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Taxonomy and geographic distribution of potential toxic cyanobacterial strains in Morocco

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In Morocco, poisoning events of fish, aquatic birds and livestock have been observed in some reservoirs and shallow lakes. In all cases, the reasons for animal mortality reasons have not been confirmed and the toxic cyanobacteria strains that were abundant in these water bodies have been suggested as their cause. Since 1994, the authors started taxonomic, ecological and toxicological studies by collecting samples from various lakes reservoirs and ponds. The results show that more than 18 out of 26 lakes reservoirs used for human water supply contained at least one species of planktonic cyanobacteria, where the genus *Microcystis* was dominant (*Microcystis aeruginosa* f. *aeruginosa*, *M. aeruginosa* f. *flos-aquae*, *M. ichthyoblabe*, *M. pulverea* f. *delicatissima*) and was associated with *Oscillatoria, Planktothrix, Anabaena, Aphanizomenon, Phormidium* and other genera. Taxonomic studies shows that among more than 150 cyanobacteria taxa identified in Moroccan freshwater ecosystems, 35 are potentially toxic. For several species, the toxicity of both natural blooms and isolated strains lyophilized material was confirmed by mouse bioassay and the microcystins content evaluated by the Enzyme-linked immunosorbent assay (ELISA). A map of geographic distribution of *Microcystis* strains inventoried in moroccan water bodies is presented.

Taxonomie et distribution géographique des Cyanobactéries potentiellement toxiques au Maroc

Mots-clés : Cyanobactéries, blooms, taxonomie, toxicité, distribution, Maroc.

Les causes exactes des cas d'intoxications de poissons, d'oiseaux aquatiques et de bétail, souvent observés dans quelques réservoirs et lacs peu profonds du Maroc, n'ont pas été définitivement établies. Les cyanobactéries qui prolifèrent régulièrement dans les plans d'eau concernés ont été cependant largement suspectés. Les études taxonomiques, écologiques et toxicologiques menées depuis 1994 sur plusieurs lacs de barrages montrent que parmi les 26 lacs utilisés pour l'approvisionnement en eau potable, 18 contiennent au moins une espèce de cyanobactérie potentiellement toxique et le genre *Microcystis* est largement dominant (*Microcystis aeruginosa* f. *aeruginosa*, *M. aeruginosa* f. *flos-aquae*, *M. ichthyoblabe*, *M. pulverea* f. *delicatissima*). Parmi 150 taxons de cyanobactéries inventoriés dans divers plans d'eau du Maroc, 35 sont répertoriés comme étant potentiellement toxiques. Pour certaines espèces, la toxicité des extraits du matériel algal lyophilisé à partir des blooms naturels ou des souches isolées a été confirmée par le test souris et les microcystines quantifiées par la technique d'analyse ELISA. Au terme de cette étude une carte de distribution géographique des souches de *Microcystis* potentiellement toxiques est proposée.

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1. Introduction

In order to reach its ever-increasing water requirements, Morocco has now an important infrastructure of dams (More than 90 reservoirs with a storage capacity of 14 billion m3). Actually, surface waters represent more than 70 % of water supply, partly provided by 26 dams some of them devoted only to the human use.

The recent studies of many moroccan reservoirs showed their rapid eutrophication due to high nutrient loads and semi-arid climate conditions (Derraz 1995, Alaoui et al 1996, Mouhri et al 1999, Cherifi & Loudiki, 1999). The main eutrophication symptom is the rapid growth of algae especially cyanobacteria which usually forms heavy waterblooms. The sanitary risks and the effects of these blooms on water quality are now well known particularly in countries where drinking water reservoirs are contaminated by toxic cyanobacteria.

In Morocco, toxic cyanobacteria blooms are common in some water bodies used for recreational and / or drinking water reservoirs. Poisoning events of fish, aquatic birds and livestock have been observed during late summer in some moroccan reservoirs and natural ponds (ONEP 1996). But in all cases, the animal mortality reasons have not been confirmed in spite of the fact that both the oxygen low concentrations and particularly the abundant cyanobacteria strains in these water bodies have been registered. In order to establish a screening of potential toxic cyanobacterial strains in Morocco and get data about toxic cyanobacteria and their potential sanitary risks in some reservoirs and shallow lakes, a research program is carried out in the laboratory of Phycology, Faculty of Sciences Semlalia, Marrakesh in collaboration with other national and international partners.

The taxonomic, ecological and toxicological studies of cyanobacteria were undertaken since 1994 by collecting environmental samples and producing isolated strains in laboratory.

This paper provides a first screening of the potential toxic strains of cyanobacteria as well as their geographical distribution in some moroccan waterbodies. Furthermore, other data about ecological characteristics and toxicity of some collected *Microcystis* natural blooms and isolated strains are presented.

2. Material and methods

The study of phytoplankton and sampling of cyanobacterial blooms were undertaken since 1994 in various freshwater reservoirs and natural lakes of Morocco. The set of 42 natural samples of planktonic cyanobacteria species was studied under the light microscope. Cyanobacteria bloom samples and isolated strains were observed, measured and identified by morphology-based taxonomy using the systems of Starmach (1966), Komarek & Anagnostidis (1986) and Anagnostidis & Komarek (1988).

In order to examine the pluriannual dynamics and the importance of cyanobacterial biomass in the phytoplankton communities and to describe the relation between environmental factors and cyanobacterial growth, a short term survey of the waterblooms was done over several years in two lake-reservoirs Lalla Takerkoust (Loudiki et al. 1997, Oudra et al. 1998) and Oued Mellah (Sabour et al. 2002). In these reservoirs, water stock is used for irrigation, watering of livestock, recreational activities, fisheries and public water supply.

To assess the toxicity of cyanobacterial, natural blooms samples were concentrated with a 27 µm mesh plankton net, freeze-dried and stored frozen at -20°C. The isolation and culture of cyanobacterial strains was made on Z8 medium under laboratory controlled conditions (Kotai 1972). The toxicity of cyanobacterium lyophilized material was confirmed by mouse bioassay according methods previously described in Oudra et al. (1998). The amount of total microcystins was determined by the Enzyme-linked immunosorbent assay (ELISA) using Millipore Microplate Kit (EnviroGard® Microcystins Plate Kit) according to methods described by An & Carmichael (1994).

3. Results and discussion

The sampling and taxonomic study of cyanobacteria species in various Moroccan stagnant waters, associated with inventory investigations of previous work, enabled us to establish a first screening of planktonic potentially toxic cyanobacteria strains. The results show that among the 150 taxa of cyanobacteria catalogued until now in moroccan freshwater bodies (Gayral 1954, Loudiki 1990), 35 are frequently quoted by the literature as being potentially toxic (Table 1). Among the inventoried cyanobacteria genera *Anabaena, Oscillatoria, Planktothrix, Aphanizomenon, Chroococcus* and *Phormidium*, *Microcystis* constitutes the most widespread one. Regularly present in many aquatic environments of Morocco, *Microcystis* often forms spectacular blooms in certain eutrophic water reservoirs. *Microcystis* blooms were reported for the first time in natural ponds (Gayral 1954) and in El Kansera and

Table 1. Distribution of potential toxic cyanobacterial strains in Moroccan reservoirs and natural ponds.

Tableau 1. Distribution des souches de cyanobactéries potentiellement toxiques dans les lacs de barrage et les plans d'eau naturels du Maroc.
Reservoirs : AF = Allal ElFassi ; AM = Al Massira ; DA = Daourat ; EK = ElKansera ; HI = Hassan I ; IM = Imfout ; LT = Lalla Takerkoust ; ME = Mansour Eddahbi ; OE = Oued El Makhazine ; OM = Oued Mellah ; SA = Sahela ; SMBA = Sidi Med Ben Abdellah ; YT = Youssef Ben Tachfine ; IB = Ibn Battouta ; MV = Mohamed V ; AB = Abdelmoumen ; MK = Mohamed ben abdelkrim Khattabi ; II = Idriss I
Natural ponds : AA = Aguelmane Azigza ; ASA = Aguelmane Sidi Ali ; TI = Tigalmamine ; DE = Dayèt Erroumi ; SB = Sidi Bou Ghaba
References : 1- Bouhaddioui (1997) ; 2- Derraz (1995) and ONEP (1996) ; 3- Loudiki (1990) ; 4- Oudra et al.(1998, 2001) ; 5- ONEP (1996) ; 6- Ghedda (1997) ; 7- Gayral (1954)

	Reservoirs																		Natural Ponds				
Locality	AF	AM	DA	EK	HI	IM	LT	ME	OE	OM	SA	SMBA	YT	IB	MV	AB	MK	II	AA	ASA	TI	DE	SB
Strains Reference	1			2	3		4	5	5		6	5	5	5	5	5	5		7				7
Anabaena flos-aquae		x										x											
Anabaena variabilis		x	x	x		x	x		x			x	x									x	
Anabaena sp.		x		x	x		x		x	x		x				x		x	x			x	
Anabaenopsis circularis				x																			x
Aphanizomenon flos-aquae		x		x	x		x	x	x	x		x	x	x			x						
Aphanocapsa pulchra																				x		x	
Aphanocapsa sp.		x																					
Cylindrospermum sp.										x													
Cy. minutissimum																			x				
Coelosphaerium naegelianum		x					x					x							x				
Chroococcus minutus		x					x		x			x							x			x	
Chroococcus turgidus		x	x	x			x	x	x			x	x						x	x			x
Gomphosphaeria aponina		x			x							x											
Gleocapsa sp.				x																			x
Lyngbya majuscula																							x
Lyngbya sp.		x					x															x	x
Microcystis aerug. f. aerugi.	x	x	x	x		x	x		x		x	x	x	x				x	x	x		x	x
M. aeruginosa f. flos-aquae		x		x		x	x		x			x											x
M. ichtyoblabe										x													
M. pulverea																							x
M. pulverea f. delicatissima										x													
M. wesenbergii																					x		
Microcoleus minimus																			x				
Nodularia spumigena																							x
Plankthothrix agardhii		x		x					x	x		x										x	
Oscillatoria Formosa				x																			
Plankthothrix Mougeotii		x		x			x	x	x	x		x	x	x	x	x		x					
Oscillatoria Tenuis				x						x													
Pseudanabaena muscicola	x	x	x	x		x	x																
Pseudanabaena balatonica																						x	
Spirulina sp.	x	x					x																
Synechococcus sp.											x												
Synechocystis sp.											x												
Schizothrix pulvinata															x					x			
Tolypothrix lanata																			x				

SMBA reservoirs (Gayral 1954, Abouzaid et al. 1984) and then in other eutrophic dams (Loudiki 1995, Derraz 1995, Sbiyyaa et al. 1998, Oudra et al. 1998). Actually, several *Microcystis* species cause regularly blooms during the summer and autumn in different drinking and/or recreational water reservoirs. More than 18 out of 26 lakes reservoirs used for human water supply contained at least one species of planktonic cyanobacteria, where the genus *Microcystis* was dominant (Table 1). The first recorded *Microcystis* species were reported in Middle Atlas natural lakes and El Kansera reservoir by Gayral (1954). Three taxa of *Microcystis* were identified : *Microcystis aeruginosa* Kütz., *M. flos-aquae* (Wittr.) Kirschn. and *M. pulverea* (Wood) Forti.

For a better understanding of the taxonomic entities of each species of the bloom-forming *Microcystis*, we collected samples from different reservoirs, natural lakes and ponds. The identification of the materials samples showed that at least six taxa of *Microcystis* are present in Morocco : *Microcystis aeruginosa*, f. *aeruginosa* Kütz., *M. aeruginosa*,. f. *flos-aquae* (Wittr.) Elenkin, *M. ichthyoblabe*, *M. wesenbergii* Kom., *M. pulverea* and *M. pulverea* f. *delicatissima* (West) Elenkin (Table 1). Among the five *Microcystis* taxa, two species were for the first time recorded in Morocco : *Microcystis wesenbergii* which was identified at Tigalmamine natural lake (Middle Atlas) in October 1998 and *Microcystis ichthyoblabe* which was identified at Oued Mellah brackish and hypertrophic reservoir in

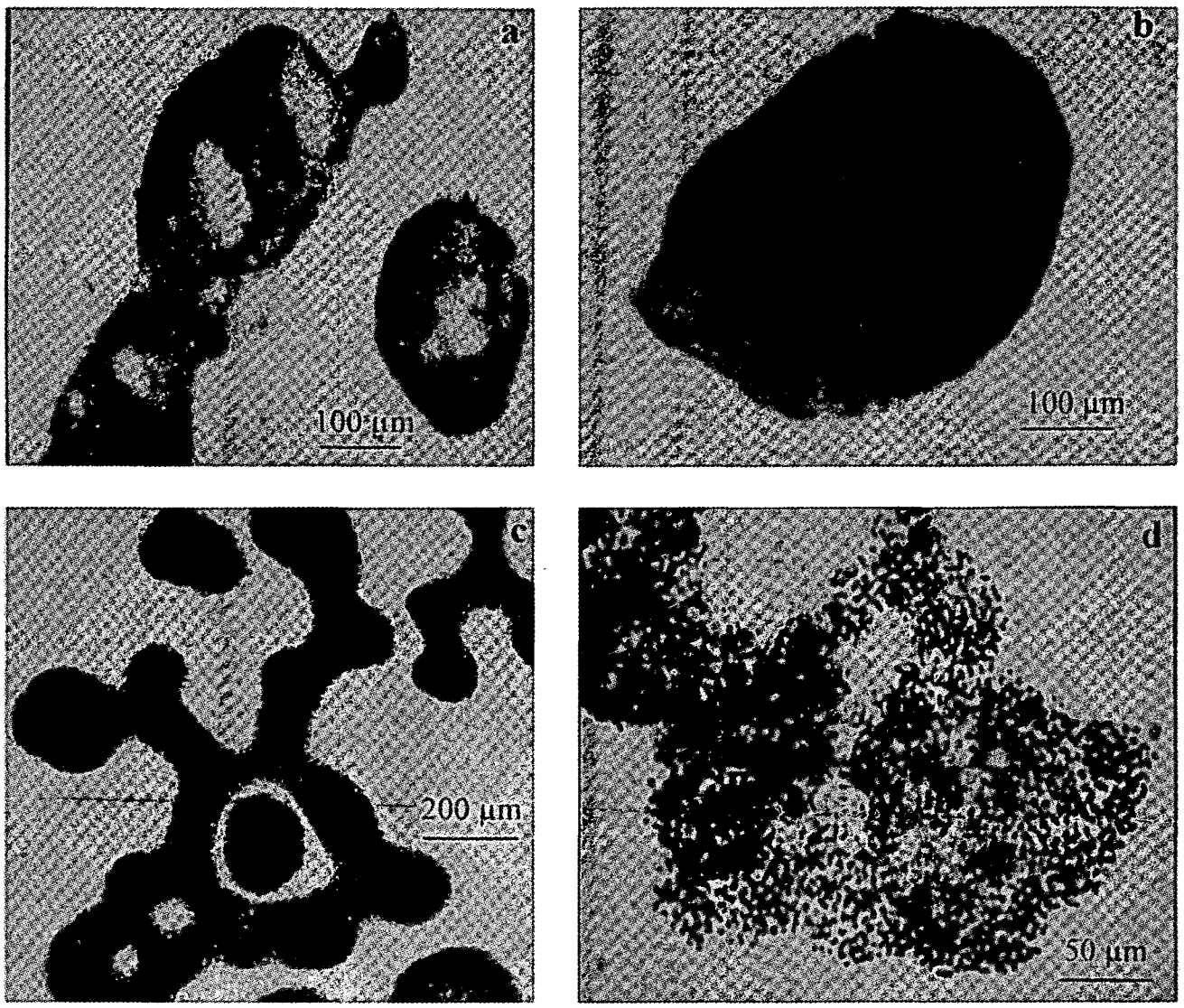

Plate 1. Light microscopy photographs of four *Microcystis* species : a) *M. wesenbergii* Kom. ; b) *M. ichthyoblabe* Kütz. ; c) *M. aeruginosa* f. *aeruginosa* Kütz. ; d) *M. aeruginosa* f. *flos-aquae* (Wittr.) Elenkin).

Planche 1. Photographies au microscope optique de quatre espèces de *Microcystis* : a) *M. wesenbergii* Kom. ; b) *M. ichthyoblabe* Kütz. ; c) *M. aeruginosa* f. *aeruginosa* Kütz. ; d) *M. aeruginosa* f. *flos-aquae* (Wittr.) Elenkin).

June 1999 (Plate 1). Some physico-chemical characteristics are given in table 2 for the two samples localities during *Microcystis* bloom-forming.

The colonies of M. wesenbergii are generally spheric or irregularly lobbed. Small cells (3 to 4 µm in diameter) are often gathered in one site of the colony's envelope and rich of gas vesicles. This explains the buoyancy of colonies on the lake surface as well as accumulation of scums especially in the sheltered areas. Whereas in *M. ichthyoblabe*, the colonies are rather soft, spongious and amorphous. The small cells (3 to 4 µm in diameter), rich of gas vesicles, are generally more pressed in the borders of colony and sometimes groups of cells are localised in mass of scarcely distribute cells. These observations correspond to the description given by Watanabe (1996) of these two species.

The analysis of the geographical distribution of the *Microcystis* strains shows that this cyanobacteria presents a large spatial distribution and it proliferates in all water bodies of Morocco (Fig.1). This observation confirms on the one hand the ubiquist character of the *Microcystis* genus and on the other hand the high trophic state of the majority of freshwater systems. The *Microcystis* proliferation shows favourable heliothermic and nutritional conditions which exist in these areas.

The toxicological study of *Microcystis* blooms that were collected in Takerkoust, Almassira and Oued Mellah reservoirs shows a positive hepatotoxicity. This toxicity was quantified both by LD_{50} and total amount of microcystins determination (Table 3). The obtained results show a great annual (case of Takerkoust lake) and spatial variability of toxicity and mi-

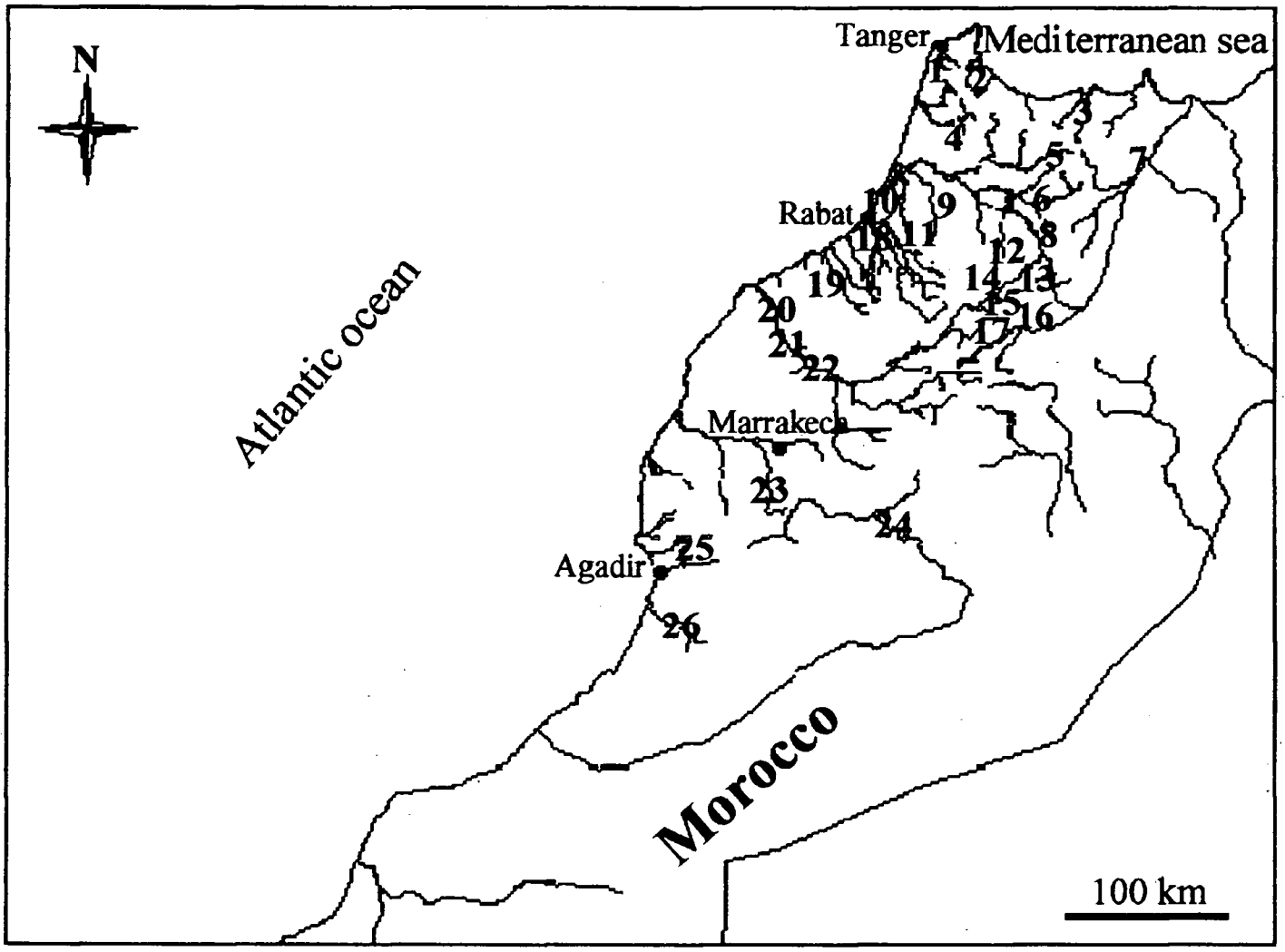

Fig. 1. Geographic localisation of some Moroccan reservoirs, natural lakes and ponds where *Microcystis* strains have been inventoried.
Reservoirs : 1- Ibn Battouta, 2- Smir, 3- M.B.A. Khettabi, 4- O. El Makhazine, 5- Sahela, 6- Idriss I, 7- Mohammed V, 8- Allal El Fassi, 9- El Kansera, 18- S.M.B. Abdellah, 19- Oued Mellah, 20- Daourat, 21- Imfout, 22- Al Massira, 23- Lalla Takerkoust, 24- M. Eddahbi, 25- Abdelmoumen, 26- Y.B. Tachfine.
Natural lakes and ponds : 10- Sidi Boughaba, 11- Dayèt Erroumi, 12- Dayèt Aoua, 13- Dayèt Affourgah, 14- Dayèt Ifrah, 15- Aguelmane Azigza, 16- Aguelmnae Sidi Ali, 17- Tiguelmamine.

Fig. 1. Localisation géographique des lacs de barrage et plans d'eau naturels du Maroc où des souches de *Microcystis* ont été inventoriées.
Lacs de barrage : 1- Ibn Battouta, 2- Smir, 3- M.B.A. Khettabi, 4- O. El Makhazine, 5- Sahela, 6- Idriss I, 7- Mohammed V, 8- Allal El Fassi, 9- El Kansera, 18- S.M.B. Abdellah, 19- Oued Mellah, 20- Daourat, 21- Imfout, 22- Al Massira, 23- Lalla Takerkoust, 24- M. Eddahbi, 25- Abdelmoumen, 26- Y.B. Tachfine.
Plans d'eau naturels : 10- Sidi Boughaba, 11- Dayèt Erroumi, 12- Dayèt Aoua, 13- Dayèt Affourgah, 14- Dayèt Ifrah, 15- Aguelmane Azigza, 16- Aguelmane Sidi Ali, 17- Tiguelmamine.

crocystins content. The *M. aeruginosa* blooms of Takerkoust reservoir appear to be the most toxic ones with a LD_{50} ranged between 2 and 83 $mg.kg^{-1}$ and microcystins content ranging also between 496 and 8800 $\mu g.g^{-1}$. The relationship between toxicity and amount of hepatotoxins have been previously discussed in Oudra et al. (2001). Whereas the extract of *M. ichthyoblabe* that occurred in Oued Mellah lake are less toxic with LD_{50} up to 500 $mg.kg^{-1}$ and total microcystins of 0.79 $\mu g.g^{-1}$. For the mesotrophic Almassira reservoir *Microcystis* bloom showed a medium toxicity level with LD_{50} of 142 $mg.kg^{-1}$ and 0.37 $\mu g.g^{-1}$ of Microcystins. These results agree with literature data which made many important spatio-temporal fluctuations of *Microcystis* strains toxicity only expressed under well determined conditions (Carmichael 1996, Watanabe 1996).

The toxicological study of the *Microcystis* isolated strains cultivated on Z8 medium shows that all studied strains are also hepatotoxic. The spatio-temporal fluctuations remain less important than those observed for the natural blooms (Table 4).

Table 2. Physical and chemical caracteristics of Oued Mellah and Tigalmamine lakes during *Microcystis* bloom-forming.

Tableau 2. Caractéristiques physiques et chimiques des lacs Oued Mellah et Tigalmamine en période de bloom à *Microcystis*.

	Oued Mellah reservoir	Tigalmamine lake
Elevation (m)	92	1626
Date of construction	1928-1931	natural
Geographic situation	33°30'N – 07°20'W	32°54'N – 05°21'W
Substratum	Primary schist with triasic saliferous clays	Lower Lias dolomite
Bioclimat	Semi-arid	Humide
Surface (ha)	250	6
Maximum depth (m)	9	16
Total capacity (Mm^3)	18	
Water temperature (°C)	25	18
pH	8.3	8.6
Dissolved oxygen ($mg.L^{-1}$)	8.1	8.8
Conductivity ($\mu s.cm^{-1}$)	4000	794
Chlorides ($g.L^{-1}$)	4.8	0.070
Salinity (‰)	6.6 - 9	

The toxicity studied by mouse bioassay and ELISA analysis showed that the *Microcystis* natural blooms were generally more toxic than the culture of isolated strains. This difference was due to the cyanobacteria growth conditions (Watanabe & Oishi 1985) or to the natural bloom contamination (containing also other cyanobacteria toxic species like *Phormidium, Pseudanabaena, Oscillatoria, Planktothrix*).

4. Conclusion

The results obtained during this study indicate that toxic cyanobacteria waterblooms may be common in many eutrophic reservoirs and natural ponds in Morocco. More than 18 out of 26 lake reservoirs used for human water supply contained at least one species of planktonic cyanobacteria, where the *Microcystis* species constitute the most widespread cyanobacterium. Most of the toxic blooms in drinking water reservoirs may represent an environmental health hazard to animals and humans.

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Table 3. Data on toxic *Microcystis* waterblooms in Morocco.
Tableau 3. Données sur les blooms toxiques au Maroc.

Localities	Waterblooms Dominant species	Sampling Periods	i.p. Mice bioassay LD_{50} (mg/kg)	Average amount of microcystins (µg/g)
Lalla Takerkoust reservoir (Marrakech)	*Microcystis aeruginosa* f. *aeruginosa* associated with *Microcystis aeruginosa* f. *flos-aquae*	1994	4	710
		1995	2	8800
		1996	20	2050
		1997	73	1920
		1998	83	664
		1999	34	496
Almassira reservoir (Settat)	*Microcystis aeruginosa* f. *aeruginosa* associated with *Microcystis aeruginosa* f. *flos-aquae*	1999	142	0.37
Oued Mellah Reservoir (Casablanca)	*Microcystis ichthyoblabe*	1999	502	0.79

Table 4. Data on toxic Microcystis isolated strains in Morocco.
Tableau 4. Données sur les isolats de souches toxiques de *Microcystis* au Maroc.

Localities	Cyanobacteria strains	Dates of isolate	i.p. Mice bioassay LD_{50} (mg/Kg)	Average amount of microcystins (µg/g)
Lalla Takerkoust reservoir (Marrakech)	*Microcystis aeruginosa f. aeruginosa :*			
	-FSMT-1	July 1994	33	650
	-FSMT-2	August 1997	nd	130
Almassira reservoir (Settat)	*Microcystis aeruginosa f. aeruginosa :*			
	-AM-1	September 1997	nd	190
	-AM-2	July 1998	238	nd
	- AM-3	November 1999	nd	nd
Deroua fish pond (Béni-Mellal)	*Microcystis aeruginosa f. aeruginosa :*			
	-FP-1	October 1997	nd	nd
	-FP-2	July 1998	108	943.7
Oued Mellah reservoir (Casablanca)	*Microcystis ichthyoblabe* :			
	-OM-1	July 1997	nd	8.5
	-OM-2	June 1998	nd	10.48
	-OM-3	June 1999	nd	5.4

nd : not determined

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