PRELIMINARY TLC STUDIES ON POSIDONIA OCEANICA SEAGRASS

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Abstract
Several photosynthetic pigments, i.e. chlorophylls and carotenoids, belonging to *Posidonia oceanica* seagrass, were separated by thin-layer chromatography (TLC) using a hexane/acetone solvent mixture. Additionally, densitometric measurements and spectral scanning of the TLC plates were performed using the Camag TLC Scanner 3 together with the WinCATS software.

Introduction
The use of several species of marine magnoliophytes, with the purpose of monitoring and managing the coastal ecosystems, was frequently discussed during recent years. Because of their wide geographical distribution, their longevity, the permanence of their populations during the seasons, the ease of sampling, their abundance, and their ability to concentrate a wide range of xenobiotics, marine magnoliophytes appear as very interesting organisms in the context of environmental monitoring. Among the many studies of magnoliophytes, *Posidonia oceanica* (Fam. *Posidoniaceae*) appears of particular interest.

Figure 1. *Posidonia oceanica* on the Tunisian coast

In the Mediterranean Sea, the *Posidonia oceanica* meadow is a powerful integrator of the overall quality of marine waters [1-3]. Very widely distributed throughout the coastline, which is particularly "receptive" to pollution, and connected to the sea bottom, it indicates by its presence and vitality (or its regression materialized by dead ecosystems) the quality of the water that drifts above it.

This paper represents a preliminary study regarding the chemical composition of *Posidonia oceanica* leaves. We first determine the optimal thin-layer chromatography (TLC) eluent which allows the separation of a maximum number of components, i.e. photosynthetic pigments [4-6]. In the second step, an identification of the isolated pigments was attempted by using UV/Vis densitometry directly on the TLC plate.

Experimental

The sample was collected from the Tunisian coast during May 2017. After harvesting, the sample was washed with tap water for several times, was dried and preserved on ice (-28 °C) for further processing. 50 g of seagrass were processed using a blender and extracted in methanol for 24 h using a Soxhlet. Next, the sample was filtered and the solvent was removed using a rotary evaporator. The evaporated sample was redissolved in methanol by stirring for a couple of minutes, and then placed on the TLC plate (10 by 10 cm TLC Silica gel $60F_{254}$ from Merck). The plate was eluted in a developing chamber with a mobile phase composed of a hexane/acetone mixture (70:30 v/v). After drying in an oven, the densitometric evaluation of the TLC plate was performed using the CAMAG TLC Scanner 3.

Results and discussion

The TLC plate (Fig. 2) was scanned in the 200–700 nm spectral region. Most peaks were revealed at 250 nm (11 peaks) and only a few were found at 700 nm (4 peaks). Fig. 3a shows the overlaid chromatograms obtained at various wavelengths, while the densitogram at 450 nm is illustrated in Fig. 3b. The major photosynthetic pigments identified in *Posidonia oceanica* with the R_f and the corresponding maximum absorbance are shown in table 1.

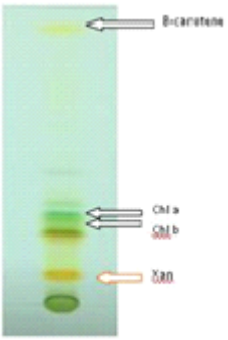

Figure 2. TLC plate with *the identified pigments*

Substance	R_f	Spectral data (nm)
Chlorophyll a	0.42	426, 662
Chlorophyll b	0.32	453, 643
β-carotene	0.81	451, 478
Xantophyll	0.31	421, 667

Table 1. The major photosynthetic pigments identified in *Posidonia oceanica* extract

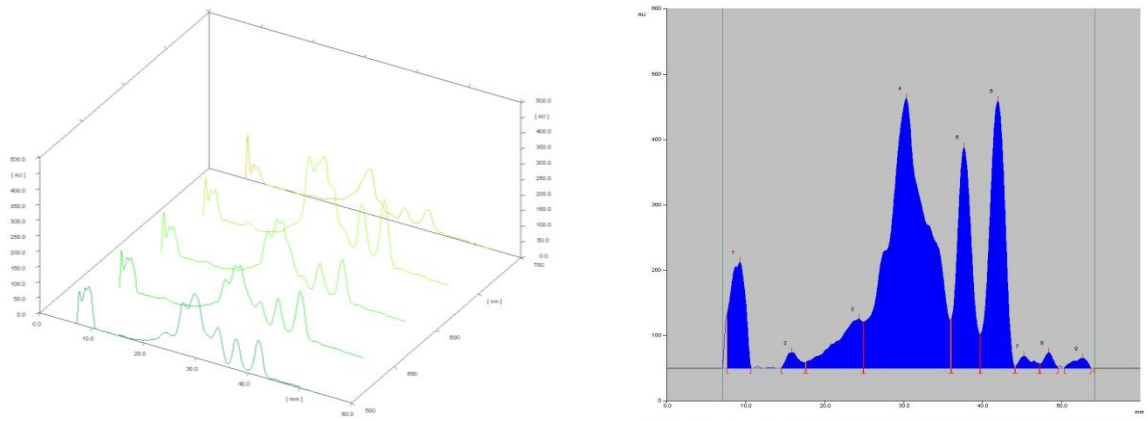

Figure 3. (a) Overlaid densitograms at various wavelengths of photosynthetic pigments, and (b) densitogram of detected peaks at 450 nm

Chlorophylls *a* and *b* (green photosynthetic pigments) are characterized by two absorption bands, located in the blue-violet and, respectively, red region of the visible domain (UV domain not shown here). Carotenoids are revealed as yellow or orange pigments. The migration of photosynthetic pigments was compared with standard samples under the same experimental conditions.

Conclusion

The densitometric analysis at different wavelengths, which reveals the presence of several peaks corresponding to various photosynthetic pigments, represents a starting point towards a more thorough chemical analysis of components found in *Posidonia oceanica*.

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