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Table of Content

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Full Length Research Paper

Characterization of cyanobacteria microcystins (cyanotoxins) blooming in the Dams of Northern Morocco

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Cyanobacteria thrive in eutrophic freshwaters and impose a serious problem for the management of water bodies. Some Cyanobacteria species impose even a risk for public health due to the production of intracellular toxins. This study is a qualitative approach to determine the degree of toxicity and the toxicological aspect of cyanotoxins in order to setup a monitoring program for cyanobacteria blooms and the management of cyanotoxins thriving in three water bodies in Northern Morocco. Water samples were collected from three major water reservoirs/dams near the city of Tétouan (SMIR, BELMEHDI and NAKHLA). These water samples were screened for possible Cyanobacteria using specific culture media (BG13 & Z8). Three cyanobacteria species (*Microcystis aeruginosa*, *Pseudanabaena galeata* and *Oscillatoria tenuis*) were isolated, purified and lyophilized. Using gas chromatography coupled with mass spectrometry, nine types of microcystins were characterized namely: (MC-LR); (MC-YR); (MC-LA); (MC-FR); (MC-RF), [Mser[7]]MC-LR; [Dha[7]]MC-LR; MC-YAba; and [Mser[7]]MC-YR. Our results strongly recommend and urge different stakeholders to consider the various health risks potentially generated by these toxins during water use and management. In addition, this study is a contribution to raise awareness of the toxicological aspect of the cyanobacteria inhabiting the water bodies of Northern Morocco.

Key words: Blue algae, gas chromatography coupled to mass spectrum, bio-toxins, water dam.

INTRODUCTION

The problem of water quality degradation in dams and reservoirs is due essentially to different sources of pollution that cause nutritive elements (nitrogen and phosphorus) enrichment causing the anarchic

development of algae, which indicates an advanced state of water quality degradation. Furthermore, soil erosion brings additional elements that may accelerate the alteration of water quality (Issaka and Ashraf, 2017; Rose et al., 2010). The ecosystem imbalance caused by such phenomenon promotes the development of algae, in particular blue algae (cyanobacteria) which are responsible for the organoleptic and esthetic alteration of water, as well as the production of cyanotoxins within these waters (self-purification phenomenon).

Previous works on lakes and reservoirs located in warm climate zones, such as dams in the Mediterranean, show that the latter are distinguished by particular hydrological, physico-chemical and biological characteristics (Loudiki, 1990; Loudiki et al., 1994; Cherifi and Loudiki, 2002). Among the determining factors, the unpredictability of the climate (flash floods, droughts and very variable low water levels) and the irregularity of rainfall and erosion materials play a predominant role.

Morocco is a Mediterranean country characterized by a semi-arid climate (Perrin et al., 2014, Ouhamdouch et al. 2019), with clear spatiotemporal disparities in rainfall towards the southern region of the country. Moreover, the country is likely to experience 20% on average net reduction in rainfall by the end of this century (IPCC, 2007); this will boost cyanobacteria in inland water bodies such as dams (Gophen, 2021).

In Morocco, the supply of drinking water is mainly ensured by rainfall collected in water reservoirs or dams. This strategic approach was adopted since the 1940s, in order to mobilize water resources through the construction of several large dams to provide drinking water and other services (El Ghachtoul et al., 2005). Nevertheless, these dams recognize in the summer period phenomena of eutrophication due to nutrient enrichment mainly nitrogen and phosphorus (El Ghachtoul et al., 2005).

In fact, recent climate change and anthropogenic impact on water environment by either intense withdrawal and diversion or chemical pollution and nutrient enrichment promoted a worldwide proliferation of cyanobacteria blooms often harmful to ecological and human health (Paerl, 2016). Certainly, the massive proliferation of cyanobacteria in dam waters is increasingly frequent phenomenon worldwide (Huisman et al., 2018), accompanied by the release of toxic substances in the form of secondary metabolites (cyanotoxins). These cyanotoxins cause harmful ecological, health and socio-economic effects leading to a degradation of water quality and a reduction in the productivity of the aquatic environment (Wiegand and Pflugmacher, 2005; Jacoby and Kann, 2007; Plaas and Paerl, 2016).

Most cyanotoxins are called Microcystins. According to some studies the first Microcystin was isolated from *Microcystis aeruginosa* (Carmichael, 1992a; Namikoshi et al., 1992, 1995). Dittmann and Börner (2005) and Hotto et al.

(2007) indicated that Microcystins are toxins produced by the most common cyanobacteria and are present in most of the world's water reservoirs. These cyanotoxins are structurally cyclic heptapeptide amino acids (Van Apeldoorn et al., 2007; Shimizu, 2014). According to the structure of Microcystins established by Chorus et al. (1999) the toxicity role of the acid group (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) recognized under the name Adda is primordial. In addition, several studies reveal the carcinogenic potential of Microcystins under long-term exposure (Yu, 1995; Codd, 2000; Carmichael et al., 2001; ADH, 2006).

Different Microcystins have been isolated from several species of cyanobacteria and several of them can be produced by a single bloom (Zastepa et al., 2017). Studies to date have focused solely on assessing the level of cyanotoxins in blooms rather than characterizing the water-soluble fractions of the toxins (Ai et al., 2020). In this context, this study is a qualitative approach to determine the degree of toxicity and the toxicological aspect of cyanotoxins in the studied reservoirs in order to setup a monitoring program for cyanobacteria blooms and the management of cyanotoxins produced in water bodies in Northern Morocco. The specific objectives are to extract and characterize the intracellular Microcystins using gas chromatography coupled with mass spectrometry analysis.

MATERIALS AND METHODS

Study site

Water samples were collected from three major water bodies supplying drinking water to the city of Tetouan (northern Morocco). The three dams are Smir (35°40'46.9 "N 5°23'33.8 "W) (Figure 1a), Belmehdi (35°42'33.2 "N 5°30'28.0 "W) (Figure 1b) and Nakhla (35°42'01.9 "N 5°30'11.7 "W) (Figure 1c). Their characteristics are noted in Table 1. These reservoirs are experiencing the phenomenon of eutrophication due to the accumulation of two highest nutrients (nitrogen and phosphorus), which lead to the proliferation of cyanobacteria blooms, a sign of water quality degradation.

Sampling method

Sampling was performed in the morning (around 10:00 am) on a monthly basis, from June to December 2017. Three sampling points were selected separately following the long transect of the reservoir lake. We collected approximately 50 liters of surface water from each water reservoirs. The water samples were carried in sterilized special plastic laboratory bottles of 5 L. To allow a good conservation, these bottles were transported in a cooler box of 4°C. These raw water samples were used for isolation and purification of cyanobacteria, which requires a preliminary culture in synthetic media (Saoudi, 2008).

Culture media and Isolation conditions

Two culture media BG13 (Ferris and Hirsch, 1991) and Z8 (Kotai, 1972; Rippka et al., 1979) were prepared in liquid and solid forms,

Figure 1. Map showing geographic location of the three dams SMIR, BELMEHDI et NAKHLA in city Tetouan Northen morocco. Geographical Distribution and Location of Water Bodies and Sample Site (**a)** SMIR (**b)** Belmehdi (**c)** Nakhla sampling locations.

Table 1. Morphometric and hydrological characteristics of the study reservoir lake.

Characteristics	Water reservoirs		
	Nakhla	**Smir**	**Belmehdi**
Filling reservoir with water	1961	1991	2005
Reservoir Headwater Level	-	43,5	49
capacity (mm3)	4,3	43	28,8
Annual flow of water (Mm3)	10	31	16
Area (Km²)	110	4	4,8
Maximum depth of reservoir (m)	15	30	50
Function	Drinking water	Drinking water	Drinking water
Stream of water	Nakhla	Smir	Raouz
City	Tétouan	Tétouan	Tétouan

autoclaved for 20 min at 120°C and a pressure of 1.1 kg/cm². Afterwards, we added 100 mg of cycloheximide per litter to both liquid and solid culture media using a sterile syringe with a 0.2 µm porosity acrodisc, to remove most eukaryotes and obtain a monoalgal culture.

Water samples were filtered through Millipore membranes with a porosity of 45 µm by means of a pressurized vacuum pump (Figure 2). These membranes were transferred into two liquid media BG13 (Ferris and Hirsch 1991) and Z8 (Kotai, 1972; Rippka et al., 1979), with pH adjusted to 8, and incubated for three weeks under ambient temperature (20 - 25°C), fluorescent lamps of 2000 lumens intensity and a photoperiod of 12 h. Afterwards, these membranes were transferred into Petri dishes containing solid media (BG13 and Z8) for purification phase. After three weeks, a tiny fragment of the growing strain was transferred into new solid media, and this step is repeated until purified strains were observed. These purified strains were then transferred to sterile bottles containing 100 ml of liquid culture media (BG13 and Z8). These bottles were sealed with a stopper of 0.2 µm porosity filter to allow airflow. By repeating these manipulations 3 to 4 times, we obtained an axenic strain after 12 weeks. The purified strains would then be subject to a preliminary morphological identification. Once verified, we transferred the axenic strains to a vial containing 2L of sterile culture medium (BG13 and Z8) under illumination and continuous aeration to obtain a large mass of each strain (massification phase). Two weeks later, the biomass of each cyanobacterial strains collected during the massification phase was centrifuged (4000 g, 20 min), then lyophilized and stored at -20°C f or cyanotoxins extraction.
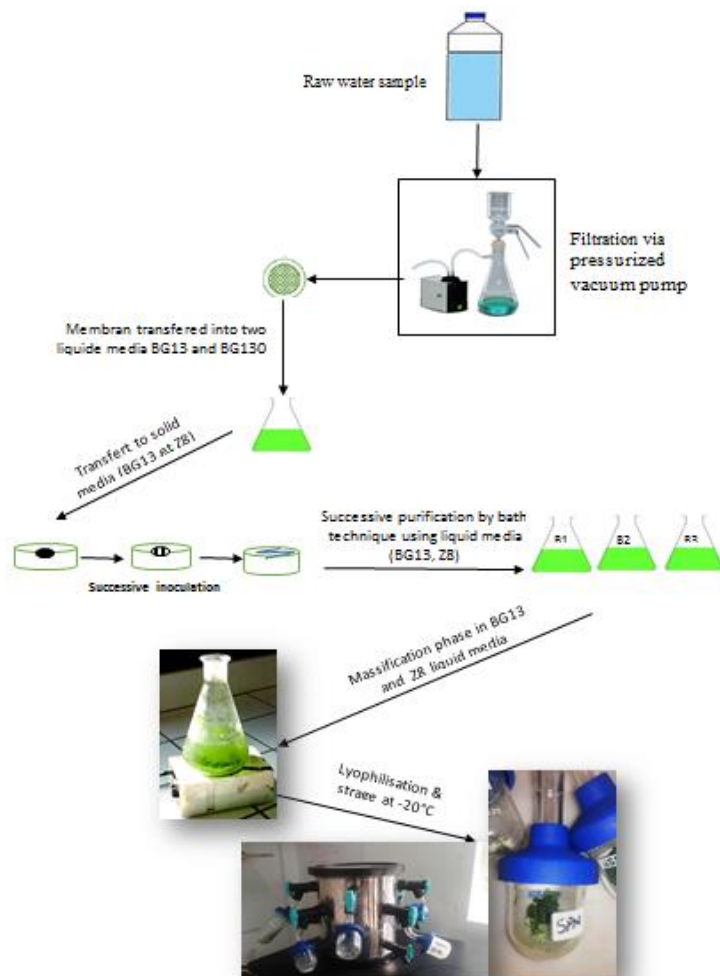
Figure 2. Culture and isolation method of cyanobacteria sampled from the studied raw water reservoirs.

All manipulations (transplantation, purification, and massification) were carried out under a laminar flow hood at 25°C. The materials necessary for these manipulations (Pasteur pipettes, platinum wire, and others) were sterilized. The species were observed, measured and morphologically identified using a light microscope according to criteria-based taxonomy using several specialized cyanobacterial florae and a multitude of works dealing specifically with these organisms (Bourrelly, 1985; Lund and Lund, 1995, Komárek and Anagnostidis, 2005; Komárek, 2016). This identification focused on the definition of many morphological criteria according to universally accepted identification keys:

(i) The color and structure of cyanobacteria (unicellular or colonial),
(ii) The shape of the colony or trichome,
(iii) The size of the cells
(iv) The presence or absence of gelatinous sheath (color, appearance and size), akinetes, heterocysts and gas vacuoles (pseudovacuoles).

Furthermore, the morphological identification was confirmed by analyzing the conservative fatty acid composition profile according to previous studies (Passaquet et al., 1989; Hayakawa et al., 2002; Thajuddin and Subramanian, 2005; Sharathchandra and Rajashekhar, 2011; Mahapatra and Ramachandra, 2013; Ouhsassi et al., 2017). The composition of fatty acid is used as a phylogenetic marker for cyanobacteria (Kenyon and Stanier, 1970; Murata et al., 1992; Sarsekeyeva et al., 2014; Los and Mironov, 2015).

Extraction and pre-purification of Microcystins

To extract and purify Microcystins, we followed the method described by Lawton et al. (1994). For each study site, the lyophilizate (250 mg) was recovered in the stationary phase from each pure culture. For each lyophilizate, the extraction is done three times with 70% methanol (Figure 3). After each extraction, the suspensions were centrifuged (4000 × g, 10 min, +4°C). The total extract was diluted with ultrapure water (milli-Q, Millipore) to obtain an extract with 20% methanol. For pre-purification of microcystins, the final extract was passed through an ODS silica gel column (Sep-Pak Vac C18, Waters Corporation, Milford, MA, USA). The last recovered fraction containing the microcystins is completely evaporated at 40°C, dissolved in 1 mL methanol /ultrapure water (50:50, v/v), and filtered through a 0.2 µm filter (Acrodisc, Nylon, Gelman Sciences Inc.) before being analyzed by gas chromatography coupled with a mass spectrometer (GC-MS).
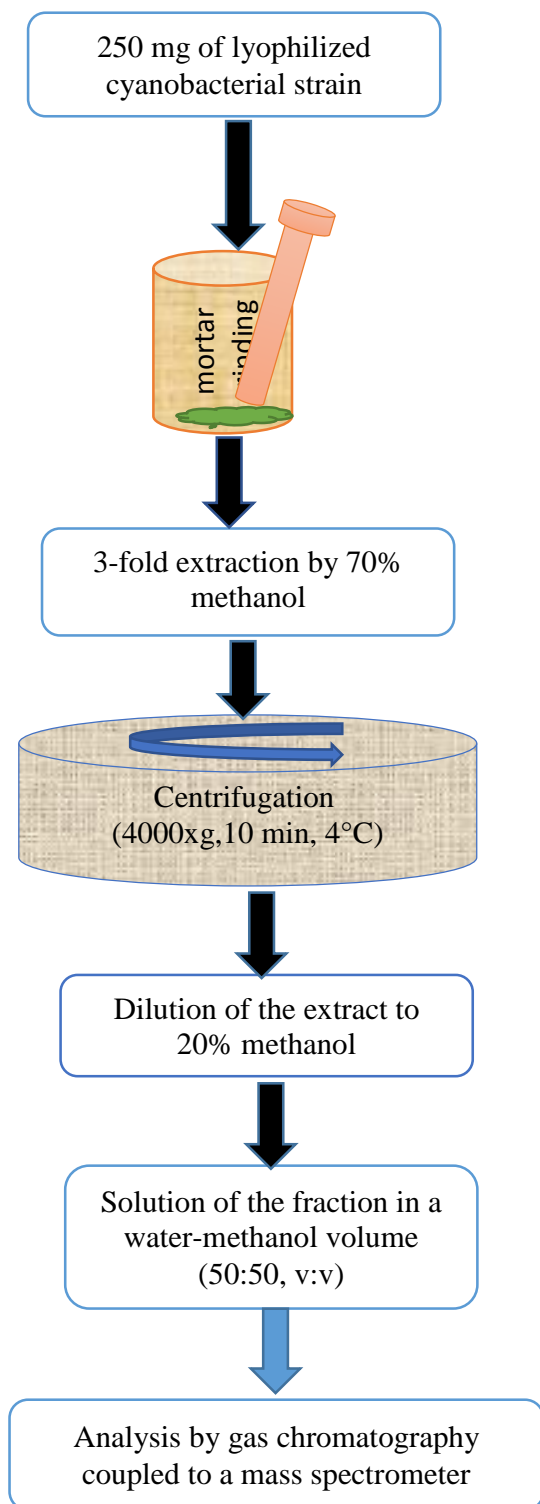
Figure 3. Protocol of the cyanobacterial microcystin extraction.

RESULTS

The morphological identification, confirmed by conservative fatty acid method, allows identifying three major cyanobacteria species with the same characteristics at the level of the studied water bodies (SMIR, BELMEHDI and NAKHLA):

Microcystis aeruginosa, belonging to Microcystaceae, isolated from Smir (1R), Belmehdi (4R) and Nakhla (7R), is a unicellular cyanobacterium with spherical colonies; it is grouped as an envelope and floated using gaseous vacuoles (Figure 4a).

Pseudanabaena galeata, belonging to Pseudanabaenaceae, isolated from SMIR (2R), BELMEHDI (5R) and NAKHLA (8R), is a filamentous cyanobacterium with solitary, mobile and without sheathing trichomes. The cells are distant from each other and joined by a gelatinous bridge. There are no kinetes or heterocysts (Figure 4b).

Oscillatoria tenuis, belonging to Oscillatoriaceae, isolated from SMIR (3R), BELMEHDI (6R) and NAKHLA (9R), is a filamentous cyanobacteria with a free, solitary and sheathless trichome (Figure 4c). The movement and helical displacement of the apex are characteristic of this genus. The analysis of GC-MS chromatograms revealed the presence of a wide variety of Microcystins in all studied cyanobacteria strains. The data (Tables 2 to 4) regroup Microcystins elaborated by *M. aeruginosa*, *P. galeata* and *O. tenuis* isolated from the three studied dams: SMIR, BELMEHDI and NAKHLA.

The chromatograms show the retention times recorded by different microcystins elaborated by *M. aeruginosa* (Figure 5), and they are:

(i) MC-LR and a demethylated form MC-YAab in the case of SMIR reservoir (Figures 6a and 6b);
(ii) MC-RF and two demethylated forms MC-YAab and [Dha^{7}]MC-LR in the case of BELMEHDI reservoir (Figures 9b to d);
(iii) MC-LA and a demethylated form MC-YAab in the case of NAKHLA reservoir (Figure 12b and c).

In the case of *P. galeata,* the retention times are shown respectively for the three studied dams (Figure 5). The elaborated Microcystins by this species are:

(i) MC-YR and a demethylated form [Mser^{7}]MC-YR (Figure 7a and b) in the case of SMIR reservoir;
(ii) MC-YR and a demethylated form MC-YAab (Figure 10a) in the case of BELMEHDI dam;
(iii) MC-LA and a demethylated form MC-YAba (Figure 13a and 13b) in the case of NAKHLA reservoir.

In the case of *O. tenuis, the* retention times recorded by different Microcystins are shown in Figure 5. The elaborated Microcystins are:

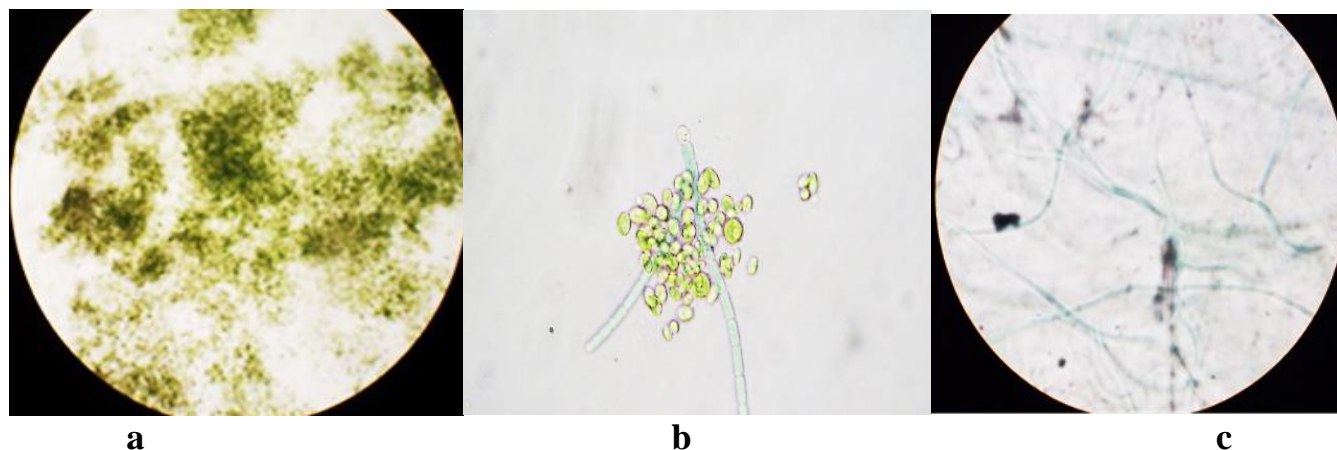(i) MC-LA and a demethylated form [Mser^{7}]MC-LR

Figure 4. Microscopic plates of cyanobacteria species: (**a**) *M. aeroginosa* (**b**) *P.galeata* (**c**) *O. tenuis* isolated from SMIR, BELMEHDI and NAKHLA dams.

Table 2. Variants of Microcystins elaborated by *Microsystis aeruginosa* on different water bodies.

Body of water	Analytical reference strain	Microcystin identified	m/z[M+H]+ calculated	m/z[M+H]+ measured
SMIR	1R	MC-LR	995.5	995.95
		MC-YAba	974.5	974.36
BEMEHDI	4R	MC-FR et MC-RF	1029.5	1029.64
		[Dha7]MC-LR	981.5	981.90
NAKHLA	7R	MC-YAba	974.5	974.36
		MC-LA	910.5	910.64
		MC-YAba	974.5	974.25

Table 3. Variants of Microcystins elaborated by *Pseudanabaena galeata* on different water bodies.

Body of water	Analytical reference strain	Microcystin identified	m/z [M+H]+ calculated	m/z[M+H]+ measured
SMIR	2R	MC-YR	1045.5	1045.06
		[Mser7]MC-YR	1063.84	1063.5
BELMEHDI	5R	MC-YAba	974.5	974.11
NAKHLA	8R	MC-LA	910.5	910.58
		MC-YAba	974.5	974.34

Table 4. Variants of Microcystins elaborated by *Oscillatoria tenis* on different water bodies.

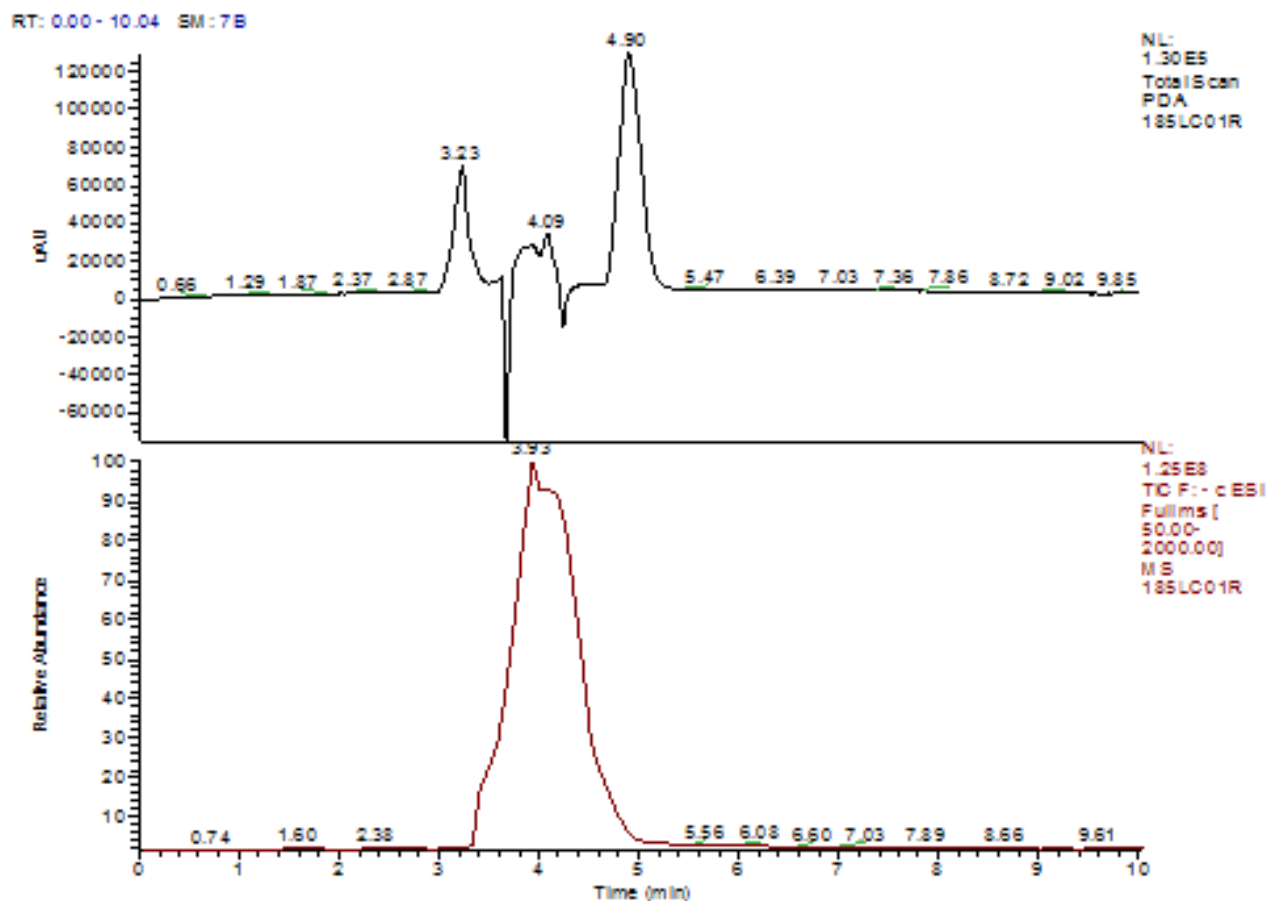| Body of water	Analytical reference strain	Microcystin identified	m/z [M+H]+ calculated	m/z [M+H]+ measured
SMIR	3R	[Mser7]MC-LR	1013.5	1013.23
		MC-LA	910.5	910.58
BELMEHDI	6R	MC-YAba	974.5	974.52
NAKHLA	9R	[Mser7]MC-LR	1013.5	1013.15
		MC-YAba	974.5	974.38

Figure 5. Chromatogram of retention time.

(Figure 8a and 8b) case of SMIR dam;
(ii) a single demethylated form MC-YAba (Figure 11a) case of BELMEHDI dam;
(iii) two demethylated forms MC-YAba and ([Mser[7]]MC-LR (Figure 14a and 14b) case of Nakhla dam.

DISCUSSION

Characterization of cyanotoxins has been performed (Poon et al., 1993; Diehnelt et al., 2005, 2006; Miles et al., 2013) using GC-MS or LC-MS or other mass spectrometry methods, which use precise mass measurements showing the connectivity of amino acids in different Microcystins. By standardizing the base peak intensity to 100%, the appearance of a mass spectrum becomes independent of the absolute amount of sample. Thus, mass spectra can be compared even when they have been generated from different sample quantities and/or different instruments. A list of m/z values (m = mass; z = atomic number) and intensity is useful for a more detailed analysis of a spectrum. The signal resulting from molecular dissociation has an ion mass (m/z) and a spectrum that normally reflects the corresponding

molecular ion, usually called a molecular ion peak. The accompanying signals represent ion fragments. For example, dissociation of MC-LR exhibits a molecular ion peak break ([M.H]+, 995.5546), dissociation of MC-RR exhibits a molecular ion peak break ([M.H]+, 1038.5), and dissociation of MC-YR exhibits a molecular ion peak break ([M.H]+, 1045.5358).

Some authors have shown that cyanobacteria blooms in Morocco appear in summer and reach their maximum proliferation in October with a significant annual variability in biomass. Sbiyyaa (1997) and Oudra et al. (1998) indicated that the main species responsible for blooms in Moroccan dams is attributed to three main species: *M. aeruginosa, M. aeruginosa flos-aquae,* and *P. muscicola.* (Malki, 1994) are reported from the Al Massira reservoir. *M. aeruginosa* proliferates regularly and dominates the phytoplankton each year between November and December with a toxic bloom of cyanobacteria. Oudra et al. (2002a) reported that several species of Microcystaceae often bloom in numerous dams, and are therefore the most studied and geographically the most distributed.

The toxicity of a single bloom is a function of time and space. In this study, the monitoring of cyanobacterial
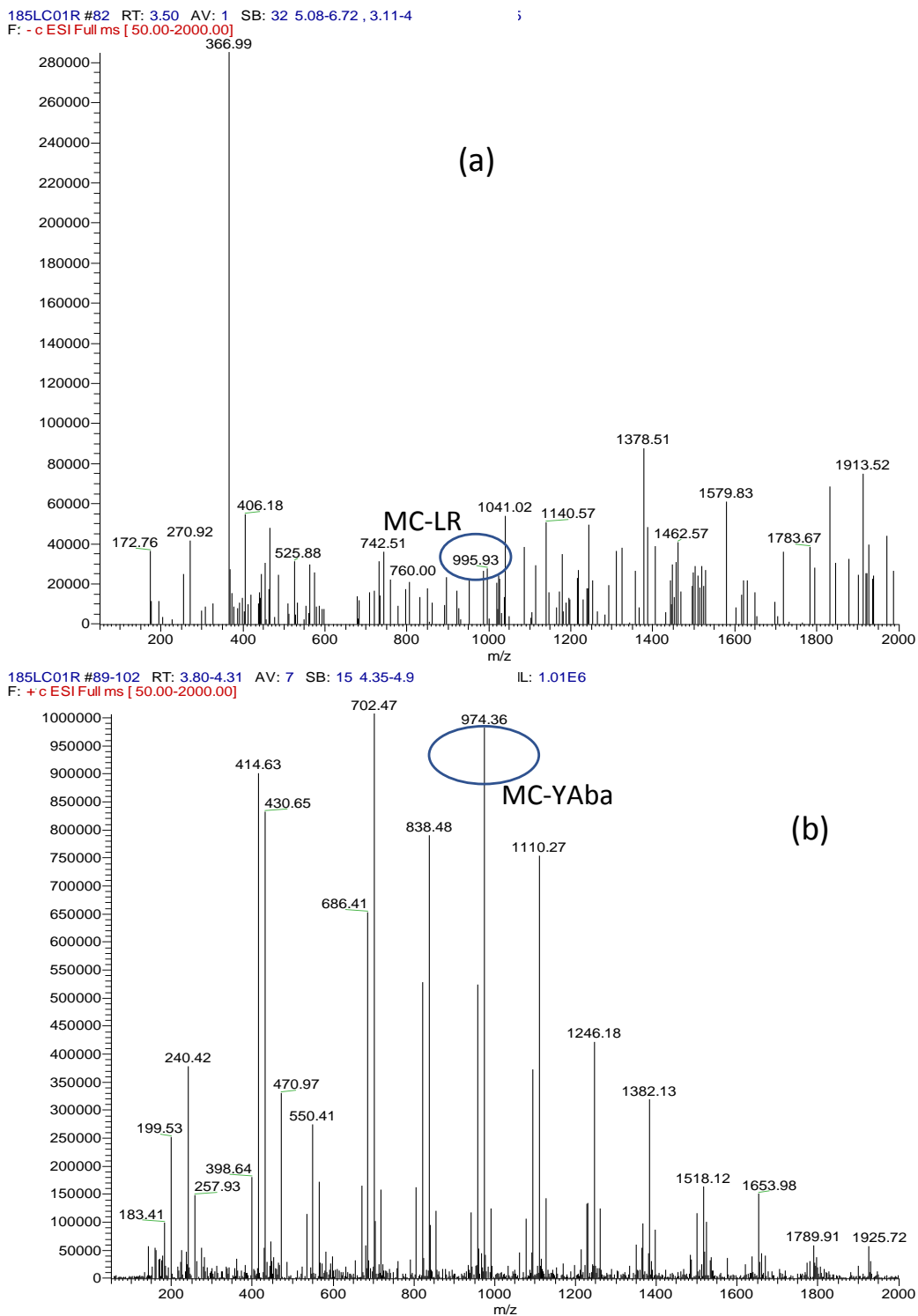
Figure 6. Analysis performed on extracts of cyanobacterial bloom *Microcystis aeruginosa* (1R) isolated from the waters of SMIR Dam. **(a, b)** Mass spectrum m/z showing the peak of the Microcystin fraction: MC-LR (995.93): [M+H]$^+$ and MC-YAba (974.36) : [M+H]$^+$.

strains toxicity revealed that the studied species generate a wide variety of cyanotoxins. Toxic blooms of the genus

Microcystis were demonstrated for the first time in the Oued el Mellah dam in 1997 (Loudiki et al., 2002). As

185LC02R #109 RT: 4.66 AV: 1 SB: 29 4.74-5.78 , 2.80~   :5
F: + c ESI Full ms [50.00-2000.00]

(a)

185LC02R #112 RT: 4.79 AV: 1 SB: 37 4.74-6.34 , 2.80~   :5
F: - c ESI Full ms [50.00-2000.00]

(b)

Figure 7. Analysis performed on extracts of cyanobacterial blooms *Pseudanabaena galeata* (2R) isolated from the waters of Smir Dam. **(a,b)** corresponding Mass spectrum m/z showing the peak of the Microcystin fraction: MC-YR (1045.06): [M+H]⁺. [Mser7]MC-YR (1063.84): [M+H]⁺.

well as in other Moroccan aquatic systems, particularly in dams intended to provide drinking water to urban populations. Species of this genus are isolated from other Moroccan water reservoirs, including Imfout, Takerkoust, and Almassira (Oudra et al., 2001a, 2001b; Loudiki et al., 2002; Sabour, 2002). This genus is known to form

cyanobacterial blooms in stagnant waters (Douma et al., 2010). In fact, in the studied water bodies, *Microcystis spp* produces more cyanotoxins compared to other species. Thus, *Microsystis aeruginsa* produces MC-LR and MC-YAba in SMIR dam; MC-FR, MC-RF, and [Dha7], MC-LR in BELMEHDI dam; and MC-YAba, MC-
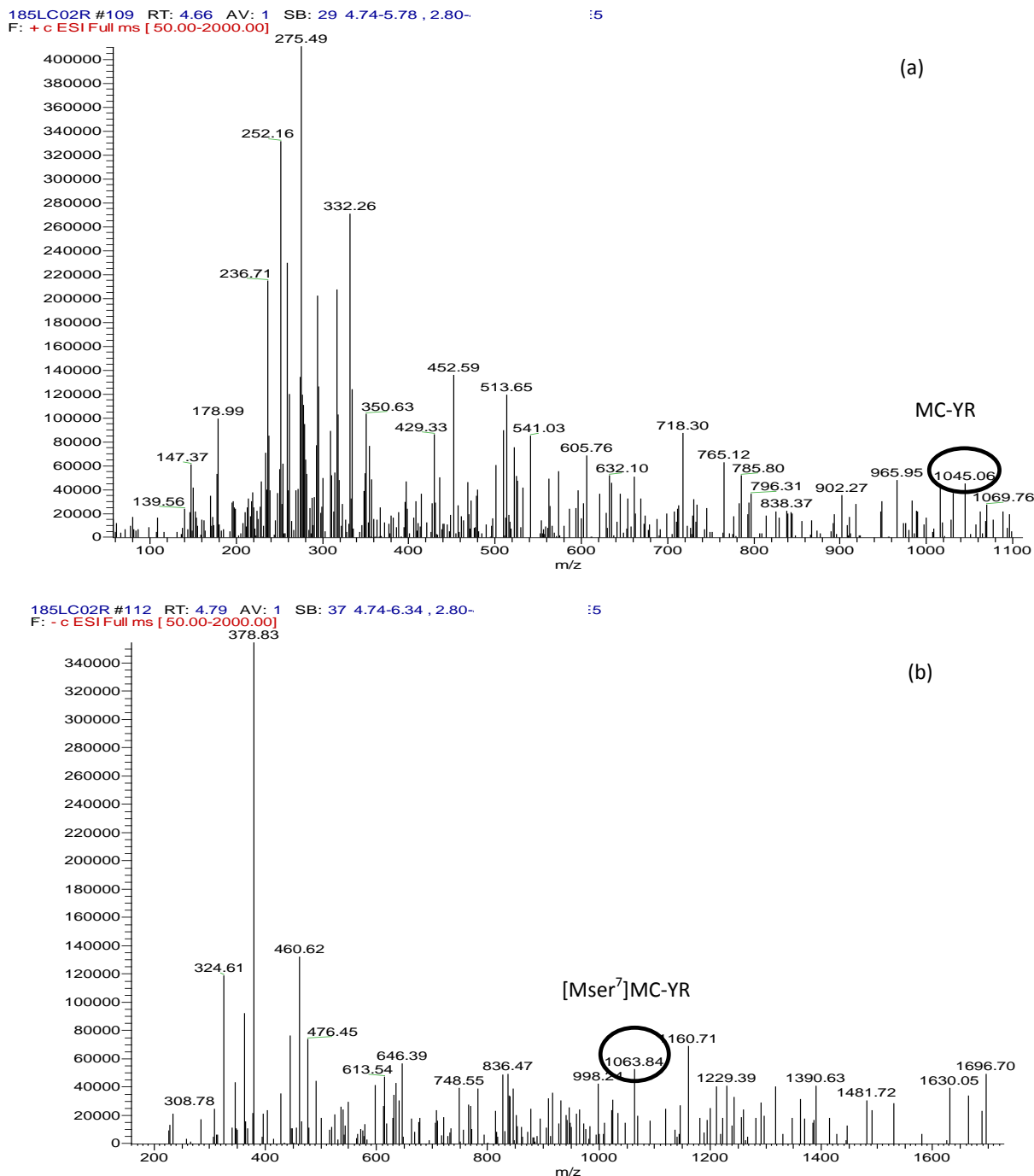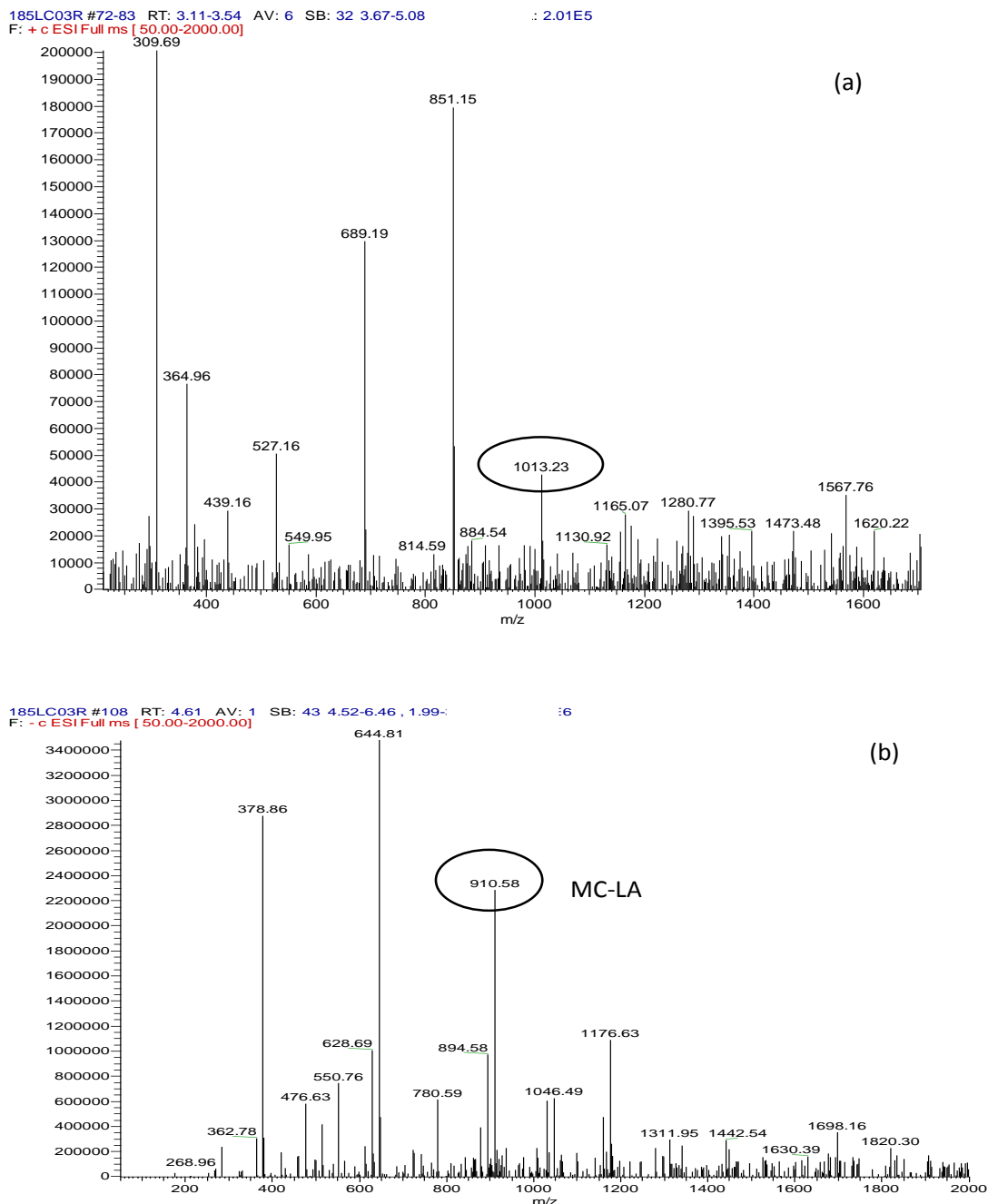
Figure 8. Analysis performed on extracts of cyanobacterial bloom *Oscillatoria tenuis* (3R) isolated from the waters of Smir Dam: **(a, b)** corresponding Mass spectrum showing the peak of the Microcystin fraction: [Mser7]MC-LR m/z 1013.23 : [M+H]$^+$ **(c)** and MC-LA m/z 910.58 : [M+H]$^+$.

LA, and MC-YAba in NAKHLA dam. These varieties of cyanotoxins are recognized by their acute toxicity. Studies have revealed that the toxicity of *Microcystis* is due to the disposition of the gene coding for microcystin (Carmichael, 1995; Rouhiainen et al., 1995; Dittmann et al., 1997).

P. galaeta filamentous species is also incriminated in dam waters and produces cyanotoxins also recognized by their acute toxicity either in SMIR (MC-YR, [Mser7]MC-YR), BELMEHDI (MC-YAba) and NAKHLA (MC-LA and MC-YAba). Studies in this context affirm that several species of Pseudanabaenaceae have been inventoried in Moroccan water bodies, including *Pseudanabaena mucicola*. This family is dominant in lakes and shallow ponds, and this dominance can sometimes persist throughout the year in turbid water

Figure 9. chromatograms of the analysis performed on extracts of cyanobacterial efflorescence *Microcystis aeruginosa* (4R) isolated from the waters of Belmehdi Dam **(a, b and c)** corresponding Mass spectrum showing the peak of the Microcystin fraction: MC-YAba m/z (974.36): $[M+H]^+$, MC-RF m/z 1029.64:$[M+H]^+$ and [Dha[7]]MC-LR m/z 981.90:$[M+H]^+$.

Figure 10. Analysis performed on extracts of cyanobacterial efflorescence *Pseudanabaena galeata* (5R) isolated from the waters of Belmehdi Dam: corresponding Mass spectrum showing the peak of the Microcystin fraction: MC-YAba m/z (974.11): [M+H]$^+$.

Figure 11. Analysis performed on extracts of cyanobacterial bloom *Oscillatoria tenuis* (6R) isolated from the waters of Belmehdi Dam: **(a)** corresponding Mass spectrum showing the peak of the Microcystin fraction: MC-YAba m/z (974.42):[M+H]+.
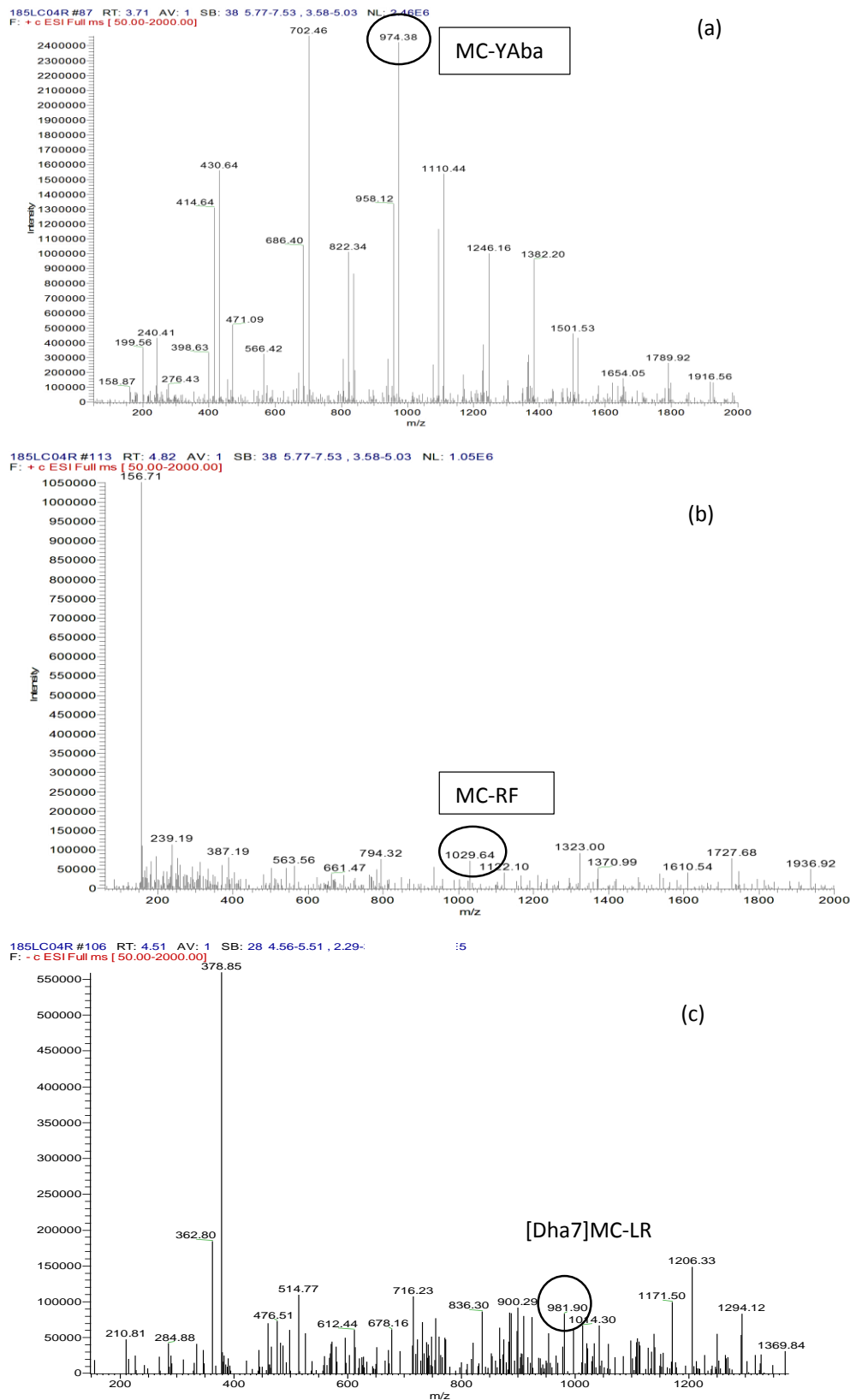
Figure 12. Analysis performed on extracts of cyanobacterial efflorescence *Microcystis aeruginosa* (7R) isolated from the waters of Nakhla Dam: (**a, b**) corresponding Mass spectrum showing the peak of the Microcystins fractions: MC-YAba m/z (974.25): [M+H and MC-LA m/z (910.64): [M+H]+.

bodies and when the winter is not very cold (Sas, 1989; Oudra et al., 2002a; Loudiki et al., 2002).

O. tenuis is also inventoried in Moroccan water bodies (Oudra et al., 2002a). This species develops variant cyanotoxins also recognized by its acute toxicity. In our study, it is found in SMIR ([Mser7]MC-LR, MC-LA), in

BELMEHDI (MC-YAba), and in NAKHLA ([Mser7]MC-LR and MC-YAba). This species is less frequent than the other species but it also has the capacity to generate large varieties of cyanotoxins.

Microcystis, Pseudanabaena and *Oscillatoria* species are the most common and potentially toxic cyanobacteria
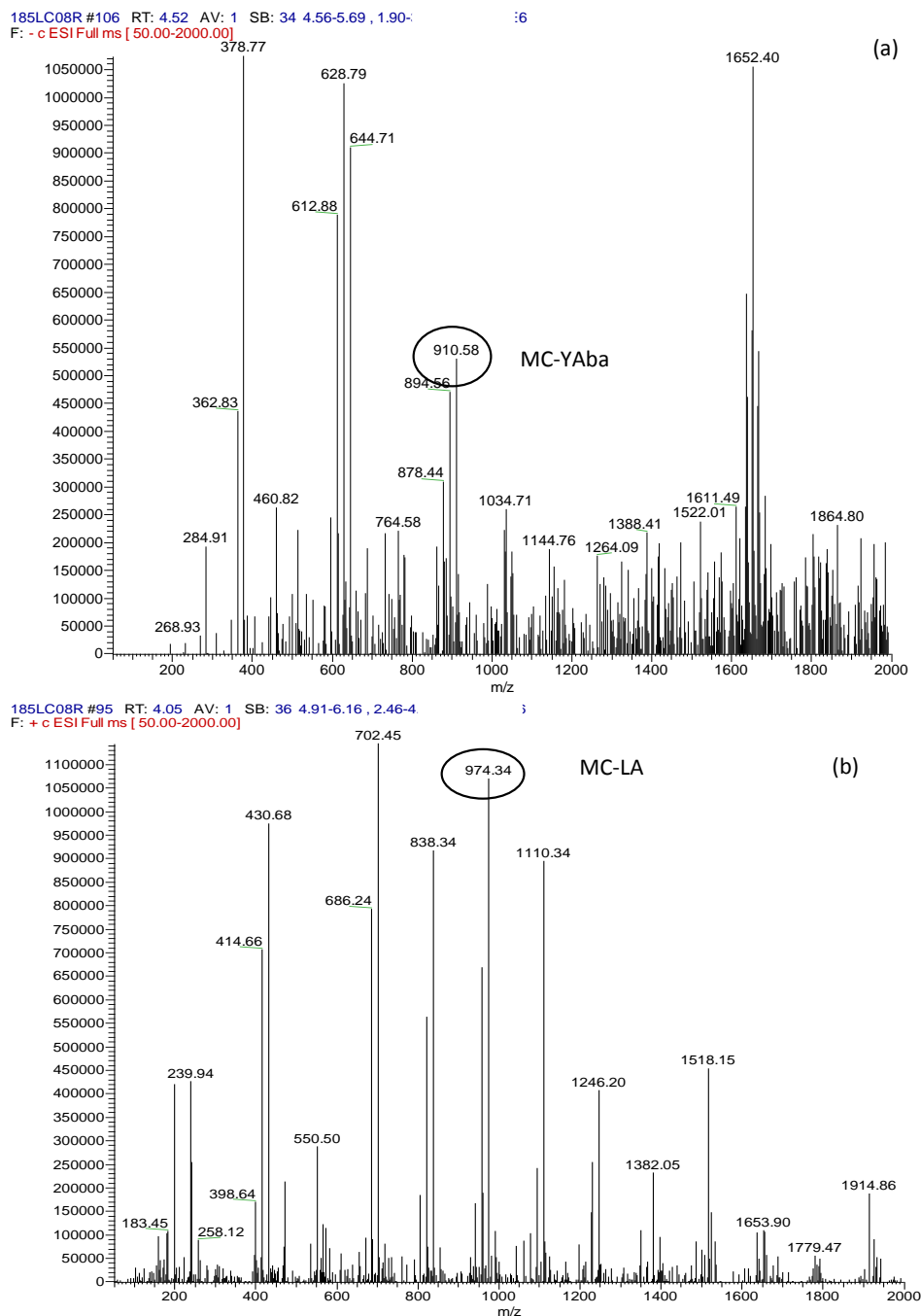
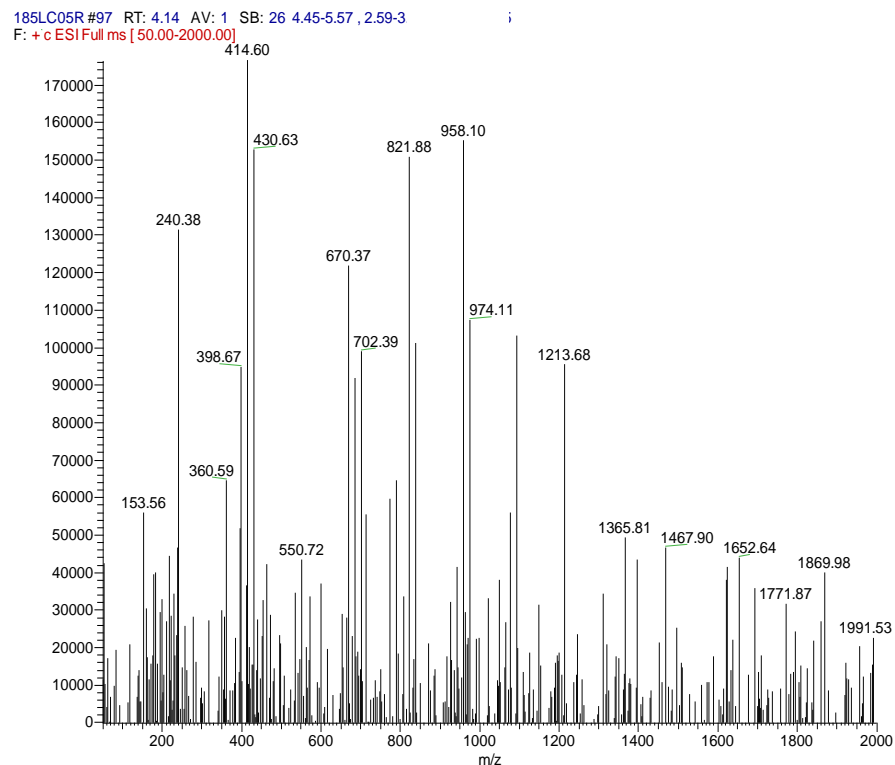**Figure 13.** Analysis performed on extracts of cyanobacterial efflorescence *Pseudanabaena galeata* (8R) isolated from the waters of Nakhla Dam: (**a, b**) corresponding Mass spectrum showing the peak of the Microcystin fraction: MC-YAba m/z (974.34): [M+H]$^+$ and MC-LA m/z (910.58): [M+H]$^+$.

found in Moroccan freshwater and are the main producers of cyanotoxins. This is also evident in Euro-Mediterranean countries (Filatova et al., 2020), such as in France (Lac de Grand-Lieu), Portugal (Vasconcelos et al., 1996; Moreira et al., 2020), Spain (Quesada et al., 2004), Greece (Christophoridis et al., 2018), and Italy (Bruno et al., 1992), African countries such as Algeria (Saoudi et al., 2017), Ethiopia (Major et al., 2018), and Nigeria (Kadiri et al., 2020).

The presence of microcystins in freshwater species of the genus *Oscillatoria* is not only an ecological problem, but also presents a health risk when water is used for
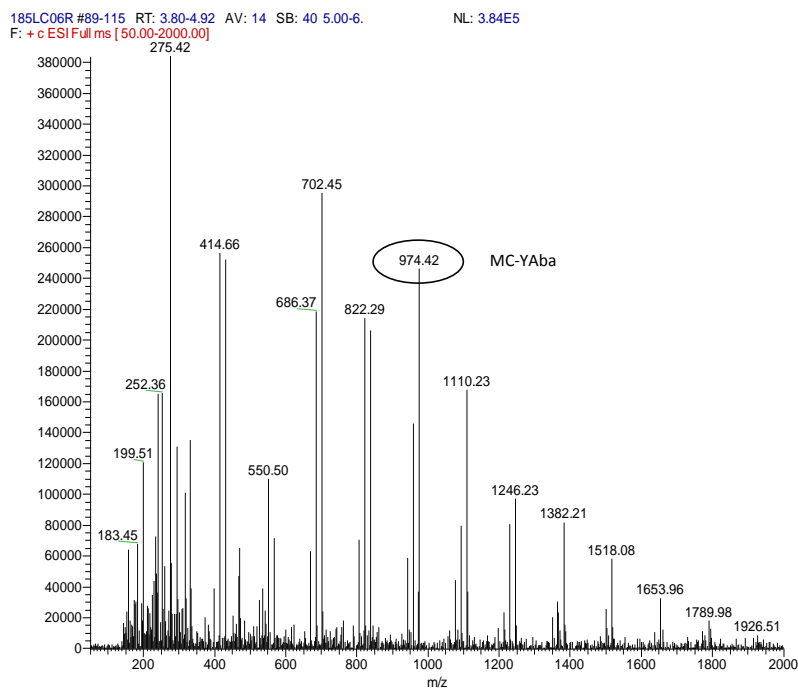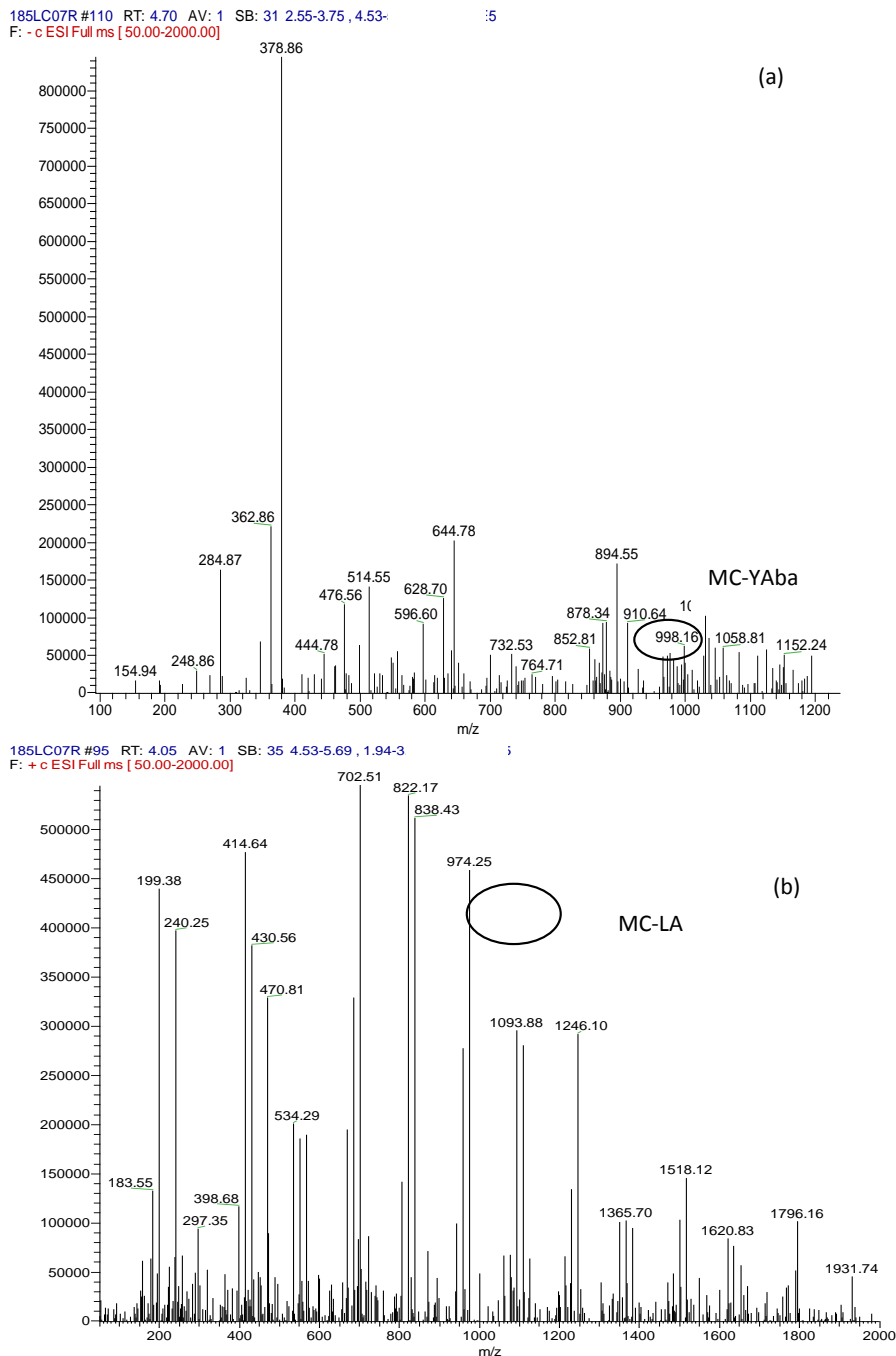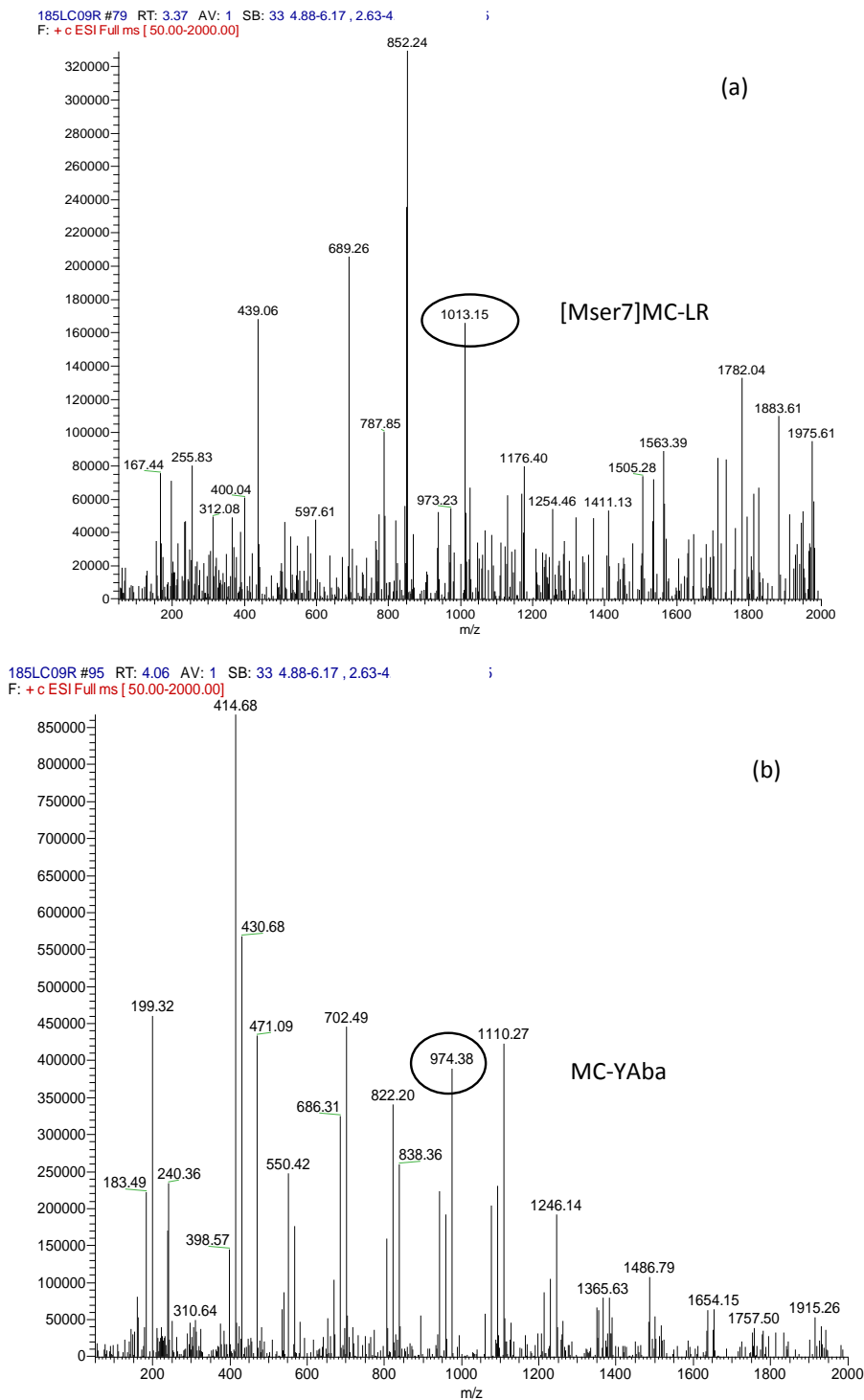
Figure 14. Analysis performed on extracts of cyanobacterial bloom *Oscillatoria tenuis* (9R) isolated from the waters of Nakhla Dam: **(a, b)** corresponding Mass spectrum showing the peak of the Microcystin fractions: [Mser[7]]MC-LR at m/z 1013.15:[M+H]+ and : MC-YAba à m/z 974.38:[M+H]+.

drinking purposes (Lindholm et al., 1989). On the other hand, some strains, known for their toxicological potential, are considered to be of biotechnological

interest, such as *Anabaena variabilis*. This species is used for its hydrogen production (Yoon et al., 2006) and can remove phenols and its derivatives (Hirooka et al.,

2003) as well as heavy metals (Nagase et al., 2005) from the environment and industrial wastewater (Yoon et al., 2006).

Conclusion

This study contributes to the knowledge of the systematics and biogeography of toxic cyanobacteria and Their toxins quality in the water bodies of Northern Morocco. It is a qualitative analysis of cyanotoxins produced by cyanobacteria species thriving in three water reservoirs near the City of Tetouan, namely SMIR, BELMEHDI and NAKHLA. The results show that the water bodies of Northern Morocco are exposed to cyanobacterial proliferation exposing these water bodies to numerous variants of Microcystins (MC-YR, [Mser7]MC-YR, MC-YAba, MC-LA, [Mser7]MC-LR, MC-FR, MC-RF, [Dha7]MC-LR) produced by the three major species (*Microsystis aeruginosa, Pseudanabaena galeata, Oscillatoria tenis*). These cyanotoxins are recognized by their acute toxicity and reflect a permanent threat to the health of the consumer (drinking water) mainly in late summer time. Thus, the need for action plan to monitor the levels and seasonal variations of these toxins and the biochemical factors involved directly or indirectly in the proliferation of cyanobacterial blooms.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Speciation of trace metal elements in sediments of Alibori River, North-West of Benin

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Alibori River located in northwestern Benin is subject to contamination by trace metal elements (TME: Zn, Cu, Pb, Cd) due to the use of agricultural inputs in its vicinity. This study focuses on the speciation of TME elements in the sediments of this river. The sediments were sampled during the rainy and dry seasons, dried, crushed and then sieved. The sediments were then mineralized and subjected to sequential fractionation. The total extraction and sequential extractions were analyzed using a spectrophotometer UV. The sediments of Alibori River had an acid pH and were contaminated by TME during the dry season. The zinc and copper had an affinity with the different fractions with a high proportion in the fractions F1 and F2 during the rainy season and a high proportion in the F5 fraction during the dry season. The lead and cadmium had an affinity with the F5 fraction during the rainy season, and the F3 and F4 fractions during the dry season. The calculated contamination index, individual contamination factor and risk assessment code (RAC) demonstrated that the river presented a potential risk of water contamination. The aforementioned parameters also showed that the river induced risk of bioavailability ranging from medium to very high for organisms. The TMEs found in the sediments of the Alibori River were linked to the exchangeable and acid-soluble fractions which made them more mobile and bioavailable to organisms.

Key words: Speciation, Alibori River, trace metal elements (TME), bioavailability, fraction.

INTRODUCTION

Rivers are natural moderately important water course, flowing continuously or intermittently following a defined route towards an ocean, sea, lake or another river. The Alibori River is an ecosystem located in the Sudanian

zone between 10° 30 "and 12° North latitude and 1° 32" and 3° 50 'East longitude in North Benin. It extends in the department of Alibori with many tributaries scattered in the municipalities of Kandi, Gogounou, Banikoara, Ségbana, Karimama and Malanville before flowing into the Niger River (Le Barbe et al., 1993; Thierry et al., 2016).

Alibori River's waters are used by the populations for daily needs (drink, cooking, laundry, dishes) and the processing of agricultural products as adequate local water supply facilities are not available in the region (PEA) (Tachégnon and Ibrahim, 2012; Chitou et al., 2019). Intensive agriculture is also developed around the river and is dominated by cash and food crops such as cotton, and corn (Agagbé, 2008; Thierry et al., 2016; Tachégnon and Ibrahim, 2012). It is characterized by an intensive use of agricultural inputs (chemical fertilizers) and phytosanitary products.

One of the consequences of the use of these products (chemical fertilizers and phytosanitary products) is the contamination of waterways by various pollutants such as pesticides and trace metal elements (TME). These TMEs can lead to morphological abnormalities and neurophysiological disorders, and affect enzymatic and hormonal activities in living organisms (Chitou et al., 2019; Wang et al., 2017).

There is a dearth of information on the effects of human activities on the Alibori River (Thierry et al., 2016; Chitou et al., 2019). Our previous study showed that sediments of the Alibori River were contaminated by TMEs (Zn, Cu, Pb, Cd) due to the use of agricultural inputs in its surroundings (Chitou et al., 2019). This study also determined the total concentration of TMEs in the river's sediments.

The total quantification of TMEs available in sediments remains insufficient to predict (or evaluate) their toxicity, reactivity, mobility and bioavailability (Zhou, 2009; Daldoul et al., 2015; Bashar, 2016). These parameters are rather evaluated through the chemical speciation of ETMs. The current study is therefore initiated to assess the toxicity, reactivity, mobility and bioavailability of TME in sediments of the Alibori River. Its objective is to determine the different chemical fractions of Zn, Cu, Pb, and Cd available in the sediments of this river during each season.

MATERIALS AND METHODS

Collection of sediment samples

Seven (7) sediment-sampling points were chosen in Alibori River based on the position of the crop fields and various human activities in its vicinity (Figure 1), and the uses of its waters (Table 1). The river's sediments were sampled during each of the two seasons (rainy and dry) prevailing yearly in the Alibori region. The sampling

of sediments was performed using a Schipeck grab or manually with a gloved hand depending on the water level.

Sediment analysis

The seven (7) sediments were dried to a constant weight using an oven at 105°C, crushed, while preserving the shells, and then sieved at 2 mm. The obtained product was stored at 18°C for analysis.

The pH of the processed sediments was measured as described by standard AFNOR NF X-31-103, 1998. The sediments were then mineralized by adding 20 ml of a mixture of hydrochloric acid (HCl) 6 mol.L^{-1} and nitric acid HNO_3 7 molL^{-1} (3: 1) to 1 g of each sample. The mineralized sediments were brought to the boil on a hot plate for 1 h at 120°C, cooled, then centrifuged, and the resulting supernatant was recovered. The collected supernatant was combined with the appropriate volume of distilled water to obtain a solution of 25 ml, and then analyzed using a spectrophotometer UV to determine the total content of TME.

Sequential extractions of TME

The speciation of TME was carried out according to the sequential method of Tessier et al. (1979) modified for 1 g of sediment. The sediments were thus subjected to fractionation according to the sequential extraction as described in Table 2. The extracted fractions were then analyzed using a spectrophotometer UV.

Given the spatial distribution of the seven (7) sites, and the contamination index CI of these sites, only the sediments from the sites 2, 3, 5 and 6 underwent fractionation (speciation).

Procedures

Data processing

The levels of variations of the physicochemical parameters and the concentrations of copper, zinc, cadmium and lead in the sediments, as well as extractions' results, were collapsed into histograms using the Excel software.

The Risk Assessment Code (RAC) and the Individual Contamination Factors (ICF) were estimated and used to assess environmental risks and possible impacts on the river's benthic organisms.

The heavy metal data were subjected to principal component analysis (PCA) and correlation calculation using X-Lstat software. The correlation coefficient was used to determine the relationship between two variables and to measure the strength of this link. The calculation of the correlation coefficient was used for screening of the possible relations between metals with regard to common origin, homogeneous distribution, identical behavior concerning physicochemical processes, and similar reciprocal fractionation.

The contamination index "CI" was estimated following Equation 1 and used to assess the degree of pollution of the sediments in line with Table 3 (Waris et al., 2018).

$$CI = \frac{\text{TME content}}{\text{TMEn}} \tag{1}$$

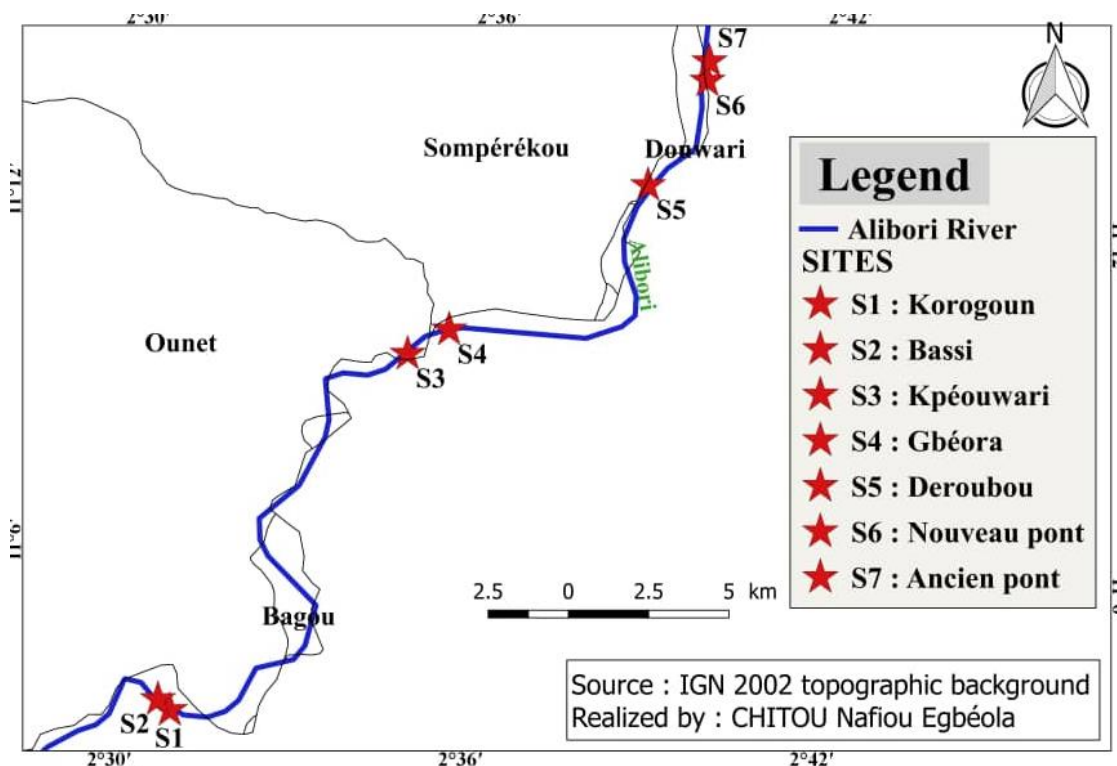where TMEs: concentration of TME in the Alibori River sediments,

Figure 1. Sampling map.

Table 1. Sediment sampling site from the Alibori River.

Site	Geographical coordinates
S1: KOROGOU-ALIBORI	N11°4'2,08082" E2°31'1,45357″
S2: BASSI-ALIBORI	N11°4'0,66592"E2°31'3,95476"
S3: KPEWARI	N11°3'39,47352" E2°30'14,59444"
S4: GBEWORA	N11°10'15,97499"E 2°35'25,31514"
S5: DEROUBOU-ALIBORI	N11°12'7,18531" E 2°38'27,88144"
S6: NOUVEAU PONT-ALIBORI	N11°14'31,11512"E 2°39'37,22355"
S7: ANCIEN PONT-ALIBORI	N11°14'50,86185"E 2°39'37,79717"

mg.kg^{-1}; TMEn: concentration of TME considered as normal, mg.kg^{-1}.

The risk of water contamination by a pollutant was examined using the individual contamination factor (ICF) of sediments by TME, which was estimated following Equation 2. For $0.14 \leq ICF \leq 69.42$, there are potential risks of water contamination by TME (Lawani, 2013).

ICF = Concentration of non-residual TME fractions / concentration of residual TME fraction

$$ICF = \frac{F1+F2+F3+F4}{F5} \qquad (2)$$

The concentration of non-residual fractions is the sum of the fractions F1, F2, F3, and F4. Fraction F5 is the residual fraction.

The availability of metals in solution was assessed using the classification of the Risk Assessment Code (RAC) outlined in Table 4 (Huajun et al., 2011; Abdallah, 2012). The RAC is based on the percentage scale to the exchangeable and carbonate fractions of TME in sediments and is the sum of the exchangeable and carbonate (acid-soluble) fractions.

RESULTS

Sediments' pH

The sediments' pH varied from 5.86 (S5) to 6.78 (S6)

Table 2. Sequential extraction protocol.

Extracted fraction	Reagent(s)	Operating mode (Volume, Duration, Temperature)
F1 :Exchangeable fraction	$MgCl_2$ (1 mol.L^{-1}), pH = 7	8ml, 1-hcontinuous shaking ambient temperature
F2 : Acid-soluble fraction	CH3COONa (1 mol.L^{-1})/CH_3COOH pure, pH = 5	8 mL, 5-h continuous shaking ambient temperature
F3 :Reducible fraction	$NH_2OH.HCl$ (0.04 mol.L^{-1}) in CH_3COOH 25%, pH = 2	20 mL, 6-h intermittent shaking, water-bath 96°C
F4 :Oxidisable fraction	HNO_3(0.02 mol.L^{-1})/H_2O_2 (30%, pH =2 with HNO_3)	3 mL HNO_3 + 15 mL H_2O_2, 2-h intermittent shaking, water-bath to 85°C
	CH_3COONH_4 (3.2 mol.L^{-1})	5 mL, 3-h continuous shaking + distilled water
F5 :Residual fraction	HNO_3 + HClaq (1:3)	10 mL, heating to 120°C, let cool Filter, wash

during the rainy season and from 4.54 (S2) to 5.70 (S4) during the dry season (Table 6).

Organic matter (OM) in the sediments

Organic matter was more abundant in the river during the rainy season than the dry season and varied from 17.34 mg.kg^{-1} sediment (S7) to 85.16 mg.kg^{-1} (S5) and 2.42 mg.kg^{-1} (S3) to 20.3 mg.kg^{-1} (S1), respectively (Table 7).

The total content of TMEs in the sediments

Zinc and copper

The zinc and copper contents varied in the sediments at each site according to the seasons (Figures 2 and 3). The concentrations of both metals were higher in the sediments during the rainy season than the dry season except the site 6 for zinc and sites 3 and 4 for copper. The high zinc content was observed at site 7 during the rainy season and site 6 during the dry season.

The highest copper's content was observed at site 7 during the rainy season and site 3 during the dry season.

The zinc and copper contents varied in the sediments of each site according to the seasons (Figures 2 and 3). The concentrations of both metals were higher in the sediments during the rainy season than the dry season except the sites 3 and 4 for copper. The highest zinc's content was observed at site 7 during both seasons. The highest zinc's content was observed at site 7 during the rainy season and site 3 during the dry season.

Lead and cadmium

Lead's concentration was higher in the sediments during the rainy season than the dry season at the sites 1, 2 and 3 (Figure 4). From sites 4 to 7, the reverse phenomenon was observed.

Cadmium was almost absent in the sediments during the rainy season except the site 7 which had a high content (52.5 mg.kg^{-1} sediment) (Figure 5). During the dry season, cadmium's concentrations in the sediments were low.

Contamination index (CI)

The Zn and Cu contamination index (CI) was well above 3 (Table 8). The CI of Cd and Pb was less than 3 except for cadmium during the dry season and at site 7 during the rainy season.

TME speciation

Fractions of zinc

Zinc was distributed in all geochemical fractions of sediments during the rainy season. The residual fraction was less observed at the first three sites (Bassi "S2", Kpéwari "S3", Deroubou "S5"). The residual fraction was the most observed (47%) at site 6 (New bridge). At site 3, zinc was much more bound to the exchangeable and acid-soluble fractions which were the most mobile sensitive to a small change in pH. At site 5, the oxidizable fraction was dominant. At site 2, the oxidizable and reducible fractions were the most observed (Figure 6).

Zinc was predominantly present in the residual fraction during the dry season except the site 3

Table 3. Classification of the sediment according to the contamination index CI.

Contamination indice	Ranking	Interpretations
Ic< 3	Class1	Normal area
$3 \leq Ic< 10$	Class 2	Polluted area
$10 \leq Ic$	Class 3	Risk area

Table 4. Classification of the Risk Assessment Code (RAC) (Huajun et al., 2011, Abdallah, 2012)

Criteria related to sediment (%)	Risks
<1	No risk
1-10	Low risk
11-30	Medium risk
31-50	High risk
>50	Very high risk

Table 5. Standard for assessing the quality of freshwater sediments Environment Canada 2007**.**

TMEs	mg/kg
Copper	22
Zinc	80
Cadmium	0.33
Lead	25

Environnement Canada et ministère du Développement durable, de l'Environnement et des Parcs du Québec (2007).

Table 6. pH values of sediments.

pH/Site	S1	S2	S3	S4	S5	S6	S7
pH dry season	5.48	4.54	5.64	5.70	5.60	4.87	5.45
pH rainy season	6.28	6.02	5.82	6.00	5.86	6.78	6.01

Table 7. Values of organic matter in the sediments.

MO mg.kg^{-1} sediment	RS	DS
S1	77.31	20.3
S2	31.72	15.83
S3	22.6	2.42
S4	20.6	18.07
S5	85.16	10.26
S6	60.14	8.47
S7	17.34	19.19

DS: Dry season; RS: rainy season.
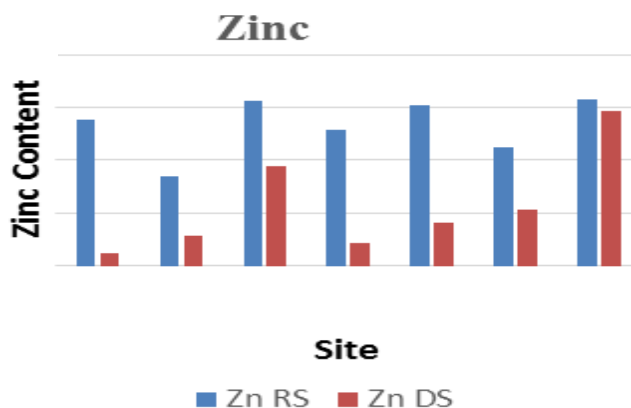
Figure 2. Zinc content in sediment (mg.kg^{-1} sediment).
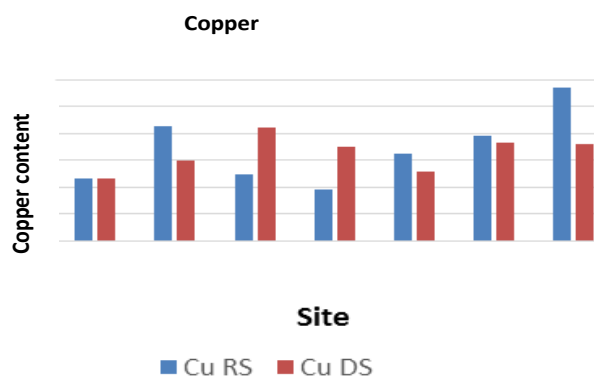

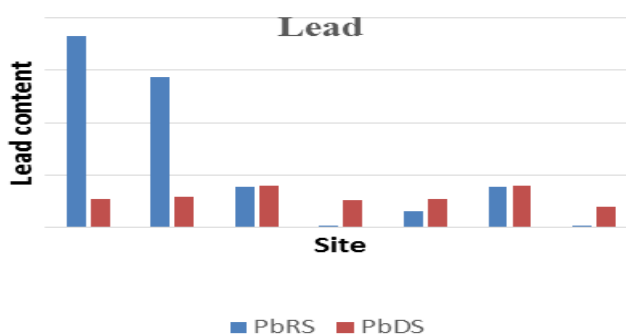**Figure 3.** Copper content in sediment (mg.kg^{-1} sediment).

Figure 4. Lead content in sediments.

(Kpéwari) where zinc was more related to the reducible fraction (Figure 7).

Fractions of copper

Figure 8 shows the distribution of copper in the different geochemical fractions of the Alibori River's sediments during the rainy season. Except for the residual fraction, the copper was much more bound to the reducible and acid-soluble fraction at the sites 2 (Bassi) and 3 (Kpéwari). At site 5 (Dèroubou), all the fractions were moderately observed. At S6 site (New Bridge), the copper was much bound to the oxidizable and reducible
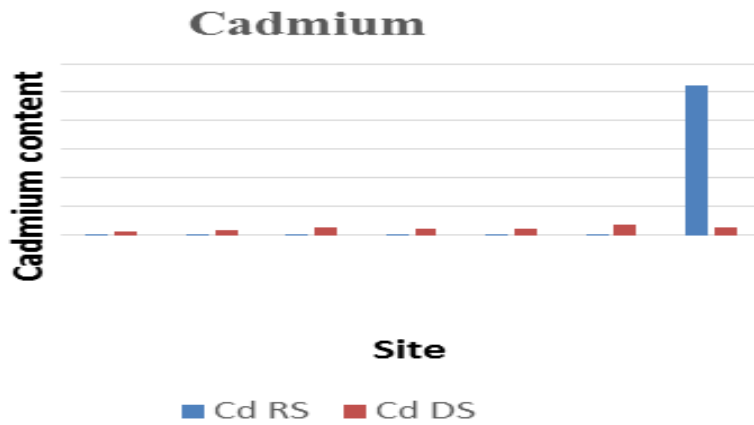
Figure 5. Cadmium content in sediments.

Table 8. Sediment contamination index values.

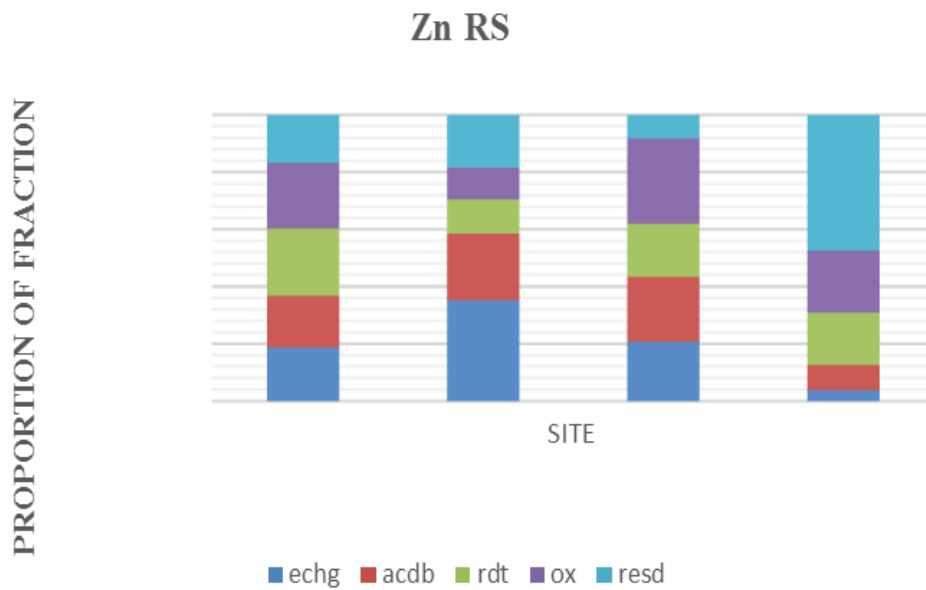| Site	ZnRS	ZnDS	CuRS	CuDS	CdRS	CdDS	PbRS	PbDS
S1	17.33	1.43	8.88	8.84	0.30	4.00	0.73	0.11
S2	10.55	3.6	16.34	11.46	0.30	4.75	0.57	0.11
S3	19.54	11.75	9.42	16.27	0.30	8.21	0.15	0.16
S4	16.14	2.68	7.24	13.49	0.30	6.24	0.004	0.10
S5	19.01	5.09	12.38	9.84	0.30	7.12	0.058	0.11
S6	14.06	27.77	15.01	14.09	0.30	10.42	0.15	0.16
S7	19.68	18.32	21.91	13.84	159.09	7.54	0.004	0.08

DS: Dry season; RS: rainy season.

Figure 6. Zinc fractions in the rainy season. SP: Rainy season, echg: exchangeable (F1), acdb: acid-soluble (F2), rdt: reducible (F3) ox: oxidizable (F4), resd: residual (F5).
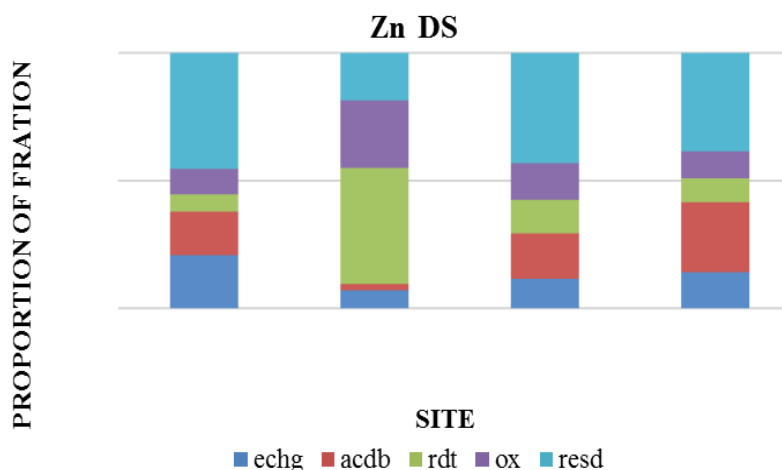
Zn DS

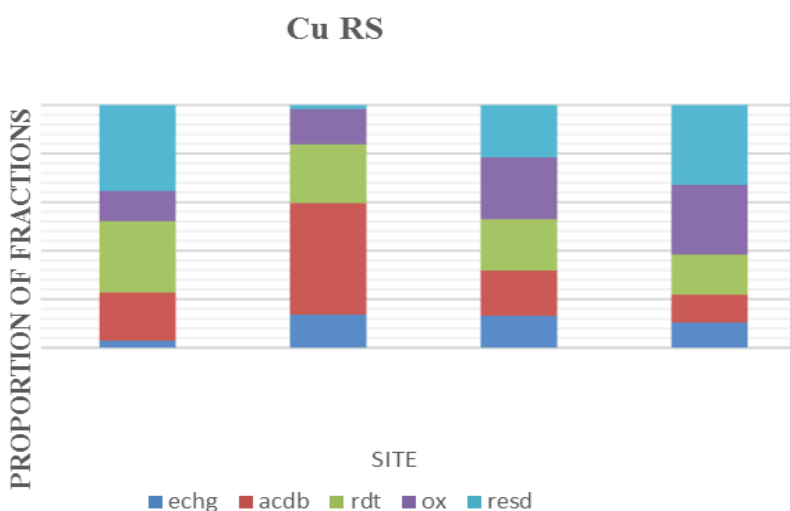**Figure 7.** Zinc fractions in the dry season.

Cu RS

Figure 8. Copper fractions in the rainy season.

fractions.

During the dry season, the residual fraction was the most observed with a percentage of 68% (site 2) (Figure 9). At site 3, zinc was weakly bound to the exchangeable and residual fraction. Site 5 was dominated by the oxidizable and exchangeable fractions. At site 6, the share of the acid-soluble fraction was the highest (53%).

Fractions of lead

Figure 10 shows the distribution of lead in all geochemical fractions during the rainy season. A low proportion of the residual fraction was observed at the sites 2, 5 and 6 and

a high proportion (51%) at site 3. The lead was much more bound to the oxidizable fraction (46%) at site 6. At site 2, lead was bound to all fractions, unlike other sites.

During the dry season, an increase in the oxidizable fraction was observed up to 52% (sites 2 and 3) for the lead (Figure 11). The lead also exhibited affinity with the reducible and acid-soluble fraction during this period at the level of the sites 5 and 6.

Fractions of cadmium

From Figure 12, cadmium was much bound to the residual fraction. Besides, it was linked to the
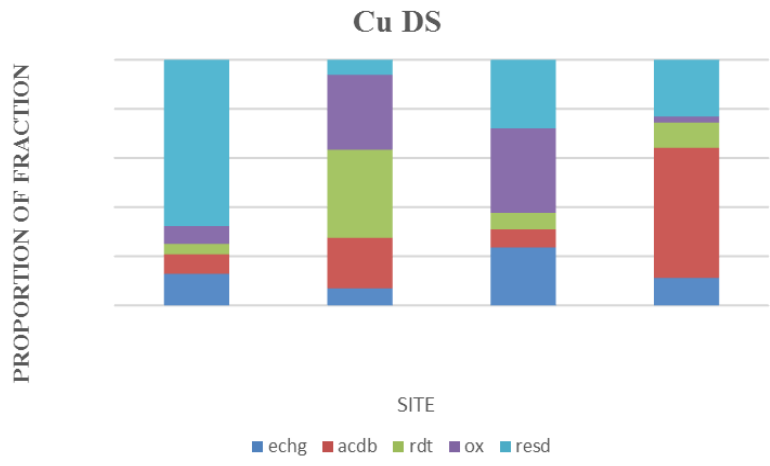
Cu DS

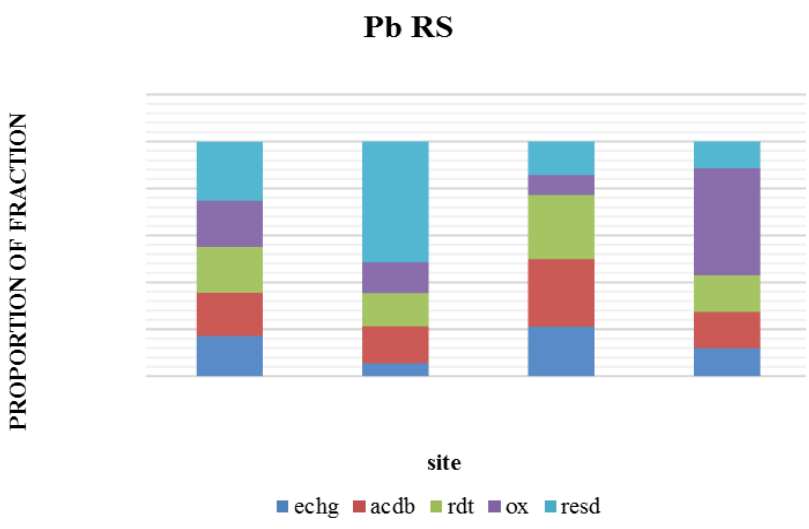**Figure 9.** Copper fractions in the dry season.

Pb RS

Figure 10. Fraction of lead in the rainy season.

exchangeable fraction, especially at the sites 3 and 6, and to the acid-soluble fraction at site 2.

Cadmium was strongly bound to the reducible and oxidizable fractions at all sites and the acid-soluble fraction at the sites 2, 5 and 6 (Figure 13). The exchangeable fraction was weakly observed.

Individual contamination factor (ICF) of TMEs

The calculated contamination factor varied from 4.96 to 11.16 for zinc, 1.82 to 57.47 for copper, 0.96 to 7.76 for lead and 0.3 to 1.66 for cadmium. The highest ICF value was observed at site 5 for zinc, site 3 for copper and site

6 for lead and cadmium during the rainy season. During the dry season, the calculated contamination factor was between 1.2 to 4.36 for zinc, 0.47 to 15.31 for copper, 1.63 to 8.99 for lead and 2.02 to 15.1 for cadmium. The highest value of the ICF of copper and zinc was observed at site 3.

Risk assessment code (RAC)

The calculated RAC for zinc and copper were in the range 10% <RAC≤65% with high risk at sites 2, 3 and 5 during the rainy season and the site 6 during the dry season. The calculated RAC for lead and cadmium were
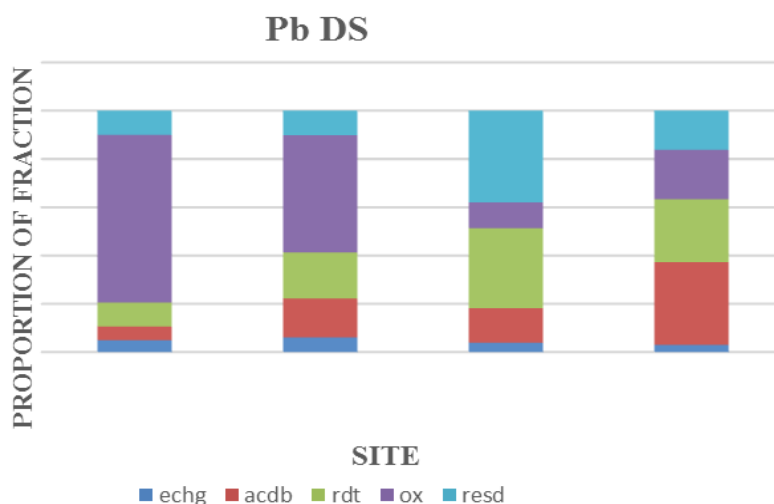
Figure 11. Fractions of lead in the dry season.
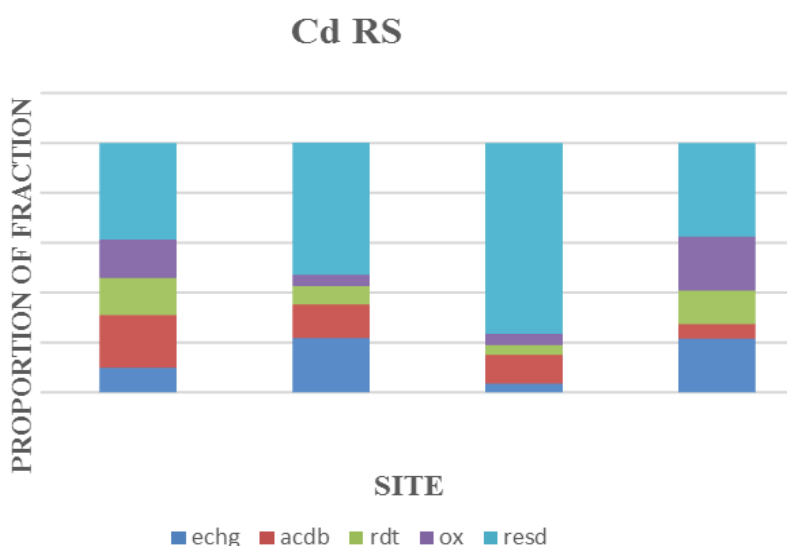
Figure 12. Cadmium fractions in the rainy season.

in the range of 10% <RAC≤49.85%. The highest RAC was observed at the sites 5 for Pb and 3 for Cd during the rainy season and the site 6 during the dry season for Cd and Pb.

Principal component analysis (PCA)

Figure 14 shows the results of the PCA during the rainy season. The majority of the parameters were well observed because they were close to the correlation circle with a proportion of 81.14% including 43.48% for the F1 axis and 37.66 for the F2 axis. It was observed a strong correlation between the oxidizable fraction of zinc (Znspox) and organic matter (OM), an average correlation between the oxidizable fraction of copper (Cuspox) and organic matter. It was also observed a strong correlation between the exchangeable fraction of zinc (Znspech) with the F1 axis and with RAC of zinc (ZnspRAC), likewise, there was a correlation between the acid-soluble fraction of copper (Cuspacdb) and RAC of copper (Tables 9 and 10).
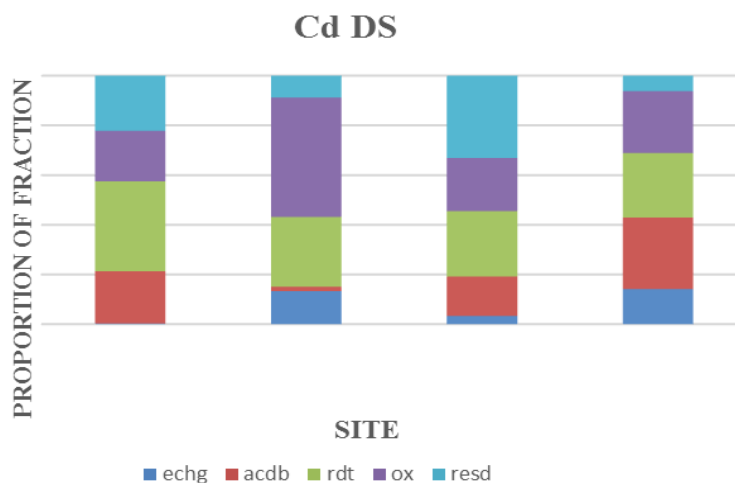
Figure 13. Cadmium fractions in the dry season.
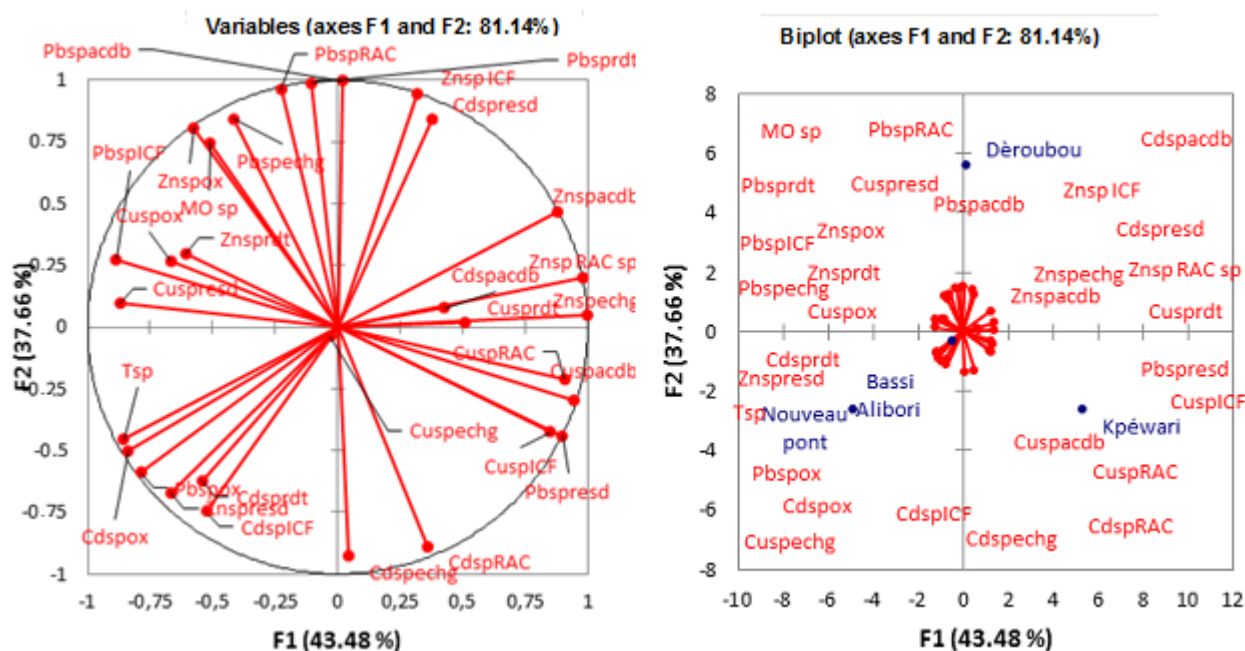
Figure 14. Main component analysis, rainy season.

It is well known that the square that accompanying the correlation circle reflects various dominant variables responsible for the pollution at each site. This suggests that the site of the new bridge was dominated by the oxidizable fraction of cadmium and lead. The Kpéwari site was dominated by the exchangeable fraction of cadmium and the acid-soluble fraction of copper. The Bassi-Alibori site was very close to the centre therefore it was dominated by the exchangeable fractions of zinc,

acid-soluble zinc and copper, and the oxidizable fraction of copper. The Dèroubou site was dominated by the acid-soluble fraction of lead and the oxidizable fraction of zinc.

Figure 15 outlines the results of the PCA during the dry season. The majority of the parameters were well observed during the dry season because they were close to the correlation circle with a proportion of 78.32% including 47.20% for the F1 axis and 31.12 for the F2 axis. There was a strong correlation between the

Table 9. Values of the individual contamination factor of TMEs calculated.

	Site	S_2	S_3	S_5	S_6
ICF RS	Zn	4.96	4.41	11.16	1.11
	Cu	1.82	57.47	3.65	2.03
	Pb	2.98	0.94	6.02	7.76
	Cd	1.58	0.89	0.3	1.66
ICF DS	Zn	1.2	4.36	1.31	1.59
	Cu	0.47	15.31	2.58	3.33
	Pb	8.99	8.85	1.63	5.18
	Cd	3.5	10.28	2.02	15.1

Table 10. RAC values in %.

Metal		Site			
		(S2)	(S3)	(S5)	(S6)
RAC	Zn RS	36.79**	58.52***	43.43**	12.14*
	Zn DS	37.92**	9.55*	29.32*	41.58**
	Cu RS	22.70*	59.56***	31.85**	21.88*
	Cu DS	20.83*	27.47*	30.97**	64.11***
	Pb RS	35.53**	21.00*	49.85**	27.44*
	Pb DS	10.57*	22.20*	18.28*	37.35**
	Cd RS	31.00**	35.00**	15.00*	27.00*
	Cd DS	21.27*	15.13*	19.14*	42,93**

***RAC> 50 very high risk of water contamination, **30≤RAC <50 high risk of water contamination. *RAC <30 medium or low risk of water contamination.

oxidizable fraction of cadmium and the individual contamination factor of copper and zinc as well as the F1 axis. These parameters and the axis were correlated with the oxidizable and reducible fractions of zinc (Znssox, Znssrdt). There was a strong correlation between the acid-soluble fraction of copper (Cussacdb), the acid-soluble fraction of lead (Pbssacdb) and RAC of lead. The Bassi Alibori site was dominated by the residual fraction of coppers (Cussresd), zinc (Znssresd) and cadmium (Cdssresd), and the reducible fraction of cadmium (Cdssrdt).

The Dèroubou site was dominated by the exchangeable fraction of zinc (Znssech) and the reducible fraction of zinc. The Kpéwari site was characterized by the exchangeable fractions of lead, oxidizable from cadmium and zinc, reducible from copper and zinc. The site of the new bridge was characterized by exchangeable fractions of cadmium, acid-soluble of lead and copper.

DISCUSSION

Chemical fertilizers and pesticides used in agriculture release TME into the environment, which can enter aquatic ecosystems through runoff (Nziguheba and Smolders, 2008; Yamaguchi et al., 2009; Alassane et al., 2018). Depending on the physicochemical conditions of the environment, these TME integrate suspended matter and are deposited overtime on the sediments which constitute pollutants sources.

The values recorded for the pH of the Alibori River's sediments in this study suggest that the environment was slightly acidic during the rainy season and more acidic during the dry season, and governed the behavior of TME between the solid and liquid phases. The increase in acidity was caused by the decomposition of organic matter of which concentrations have decreased from 44.98 to 13.50 mg.kg^{-1}, producing CO_2. The observed pH values were lower than those reported for sediments at the coastal lagoon of Benin (6.9 to 8.7) by Waris et al. (2017), and at the level of the Boumerzoug Algeria basin (6.15 to 8.51) by Leila et al. (2014). The divergence between our results and those of these authors could be explained by several factors including the difference between the sampling periods and the activities developed around the bodies or courses of water.
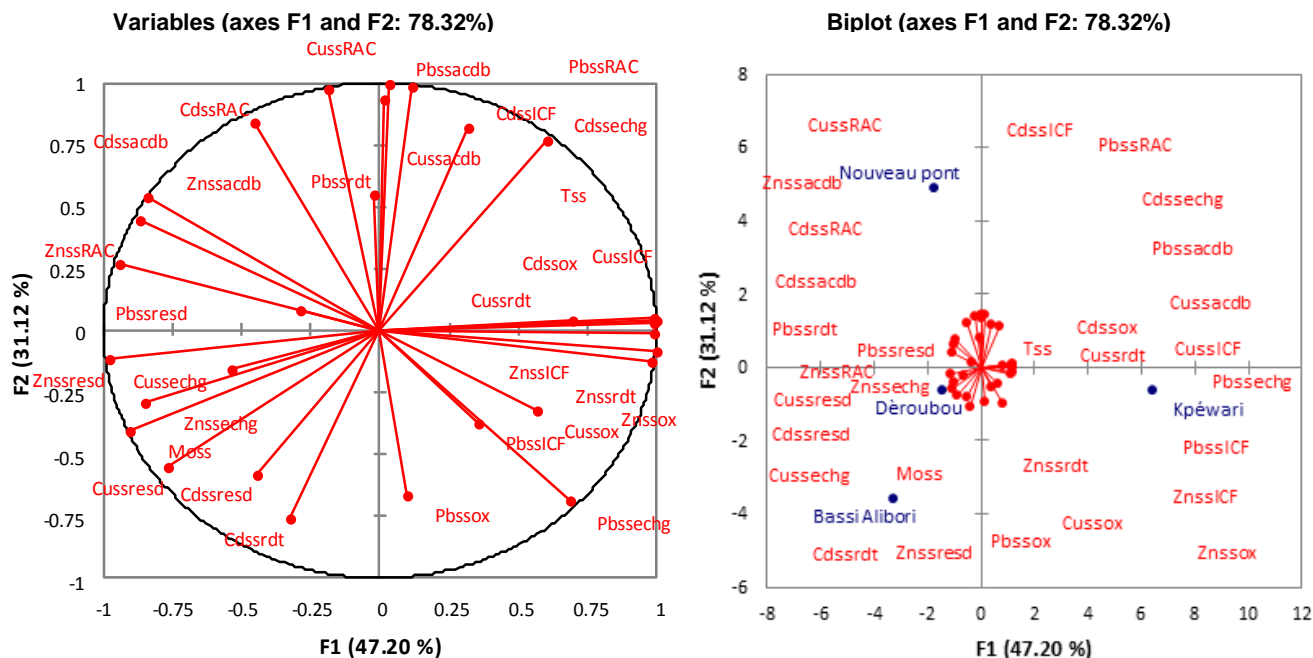
Variables (axes F1 and F2: 78.32%)

Biplot (axes F1 and F2: 78.32%)

Figure 15. Main component analysis, dry season.

Zinc, copper and lead were more concentrated in the sediments during the rainy season, which is a cultivation period, than the dry season, unlike cadmium. This observation is in agreement with Chitou et al. (2019) who reported similar variation for TMEs concentrations in Alibori River's sediments between the dry season and the rainy season in 2018.In accordance with Chitou et al. (2019), it could be concluded that the high values of TME during the cultivation period were due to the use of fertilizers and pesticides since the river is located in a rural area dominated by agricultural activities (Chitou et al., 2019). The concentration of Zinc and copper in the sediments has increased compared to that obtained in the 2018 during the dry season (Max: 15.6 mg.kg-1 for zinc, 19.1 for copper) and the rainy season (Max: 175 mg.kg^{-1} for zinc, 231 mg.kg^{-1} for copper) using a metalyser (Chitou et al., 2019). This increase could be due to the accumulation of TME in the sediments and the analysis method. This accumulation depended on the proportion of non-labile and strongly retained fractions in the sediments. The observed cadmium during the dry season may be explained by the degradation of organic pollutants (pesticides) which can last for weeks depending on environmental conditions. Therefore, agricultural activity through the use of chemical inputs and pesticides remains the main source of contamination of the Alibori River's sediments by Cu, Zn, Cd, and Pb, without however excluding domestic discharges and atmospheric

deposits from the combustion of fuels associated with road traffic.

The estimated contamination indices (CI) confirmed the contamination of the sediments by copper, zinc, lead and cadmium in the order Zn> Cu> Cd> Pb. The CI values were overall greater than 10, this suggests that the Alibori River constituted a high-risk area for living beings (abiotic factors) according to the sediment contamination assessment grid (Aouatif et al., 2016).

This contamination index remains insufficient to assess the risks of contamination of aquatic species and fauna by TME in the environment. Rather, these risks were assessed through the chemical speciation of ETMs.

The speciation analysis of the TME showed that zinc and copper were present in all fractions with varying proportions, F5> F4 > F1> F2 > F3 during the rainy season. By ignoring site 6 (new bridge) because of its position in cotton fields and other crops, the order of the fractions changes F1> F2> F4> F3> F5 for zinc and F2> F3> F5 > F4 > F1 for copper, which explains the effect of the proximity of the culture fields to these sites.

The TME linked to these different fractions (F1, F2, F3, F4) of the sediments can be released into the water depending on the physicochemical conditions of the environment to be bioavailable. The bioavailability involves more the metal species linked to the F1 and F2 fraction because they are labile and can be toxic to aquatic organisms Daldoul et al., 2015.

The F1 and F2 fractions had the highest proportions suggesting that copper and zinc were the most labile, thus bioavailable for aquatic organisms. This observation shows that the contamination of the sediments by zinc and copper constituted a risk for the aquatic fauna and flora of Alibori River. The affinity of copper with the F2 fraction is in agreement with previous study on the copper in sediments of the Ouémé delta (Rodrigue et al., 2018). The observed affinity confirms the effect of pH on the behavior of TMEs in the aquatic environment. The acid-soluble (F2) and exchangeable (F1) fractions predominated at low pH (5 - 5.86), and the reducible (F3) and oxidizable (F4) fractions appeared when the pH increased. This is in line with Bashar et al. (2016) who reported that the precipitation of cations is favorable at high pH. The retention of TME in the different fractions of the sediments as indicated in reaction Scheme 1 may explain the accumulation of TME in the sediments during the rainy season.

During the dry season, the reverse reaction was observed F5> F3 > F4> F1> F2 for zinc and F5> F4> F3> F1> F2 for copper, which could be explained by the release of the bound zinc and copper to these fractions into the liquid matrix (water). This chemical reaction which took place between the solid (sediment) and liquid (water) matrix was due to the variation in pH.

Zinc adsorbed on the particles (F1) and the part bound to carbonate (F2) were sensitive to a slight decrease in pH. The decrease in pH observed during the dry season making the environment acidic lead to a decrease in these fractions (F1, F2) at certain sites, which may justify the low zinc content in the sediments at these sites.

Indeed, according to Serpaud et al. (1994), the absorption of Cu and Zn decreases when the temperature increases (30 - 40°C). However, the pH being inversely proportional to the temperature, therefore the decrease in pH suggest to the increase in temperature of the medium, thus causing the decrease in the absorption of copper and zinc. There was likely a phenomenon of chemical release as shown in reaction Scheme 2, which explains the increase in the concentrations of the latter (TME) observed in the water in previous work in this period (Chitou et al., 2019).

There is a great affinity between organic matter and metallic trace elements (Zhao et al., 2012), which explain the strong correlation observed between the oxidizable fraction of copper, zinc and organic matter during the rainy season as shown by the circle of correlation. The proportion of the oxidizable fraction (F4) observed during the dry season was due to the gradual degradation of organic pollutants.

Lead and cadmium were also unequally distributed in all fractions as follows: F5> F2> F3> F1> F4 for lead, F5> F2> F1> F3> F4 for cadmium, with a much greater enrichment in the residual fraction during the rainy

season, unlike copper and zinc. During the dry season, the oxidizable and reducible fraction is the most dominant following the order F4> F3> F5> F2> F1 for lead, F3> F4> F5> F2> F1. The observed affinity of cadmium and lead proves that they were less mobile, unlike copper and zinc. The distribution of cadmium and lead in the different fractions was weakly influenced by pH. Metals (Cd and Pb) are more bioavailable at low salinity (Zhao et al., 2012). The salinity of the waters of Alibori River is zero, which explains the strong affinity of cadmium and lead with the F3 and F4 fractions and hence their weak bioavailability.

The individual contamination factors (ICF) of Zn, Cu, Cd and Pb recorded during this study were in the range (0.14; 69.42), which proves that the contamination of sediments by TME constituted potential risks of contamination for the river's water. The calculated RAC (Risk Assessment Code) showed a risk of bioavailability ranging from medium to very high for these TMEs. The bioavailability of these TMEs for the aquatic environment is linked to their affinity with the exchangeable and acid-soluble fractions as the binding (particles-metals) and (carbonate-metals) is weak in these fractions (Perin et al., 1985).

This affirmation supports the strong correlation between Znspech and ZnspRAC, Cuspacd and CuspRAC, Pbssacd and PdssRAC, Cdssech and CdssRAC as revealed by the circles of correlations. The problem of bioavailability risk posed by these TMEs for the aquatic environment is believed to cause the disappearance of taxa from the Alibori River as reported by Thierry et al. (2016). Indeed, according to Chouti (2011), these benthic species do not tolerate an average concentration of 0.77 mg/kg cadmium. The risk of bioavailability can lead to the disappearance of aquatic species owing to the anomalies caused by the TME in their body (Okoro et al., 2012; Wang et al., 2017).

Conclusion

The sediments of the Alibori River were contaminated with metallic trace elements. The results of our analyzes is revealed an accumulation of these metallic trace elements in the sediments with concentrations greater than the standard for Zn, Cu and Cd and high contamination indices.

Therefore, the chemical quality of the sediments constituted a risk for the aquatic resources of the Alibori River.

The study of the speciation of Zn, Cu, Cd and Pb showed that these elements were distributed in all fractions of sampled sediments with a high proportion in the exchangeable fraction and acid-soluble for zinc and copper, particularly during the rainy season. The fractions
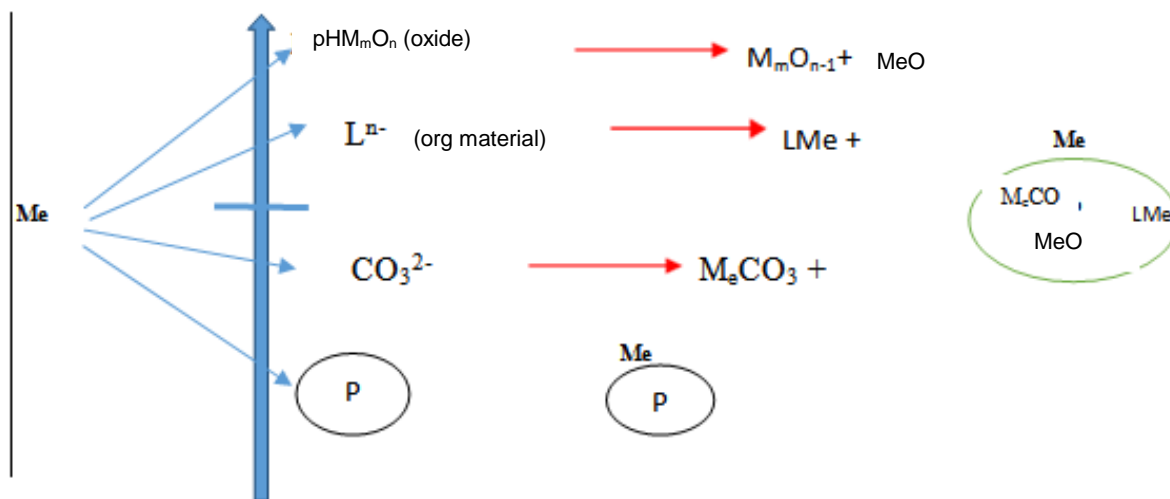
Scheme 1. Contamination of sediments by the different geochemical fractions of ETMs depending on the pH.

Contaminated sediment   pH   **Contaminated water**
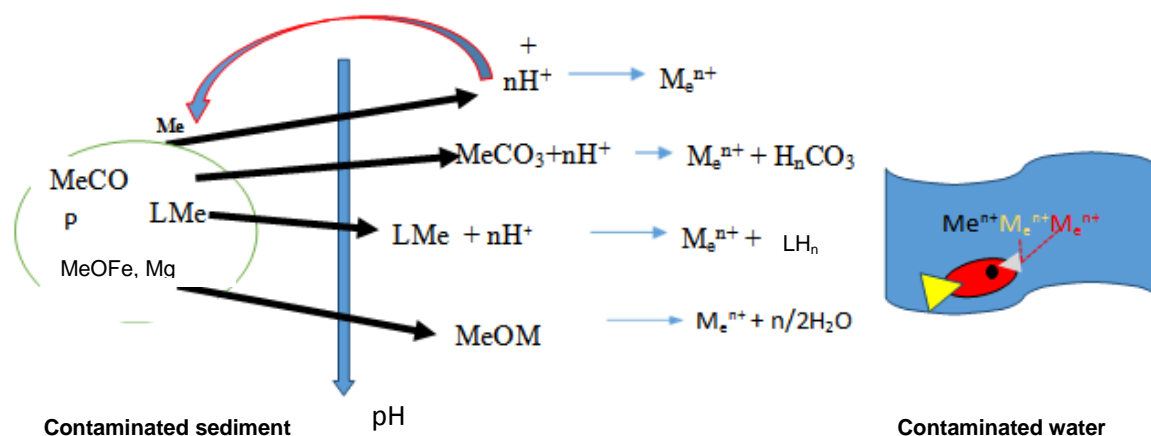
Scheme 2. Release of ETMs trapped in the different geochemical fractions of the contaminated sediments as a function of the pH.

of Zn and Cu were however quite mobile, suggesting that these elements may be readily available to organisms.
In addition to metals, pesticides also contain active molecules which are persistent toxic pollutants and very dangerous for aquatic species and humans. Thus, it will be relevant to screen the contamination of the Alibori River by active molecules from pesticides under the next stage of our work on the assessment of the impact of the use of agricultural inputs on this river.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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