MATERIAL AND METHODS USED IN THE CEAB-CSIC TEAM MISSIONS FOR THE WADI PROJECT

I. INTRODUCTION

A descriptive and ecological study offers the oportunity to apply and improve different field methodologies that can be extremely useful not only to obtain a general descriptive view of the ecosystem but also to evaluate its environmental quality and to design a possible management and monitoring plan. The two sites visited so far in the WADI project gave the scenarios needed to work with this kind of methodology: the Mariut lake (Alexandria,Egypt), a highly polluted and hypertrophic water mass, and the Oued Tahaddart (Assilah, Morocco), an estuary presenting a good environmental status. Another mission is planned in the Ghar El Melh lagoon (Tunisia), where all the methods, described below, and new ones, are going to be intensively and extensively used.

II. SAMPLING PLAN

a) Establishment of stations

For both case study sites, stations were determined following either a pollution gradient, as was the case of lake Mariut, and a salinity gradient, as for the case of Oued Tahaddart. Three sub-stations per each station, aproximately 50 meters distance from one another, were established for both study sites.

a)

b)

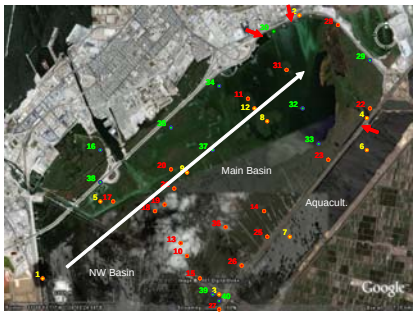


Figure 1. Situation of the different stations for both study sites. a) Oued Tahaddart. b) Lake Mariut, where the yellow dots correspond to full stations (all parameters described below were measured), green dots to intermediate stations (biomass and density were not measured) and red dots to simple stations (biomass, density and water nutrients and heavy metals were not measured).

b) Replication

In every sub-station, replication proceed as following:

- Water analysis:
  - Physical parameters: one water sample was collected in all sub-stations (full, intermediate and simple) and the different physical measures (oxygen, pH and conductivity) were carried out in the same water sample.
  - Nutrient and heavy metal content: one water sample was collected in full and intermediate sub-stations and the different analysis were developed in water aliquots taken from the same water sample.
- Vegetation samples:
  - Cover: every researcher (9 for the case of lake Mariut) evaluated the cover of the considered species within all sub-stations (full, intermediate and simple).
  - Density: for each species, three measurements were done in every full sub-stations.
  - Abundance: every researcher (9 for the case of lake Mariut) categorized the abundance of the considered species within all sub-stations (full, intermediate and simple).
  - Biomass: three individuals for every species in study (*Phragmites australis*, *Eicchornia crassipes*, *Typha domingensis* and *Echinochloa stagnina* in the case of the Mariut lake), were collected and weighted in every full sub-stations.
- Sediment samples: one sediment sample per each full sub-station was collected and they were divided into different aliquots in the laboratory for their further analysis (organic matter content, cabonates content, elemental and isotopical composition, heavy metals).
  - Redox potential: in Oued Tahaddart, six cores of sediment per each sub-station were collected, three of them in areas covered by *Zostera noltii* and the other three in unvegetated areas.

- Macrofauna and fishes: muscle tissue from three macroinvertebrate individuals from the same species was sampled in lake Mariut. Muscle tissue of four fish species was collected in a composite sample integrated by 2-3individuals from the lake Mariut main basin. Three of these species were sampled following the same protocol in the aquaculture basin near the lake. Three species of macroinvertebrates (*Carcinus sp*, *Upogebia sp* and *Palaemon sp*) were collected in each sub-station of Oued Tahaddart, keeping the whole individuals on a stove until their transportation into laboratory.

III. PHYSICOCHEMICAL WATER ANALYSIS

a) Physical parameters:
Different physical parameters of water were measured within the process of characterization of the lake Mariut body mass.

a.1) Oxygen concentration:
This parameter was measured by means of an oxymeter Oxi 340i/SET WTW. The values given were considered both as a percentage (%) and as concentration in water (mg/l).

a.2) pH:
The pH was measured directly in the water samples using a pHmeter (Crison Instruments, GLP 21).

a.3) Conductivity:
The conductivity was measured with a conductimeter (Orion Scientifics). The values were given in mS.

a.4) Temperature:
An specific instrument to measure temperature was no needed, as the three sensors descrived above (oxymeter, pHmeter and conductimeter) gave each of them a temperature value. The final value used for the data analysis is the mean value of the three meaures given in ºC.

b) Chemical parameters:
b.1) Nutrients:
A field photometer (Macherey Nagel, PF11 + Visocolor and Nanocolor – only for lead- kits) was used to measure the concentration (mg/l) of the following nutrients in the water of the lake: NH^{4+},NO^{3-},NO^{2-}, PO_4^{3-}. A sample of water was taken and mixed with one or two reactives for each different kind of nutrient analysis. The protocol steps to be followed were indicated on the spectrophotometer manual and they were all made *in situ*.

b.2) Heavy metals:
The same method was used to evaluate the heavy metal concentration (mg/l) in the lake Mariut water. The metals considered were the following:Cr^{2+}, Cu^{2+},Pb^{2+}.

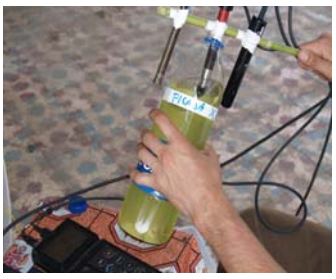
a)  b) 

Figure 2. Water analysis. a) Nutrients and heavy metals evaluation by means of a field photometer. b) Physical water conditions evaluation by the use of three different sensors (oxymeter, pHmeter and conductimeter).

IV. VEGETATION ANALYSIS

a) Cover:
The vegetation cover was evaluated visually in terms of percentage of vegetation for each studied species in all considered stations using the following classes: 0, 10, 25, 75 and 100%. In order to diminish subjectivity, one value for every species in study (*P.australis*, *E.crassipes*, *T.domingensis* and *E.stagnina* in the case of the Mariut lake) was given by each researcher participating in the mission, so that each value could be considered as one replica.

b) Density:
The vegetation density (number of shoots/m^2) was measured for the most representative species (see III, a), using replicate quadrats (100x100 cm) randomly placed over the considered station area excluding zones with zero cover. When a species became to be very productive, its density estimation was usually complex and, to avoid this difficulty, divisions of 50x50 cm in the quadrats were done. The

density value refered to a 50x50cm quadrat could be converted into a value refered to a 100x100 cm quadrat thereafter.

a)  b) 

Figure 3. Replicate quadrats (100x100cm) used to measure vegetation density. a) *Echinochloa stagnina.* b) *Eicchornia crassipes.*

c) Abundance:

In each station, visual density categories (from 1 to 5) were assigned independently by each researcher for all the species in study. Regression analysis showed a good agreement between categories and field measures (Fig. 4). The regression equation was then used to convert the visual abundance values into measured density values. Once a sufficient number of field observations (categories) have been made (to satisfy regression procedures), the visual categorization approach can be used alone in an extensive a fast way over the lake.

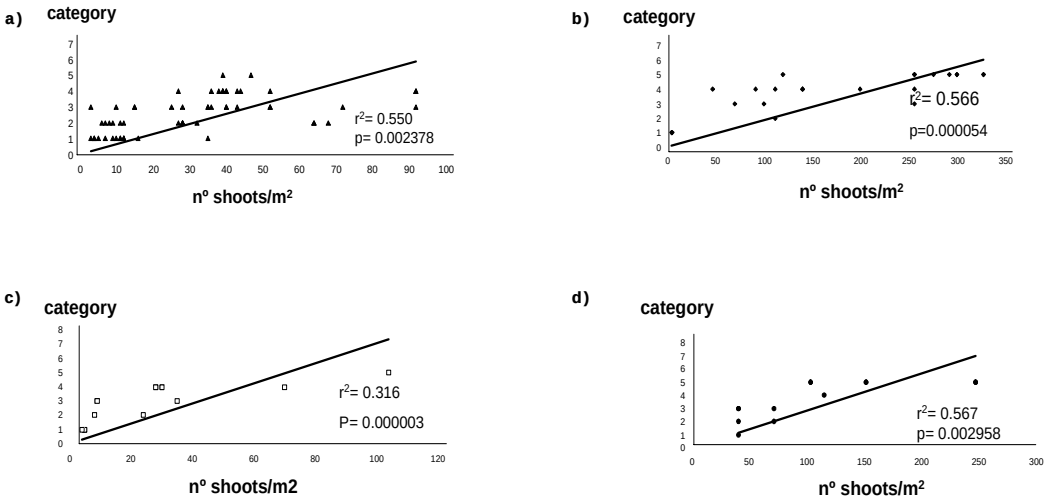


Figure 4. Regression lines between the abundance category and density (nº shoots/m^2) for the main species studied in the lake Mariut. Regression coeficients (r^2) and p value for significance are shown. a) *Phragmites australis.* b) *Eicchornia crassipes*. c) *Typha domingensis*. d) *Echinochloa stagnina*.

d) Biomass:

The studied species (*P. australis*, *E. crassipes*, *T. domingensis* and *E. stagnina* in the case of the Mariut lake) were separated in roots, leaves and steam and weighted *in situ* with a field balance. Three replicates per station were considered for each species. Some samples were oven-dried at 70ºC until constant weight and weighted again in order to calculate the fresh weight/dry weight ratio for each fraction of every species considered. The fresh weight measured in field could be converted into dry weight by means of this ratio and, after multiplying the resulting value for the corresponding density, a biomass value for the considered patch refered as gr dry weight/m^2 could be obtained. The density of a given station could be calculated by multiplying again this biomass value (gr dry weight/m^2) for the cover of each station, what would result in a biomass value for the total station (gr dry weight/m^2). Aerial image and processing will be used to estimate the total lake biomass using the Geographic Information System Program ArcGIS v.8.1. This program provides the tools needed to determine the different vegetation polygons and their surface, what could be thereafter converted into biomass values for the total lake vegetation. A cartographic characterization of the lake Mariut, including vegetation and water conditions (all data recorded was georeferenciated), is planned to be designed using the informatic program descrived above.

e) Elemental and isotopic analysis (CNP, $\delta^{13}C$, $\delta^{15}N$):

All plant material collected (10 species in total for the Mariut lake, 1 species for Oued Tahaddart) was dried at 70ºC until constant weight, ground to powder and stored in low humidity conditions prior to isotopic analysis. Elemental and isotopic composition were measured from the gasses evolved from

sample combustion in a Finnigan Delta S isotope ratio mass spectrometer (Conflo II interface) at the Scientific-Technical Services of the University of Barcelona. Isotopic values are reported in the δ notation relative to the standards Vienna Pee Dee Belemnite for carbon and air for nitrogen (δsample = [(Rsample/Rstandard) – 1] x 1000, R = 13C/12C, or R = 15N/14N). Analytical precision based on the standard deviation of internal standards (atropine, IAEA CH3, CH6, CH7, and USGS40 – analytical grade L-glutamic acid, for carbon, and atropine, IAEA N1, NO3, N2, and USGS40, for nitrogen) ranged from 0.06 ‰ to 0.11 ‰ (mean = 0.09 ‰) for carbon, and from 0.06 ‰ and 0.28 ‰ (mean = 0.16 ‰) for nitrogen. After digesting the sampled dry plant tissues in acid solution, phosphorus was also mesured by optic ICP.

f) Heavy metals:
The following metals are going to be determined in every fraction of the species in study: iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), nickel (Ni), lead (Pb), cadmium (Cd) and chromium (Cr). The dry tissues are digested in an acid solution and metals analysed by optic ICP (for Fe, Zn and Mn) and mass ICP (for the rest). Heavy metal content in aquatic macrophyte tissues is correlated to trace metal in the environment.

V. SEDIMENT CHARACTERIZATION

a) Organic matter content:
Sediment samples were collected in each station and kept freezed until the end of the mission. Once in the laboratory, sediment samples were dried at 70ºC until total dehydration. An aliquot of 200 mg was weighted and placed in the oven at 450ºC for 5 hours. After weightening again the samples, the weight loss corresponded to the sediment organic matter content, as it represents the organic matter burned in the combuestion process.

b) $CaCO_3$ content:
The sediment samples carbonates content was estimated by the acidification of an aliquot of 150 mg aprox. for each sample. Sediment aliquots were weighted, treated with hydrochloridic acid 0,25M during 15 minutes in order to remove carbonates and dried in a stove at 70ºC until constant weight to eliminate the excess of hydrochloridic acid. The samples were weighted again and the weight loss was considered as their carbonates content.

a)  b) 

Figure 5. Sediment sampling methods used. a) Plastic small container to sample the sediment from the bottom of the lake Mariut. b) Sediment sampling in the lake Mariut.

e) Elemental and isotopic analysis (CNP, $\delta^{13}C$, $\delta^{15}N$):
Elemental and isotopic composition of the sediment samples were also analysed after acidifying the samples (see V.b) to remove inorganic carbonates. The protocol followed was the same than in the case of the plant samples (see IV.e)

f) Heavy metals:
The protocol used to analyse heavy metals in the sediment samples was the same than the one descrived for the plant samples (see IV.f).

g) Redox potential:
Redox potential measurements in sediment cores were carried out in Oued Tahaddart (6 cm diameter, 30 cm long). One mm diameter holes were done in the cores from top to bottom, at 27 mm from each other following a spiral pattern. The redox potential was measured in every hole using an oxidation-reduction potential (ORP) platinum electrode connected to a voltimeter (Crison Instruments, GLP 21) and after equilibrating the electrode for a few seconds. Final readings were corrected by adding the potential (+199 mV) of a silver/silver-chloride reference electrode.

a)  b) 

Figure 6. Potential redox measurements in Oued Tahaddart. a) Measuring the potential redox profile in a sediment core. b) Potential redox sensor.

VI. MACROFAUNA AND FISH

a) Elemental and isotopic analysis (CNP, $\delta^{13}C$, $\delta^{15}N$):

Elemental and isotopic composition were measured in macrofauna (1 species for Mariut lake, 3 species for Oued Tahaddart) after acidifying the samples in order to remove inorganic carbonates (see V.b). Both analysis for macroinvertebrate and fish samples (four species for Mariut lake) were developed following the same protocol than in plant samples (see IV.e).

b) Heavy metals:

Heavy metal content in macroinvertebrates and fish tissues was evaluated through the same guidelines than in plant samples (see IV.f).

CEAB-CSIC team