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A study of the subfossil oospores of *Nitella opaca* (Charophyceae) from Megene Chitane (Tunisia)

*Najat ELKHIATI [a], Ingeborg SOULIÉ-MÄRSCHE [b], Mohammed RAMDANI [*c] & Roger FLOWER [d]*

[a] Faculté des Sciences Ain Chock, km 8, route Eljadida, BP 5366, Casablanca, Maroc

[b] ISEM, Laboratoire de Paléobotanique, UMII, Place E. Bataillon, F-34095 Montpellier, France

[c] Institut Scientifique, DZEA, BP 703, Rabat-Agdal, Maroc

[d] Geography, University College of London, 26 Bedford Way, London, UK

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Abstract — The only previous study on the charophycean flora of Tunisia was that of Corillion (1977), which described the presence of *Nitella capillaris* in the northern Tunisia. However, no site description was given and the species was not described in detail. The present study concerns the presence and the examination of charophycean oospores in a sediment core from a small soft-water Tunisian lake (Megene Chitane). The presence of *Nitella opaca* oospores in the sediment was established. This record extends the biogeographical distribution of the species in the temperate zones of North Africa (Rif in the North of Morocco and the North West of Tunisia). The distribution of the oospores down the core sediment was very heterogeneous: high abundances occurred between 33 to 45 cm sediment depth, whereas low abundances characterised the upper part of the core (0-30 cm). These sediment core results demonstrate the former abundance of this species in Megene Chitane, but lower numbers of oospores recorded in the most recent upper lake sediment suggest that the plant is becoming less common. Reduction in water levels and enrichment are thought to have degraded the site in recent years and environmental stress appears to be threatening the submerged aquatic plant communities in this lake. Acid lakes are very rare in Tunisia and the disturbance of Megene Chitane is most clearly related to increased human activity. Megene Chitane warrants conservation management to restore both water quality and water level.

acidic lake / Characeae / core sediment / freshwater algae / *Nitella opaca* / oospores / palaeolimnology

Résumé — Étude des oospores subfossiles de *Nitella opaca* (Charophyceae) de Megene Chitane (Tunisie). Le seul travail entrepris sur la flore charologique de Tunisie a été réalisé par Corillion (1977) à partir d'échantillons rapportés par des naturalistes. L'auteur a mentionné la présence de *Nitella capillaris* dans le Nord-Est du pays sans donner de précisions sur l'espèce ni sur le site. La présente étude est réalisée sur les oospores issues d'un carottage prélevé au centre du lac Megene Chitane dans le Nord de la Tunisie. Les analyses

* Correspondence and reprints: e-mail : ramdanimohamed@yahoo.fr

confirment la présence de *Nitella opaca* dans ce site à pH acide et permettent d'étendre l'aire de répartition biogéographique de l'espèce aux régions tempérées de l'Afrique du Nord (le Rif au Nord du Maroc et le Nord-Ouest de la Tunisie). La répartition des oospores le long du carottage est très hétérogène : une densité importante apparaît entre 33 et 45 cm, alors que les sédiments de la partie supérieure (entre 0-30 cm) sont caractérisés par une faible abondance. Ces résultats confirment la présence de l'espèce dans ce lac. Le petit nombre d'oospores dans la partie supérieure du sédiment témoigne que l'algue est devenue moins abondante dans ce site. La baisse du niveau d'eau et l'enrichissement en nutriment seraient à l'origine de la dégradation du milieu durant les dernières années. Le stress environnemental menace les plantes submergées dans ce lac. Les hydrosystèmes acides sont rares en Tunisie et la perturbation de ce site par le comblement du lac qui reçoit de moins en moins d'eau durant la dernière décade, doit être imputée, en majorité, à une activité humaine croissante autour de Megene Chitane. Cette zone humide nécessite un aménagement de conservation afin de restaurer à la fois la qualité et le niveau d'eau.

algues d'eau douce / carottage / Characeae / lac acide / *Nitella opaca* / oospores / paléolimnologie

INTRODUCTION

The present work arose from the pluri-disciplinary research project 'CASSARINA' (Flower *et al.*, 2001), an international collaborative investigation of nine wetland lakes in North Africa (Morocco-Tunisia-Egypt). During this study one site was found to be unusual in that the water was acidic. Megene Chitane was only about 1 m deep in 1997 and subsequent analysis of a sediment core from this lake revealed the presence of unusual charophycean oospores.

Multi-proxy analysis of sediment cores from Megene Chitane was undertaken during the CASSARINA Project and the results for diatoms (Flower *et al.*, 2001), pollen and macrofossils (Birks *et al.*, 2001), sediment dating (Appleby *et al.*, 2001), and zooplankton remains (Ramdani *et al.*, 2001 b) are described elsewhere. The aim of this study is to bring new palaeolimnological techniques to the study of aquatic plant communities in the Tunisian hydrosystem. These techniques allow for a better understanding of the distribution and persistence of Charophycean species in North African standing waters. Furthermore, the presence of oospores of these species can be regarded as excellent bioindicators for comparing past and present environmental conditions (Soulié-Märsche, 1991).

MATERIAL AND METHODS

Megene Chitane is located on Djebel Chitane at an altitude of 150 m (area of Sejnane) about 2 km from the Mediterranean Sea (Fig. 1) in NW Tunisia. It is a sub-circular lake *ca* 90 m in diameter and is 2 ha in surface area in winter, and about 1 ha in summer. The water depth (in 1997-99) varied between 40-100 cm. The geographical location co-ordinates are 37°11'N-09°10'E. The lake is fed by rainwater and by two springs located at the north-western end of the catchment area (Fig. 1). Annual average rainfall is 500 mm and the annual average temperature is 18°C (Kraiem & Ben Hamza, 2000).

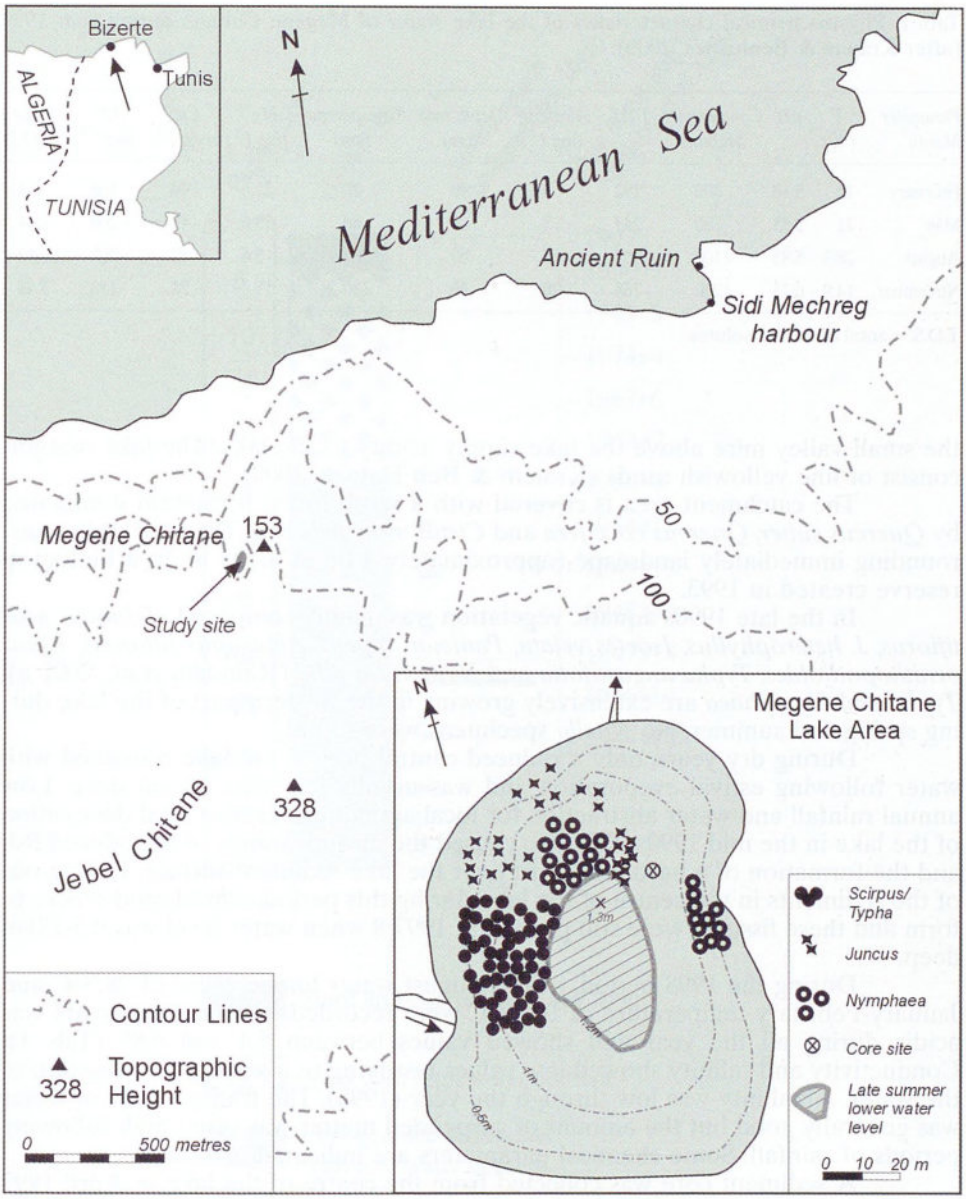

Fig. 1. Location of Megene Chitane in the Sejnane region, North of Tunisia.

The bedrock consists of Oligocene sandstone and flysch. The sandstone aquifer constitutes the principal water supply to this lake. The semi-permanent water sources are two springs, located in the north of the basin some 400 m upslope from the lake. Both the lake and the aquifers are closely linked and dependent on the quantity of the annual rainfall. The chemical nature of the aquifer and

Tab. 1. Physicochemical characteristics of the lake water of Megene Chitane during year 1998 (after Kraiem & Benhamza, 2000).

Parameter *Month*	*T* *(°C)*	*pH*	*Conductivity* *MOhms*	*T.D.S.*	*Alcalinity* *(mg l^{-1})*	*Depth max* *(cm)*	*Transparency* *(cm)*	*Mg^{++}* *(mg l^{-1})*	*Ca^{++}* *(mg l^{-1})*	*Cl^{-}* *(mg l^{-1})*	*Silica* *(µg l^{-1})*
February	12	5.38	300	192	3	90	80	21.6	40	160	3.3
May	21	5.55	380	243	3	80	80	19.6	43	200	1.44
August	28.3	5.83	1100	704	6	60	50	3.6	40	200	1.83
November	14.9	6.75	1200	768	70	40	30	11.2	28	150	3.46

T.D.S. = total dissolved solutes.

the small valley mire above the lake supply acidity to the lake. The lake margins consist of fine yellowish sands (Kraiem & Ben Hamza, 2000).

The catchment area is covered with a scrub-forest formation dominated by *Quercus suber, Quercus coccifera* and *Cistus monspeliensis*. The lake and its surrounding immediately landscape (approximately 4 ha in area) lie in a biological reserve created in 1993.

In the late 1990s aquatic vegetation was mainly composed of *Juncus acutiflorus, J. heterophyllus, Isoetes velata, Panicum repens, Aeluropus littoralis, Lotus ornithopodioides, Typha angustifolia* and *Nymphaea alba* (Ramdani *et al.*, 2001 a). *Typha* and *Nymphaea* are extensively growing in the western part of the lake during spring and summer. No *Nitella* specimens were found.

During dry years, only a reduced central part of the lake remained with water following estival evaporation and was usually less than 50 cm deep. Low annual rainfall and water abstraction for local agriculture caused total desiccation of the lake in the mid 1990s. This also caused the disappearance of introduced fish and the formation of a hardened crust over the lake sediment surface. Drying out of the sediments in the centre of the lake during this period caused mud-cracks to form and these fissures were still present in 1997/8 when water level was 0.5-1.0 m deep.

During the 1998 period, a July-August water temperature of 28.3°C and January-February temperature of 10-12°C were recorded. The lake water pH was acidic during all the year and showed values between 5.4 and 6.85 (Tab. 1). Conductivity and salinity showed low values testifying to a weak mineralisation of the water. Alkalinity was low through the year (1998). The transparency of water was generally good but the amount of suspended matter was often high following periods of rainfall. Some chemical parameters are indicated in Table 1.

A sediment core was collected from the centre of the lake in April 1997 (37.152760N-09.097810E) (Flower *et al.*, 2001; Ramdani *et al.*, 2001 b). The core was 7,5 cm in diameter and ~60 cm long. The sediments showed a hard and compact surface layer (2 cm) of a brownish colour, overlying a thick layer of black finer sediment with higher water content (Fig. 2). The core was cut every centimetre and kept in sealed plastic bags until return to the laboratory. After washing and sieving of 10 cm^3 sub-samples of sediment and washing through a 50 µm mesh, the charophycean oospores were sorted and isolated under a binocular microscope. All complete specimens were measured with a Leitz micrometer screw. Cleaning proved necessary as the outer surface of the oospores was obscured by adhering sediment particles. Several oospores were subjected to

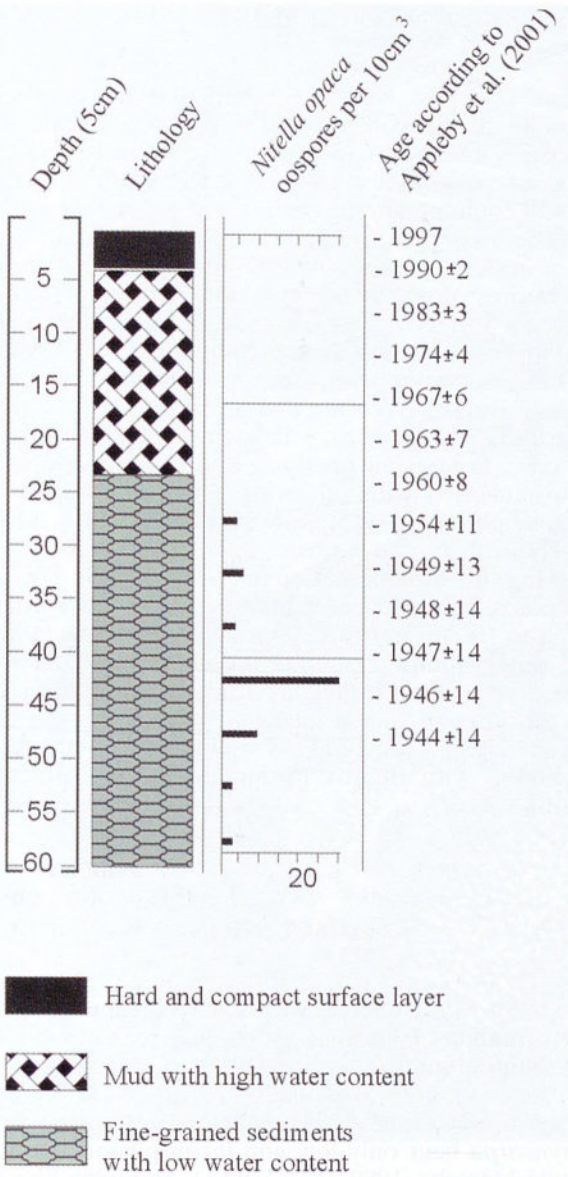

Fig. 2. Vertical distribution of the *Nitella* oospores in the core SHEE 1/A.

treatment with a 4 % potassium hydroxide for cleaning purposes. After 24 h, the oospores were washed in distilled water and ultra-sonicated for 3-5 minutes in order to reveal the fine-structure of the oospore wall. The oospore wall ornamentation was investigated using Scanning Electron Microscopy. The material is stored at the Palaeobotany Laboratory of the University of Montpellier II (Sciences and Techniques). Dating of the sediment core is described in Appleby *et al.* (2001)

RESULTS

Characeae oospores were commonly present between 25 and 60 cm depth. The sediments of this core showed a high concentration of these remains at 43 cm depth corresponding to an age of only 50 years (Fig. 2). Several megaspores of the *Isoetes* type were also identified. These probably derive from *Isoetes velata*, a species still common among the present vegetation in the lake, (Fig. 3, e).

Most of the fossil plant remains were charophycean oospores whose laterally compressed outline indicated without ambiguity that they belonged to the genus *Nitella*. Oospores of *Nitella* are generally identified according to the structure of their external surface which, for most of the species, shows characteristic ornamentation. However, the specimens from Megene Chitane display smooth oospore walls. This observation is at variance with the description of *N. capillaris* (Krocker) Groves & Bullock-Webster, the only *Nitella* species previously recorded from Tunisia (Corillion 1977, map 8), which cannot, therefore, be the source of the oospores in the core. Indeed, all previous studies described the oospore wall of *N. capillaris* as ornamented with tubercles, warts or papillae (Leonhardi, 1864; Stroede, 1933; Corillion, 1957; 1975; Roweck, 1986; John & Moore, 1987; Soulié-Märsche, 1989; Haas, 1994 and Krause, 1997). Details of the oospore wall of *N. capillaris*, showing this ornamentation are illustrated in Fig. 3, f.

The oospores from Megene Chitane are therefore attributed to another species. According to the reference keys in John & Moore (1987), Krause (1997) and to personal observations on herbarium specimens and living material, we conclude that the oospores in Chitane are from *Nitella opaca* (Bruzelius) Agardh. (Fig. 3 a-d). The oospores of *Nitella opaca* are dark-brown to russet-red, laterally compressed and 325-400 µm long and 300-350 µm wide; on average 6-7 striae are visible in lateral view with slightly prominent flanges and the surface of the oospore is smooth.

DISCUSSION

The two main *Nitella* species with smooth oospores are *Nitella opaca* and *Nitella syncarpa* (Thuillier) Chevalier. According to Krause (1997), they can be distinguished by their colour, the oospores of *N. syncarpa* being brilliant black. In addition, the presence of very prominent flanged striae of russet-red colour in *N. opaca*, was noted (Corillion, 1957; Krause, 1997). By way of contrast, the oospores of *N. syncarpa* bear only low and inconspicuous striae (John & Moore, 1987, fig. 10; Soulié-Märsche, 1989, Pl. VI, fig.1). The wing-like striae of *N. opaca* are extremely fragile and subjected to erosion when the oospores become sedimented. Nevertheless, the material from Megene Chitane still shows low striae like crests between the fossa.

These distinct remains of the striae and the dark brown colour in the Chitane material point to *N. opaca*. Further, *N. syncarpa* has never been found in North Africa (Corillion, 1957) whereas *Nitella opaca* was described from five localities in the Rif of Morocco (Guerlesquin, 1978) and has been recently found in three further Moroccan sites located at about the same latitude as Megene Chitane (Elkhiati, 1995). The Chitane core contains the first record of *N. opaca* for Tunisia.

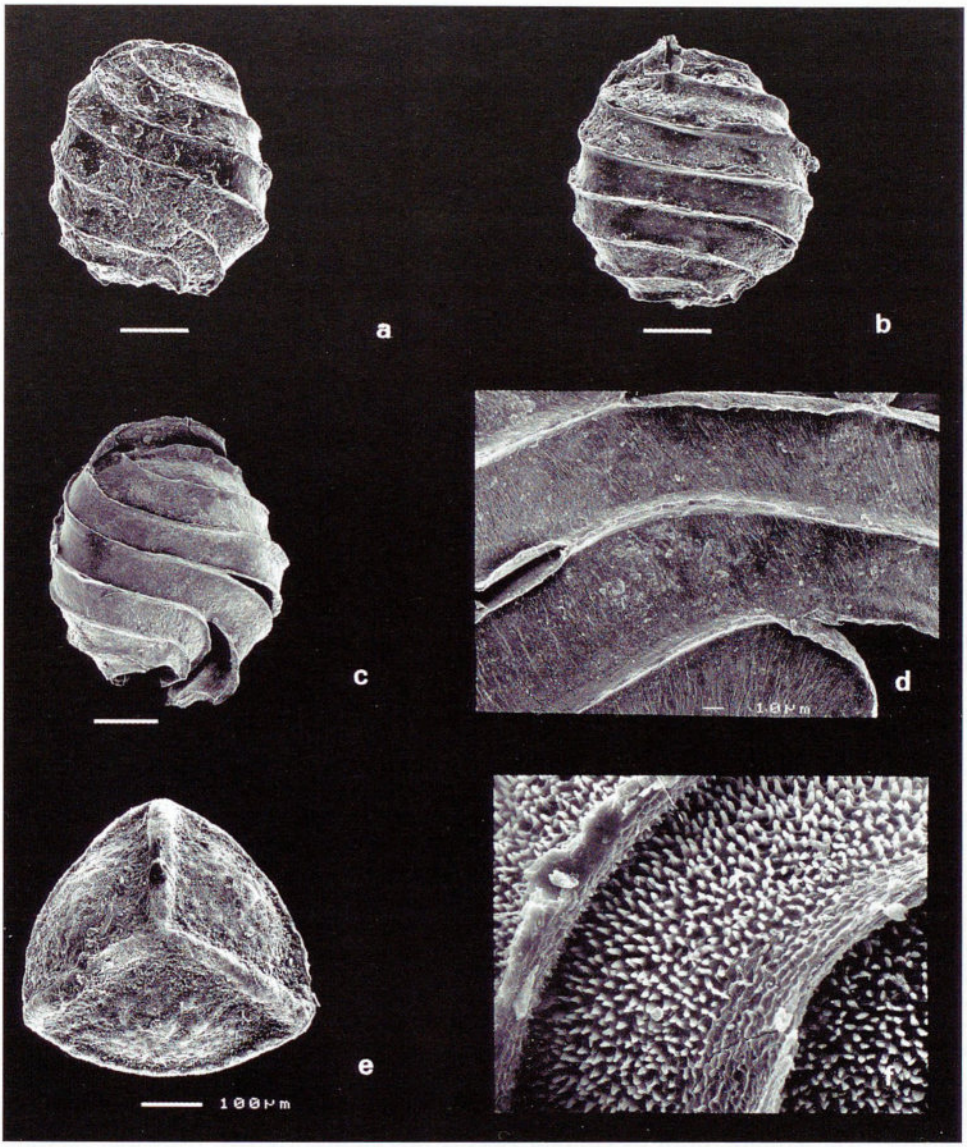

Fig. 3. Macrofossils from core SHEE 1/A. a-d, *Nitella opaca* from level 43 c; a, b, oospores before cleaning; c, oospore after chemical treatment and ultrasonication (same specimen as fig. 3a); d, *Nitella opaca*, portion of the oospore wall after treatment, showing the smooth surface; e, megaspore of *Isoetes*-type; f, *Nitella capillaris*, herbarium specimen from France, close view of the papillate ornamentation of the oospore wall (from Soulié-Märsche, 1989, Pl. VI, fig. 3). Scale bars are 100 µm for a, b, c, e; scale bar is 10 µm for figs d and f.

From a palaeolimnological point of view, the number of oospores at around 43 cm depth in the core SHEE 1/A (dated to the mid 1940s) seems to represent the optimal stage for the development of the charophycean flora in this

lake. This core section reflects a period when the lake was more oligotrophic. The presence of *Nitella* indicates the presence of acidic water during most of the earlier period represented by the sediment core. At present, the lake is still acid and this is maintained by acidity supplied by the peat bog in the upper part of the catchment and from the aquifer in the underlying catchment sandstone. The pH of the lake water was probably around 6 during most of the observation period (Table 1) and based on the acidophilous nature of the sediment core diatom assemblages (Flower *et al.*, 2001). The usual values for *N. opaca* occurrence are between pH 6-7 (Corillion, 1957; Krause, 1997). These authors also emphasise the frequent association of *N. opaca* with *Isoetes*.

At the site studied here, characean oospores were not encountered at any level above 25 cm depth in the core (corresponding to *ca* 1960 AD). This date seems to mark the disappearance of *Nitella opaca* at Chitane, whereas in a parallel core studied by Birks *et al.* (2001), oospores of *Nitella* were present up to the surface, although at low abundance. This difference could be a result of a slightly different position of the core studied by Birks *et al.* (2001), or to sediment disturbance. This latter core was nearer the centre of the lake, where standing water may persist for longer periods. In addition, the much greater quantity of sediment (50 cm^3) used for analysis by Birks *et al.* could affect the results. In any case, *Nitella opaca* is certainly much scarcer in the lake today as compared with a few decades ago. The species may now even be extinct at the site, since no specimens were found by modern surveys in 1997-1999 (Ramdani *et al.*, 2001 a).

CONCLUSIONS

Megene Chitane represents an unusual ecosystem for Tunisia. This is due to the physico-chemical characteristics of the water, its water supply coming from two acidic sources. *Nitella opaca* is a species rare in North Africa and its presence in North Tunisia is noteworthy. Its former abundance in Megene Chitane would have been entirely overlooked without the application of paleaolimnological techniques. According to the sediment core studied here, the species has disappeared from the littoral zone of the lake during the last 40 years. The sedimentation rate (of about 1 mm $year^{-1}$ calculated from sediment core dating) points to the progressive disturbance of the lake and reduction in water supply during the 20^{th} century.

The aquatic flora of Chitane seems threatened by two principal factors: firstly, the phenomenon of natural filling and, secondly, the acceleration of environmental change by anthropogenic influences. Use of fertilisers and water for agricultural practices around the lake affects both lake water level and water quality. These practices favour the proliferation of macrophytes such as *Lemna minor*, *Nymphaea alba* and *Typha angustifolia*, plants that are indicators of eutrophication. Their increasing abundance during the recent decades (Birks *et al.*, 2001) suggests that this ecosystem is being rapidly degraded by human activity, with a consequent loss of natural biodiversity.

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