis* sauv.1892, *Synechcoccus* Nag. 1849 and *Cyanothece* gen.nov. (Cyanophyceae). *Arch. Protistenk. Bd.* **118**, 119-179

Kotai, J., (1972). Instruction of preparations of modified nutrient medium Z8 for algae. *Publication* B-11/69. Norsk Institut for Vannforsk-ning, Oslo, Norway. 5pp.

Kotak, B.G., Zurawell, R.W., Prepas, E.E. and Holmes, C.F.B. (1996). Microcystin LR concentration in aquatic food web comportment from lakes of varying trophic status. *Can.J. Fish.Aqua. Sci.*, **53**(9), 1974-1985.

Lange, S., Andersson, G.L., Jennishe, E., Lönmoth, I., Li, X.P. and Edebo, L. (1990). Okadaic acid produces drastic histopathologic changes of the rat intestinal mucosa and with concomitant hypersecretion. In: *Toxic Marine Phytoplankton*, E. Graneli, L. Sundstron, D.H. Edler, A. Andersson, (eds.), Elsevier-Amsterdam, p 356-361.

Lincoln, E.P. and Carmichael, W.W. (1981). Preliminary tests of toxicity of Synechocystis sp. grown on wastewater medium. *The Water Environment: Algal Toxin and Health*. W.W. Carmichael (ed.), Plenum Press, New York, 223-230.

Lincoln, E.P. and Hill, D.T. (1980). An integrated microalgae system. In: *Algae Biomass*. G. Shelef, and C.J. Soeder (Eds), North- Holland, Biomedical Press., 224-244.

Meada, H., Kawia, A. and Tizler, M.M. (1992). The water bloom of cyanobacterial picoplankton in lake Biwa, Japan. *Hydrobiologia*, **248**, 93-103.

Mezrioui, N. and Oudra, B. (1998). Dynamics of picoplankton and microplankton flora in the experimental wastewater stabilisation ponds in the arid region of Marrakech, Morocco and Cyanobacteria effect on *Escherichia coli* and *Vibrio cholerae* survival. In: *Wastewater Treatment with Algae*. Wong Y.S. and Tam N.F.Y. (eds): Springer-Verlag Landes Bioscience Georgetown, TX, USA, 165-188.

Mezrioui, N., Oudra, B., Oufdou, K., Hassani, L., Loudiki, M. and Darley, J. (1994). Effect of microalgae growing on wastewater batch culture on Escherichia coli and Vibrio cholerae survival. *Wat. Sci. Tech.* **30**(8), 295-302.

Mitsui, A., Rosner, D., Goodman, A., Reyes-vasquez, G., Kusumit, T., Kodama, T. and Nomoto, K. (1987). Hemolytic toxins in marine cyanobacterium *Synechococcu*s sp. *Proc. Int. Red. Tide Symp.* Takamatsu, Japan.

Nizan, S., Dimentman, C. and Shilo, M. (1986). Acute toxic effects of the cyanobacterium *Microcystis aeruginosa* on *Daphnia magna* . *Limnol. Oceanogr.* **31**, 497-502.

Oudra, B., Loudiki, M., Sbiyyaa, B., Vasconcelos, V., Zerrouk, H., El andaloussi, M.D. and Darley, J. (1998a). Occurrence of hepatotoxic *Microcystis aeruginosa* blooms in eutrophic Moroccan lake – reservoir. *Harmful Algae*, Reguera, B., Blonco, J., Fernadez, M.L. and Wyatt, T. (eds.). Xunta de Galicia & Intergovernmental Oceanographic Commission of UNESCO, 29-31p.

Oudra, B., El andanloussi, D.M., Franca, S., Martins, R., Vasconcelos, V., Oufdou, K., Sbiyyaa, B., Loudiki, M. and Mezrioui, N. (1998b). Toxicological study of Picocyanobacterium *Synechocystis* isolated strain. *4th International conference on Toxic Cyanobacteria (Compilation of Abstracts)*, 27 September-1 October 98, Beaufort, North Carolina, USA.

Oufdou, K., Mezrioui, N., Oudra, B., Barakate, M. and Loudiki, M. (1998a). Effect of extracellular and endocellular products from cyanobacterium, *Synechocystis* sp., on the growth of some sanitation system bacteria. *Arch. Hydrobiol. (Algological Studies 90)*, 139-148.

Pearl, H.W. (1988). Growth and reproductive strategies of freshwater blue-green algae (cyanobacteria). –In: *Growth and Reproduction Strategies of Freshwater Phytoplankton*. D. Craig, Sandgren, (Ed.), Cambridge Univ. Press., 261-315. Pick, F.R. (1991). The abundance and composition of freshwater picocyanobacteria in relation to light penetration. *Limnol. Oceanogr.*, **36**, 1457-1462.

Reinikainen, M., Kiviranta, J., Ulvi, V. and Niku-Paavola, M.L. (1995). Acute toxic effects of a novel cyanobacerial toxin on crustaceans *Artemia salina* and *Daphnia pulex*. *Arch. Hydrobiol.*, **133**, 61-69.

Sbiyyaa, B., Oudra, B., Loudiki, M., Bouguerne, A. and Tifnouti, A. (1997). Acute toxicity of *Microcystis aeruginosa* KÜTZ. to three cladoceran species. In: *Harmful Algae*, Reguera, B., Blonco, J., Fernadez, M.L. and Wyatt, T. (Eds) Xunta de Galicia & Intergovernmental Oceanographic Commission of UNESCO,382-385.

Stramach, K., (1966). Cyanophyta-Sinice Glaucophyta-Glankofity. *Flora slodkowodna Polski*, Tom2, Warszawa p.807

Tero, K., Ito, E., Yanagi, T. and Yasumoto, T. (1986). Histopathological studies on experimental marine poisoning. I-Ultrastructural changes in small intestine and liver of suckling mice induced by dinophysitoxin-1 and pectenotoxin-1 . *Toxicon.*, **24**, 141-1151.

Tifnouti, A. and Pourriot, R. (1989). Dynamique d'une population de *Moina micrura* (Crustacea, Cladocera) dans un bassin de lagunage à Marrakech (Maroc). *Rev. Hydrobiol. Trop.* **22**, 239-250.

Watanabe, M.F., Harada, K., Carcichael, W.W. and Fujiki, H. (1996). *Toxic Microcystis*. CRC Press, Inc. USA.

Wong, Y.S. and Tam, N.F.Y. (1998). *Wastewater Treatment with Algae*. Springer-Verlag Landes Bioscience Georgetown, TX, USA.