## **Evolution of Enterobacterial Populations along the Aljezur Costal Stream** LABORATÓRIO NACIONAL DE ENGENHARIA CIVIL



# as Correlated to the Occurring Physical-Chemical Characteristics of the Water

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## INTRODUCTION

Fecal coliforms (FC) and fecal enterococci (FE) are indicators of enteric pathogenic organisms in aquatic environments, including bathing waters. There, sewage effluents, agricultural runoff and wildlife are important sources of such surrogates. After being discharged into the water their numbers decrease with time due to sedimentation, dilution and death. While solar UV-irradiation is believed to be the major cause of bacterial death in stream and marine waters, indicator die-off rates also depend on salinity, temperature, nutrient availability and predation. Owing to their potential to release hepato-, neuro- and dermato-toxic cyanotoxins, the occurrence of cyanobacterial blooms are added hazards to bathers.



With the objective of understanding the behaviour of fecal indicators populations, as well as the associated factors, the evolution of FC and FE numbers, and of a number physical) chemical parameters was studied along the Aljezur stream. In addition, the occurrence of algal blooms was assessed by determining chlorophyll a in water samples.

A series of surveys, which covered different seasons and samples collected over entire tidal cycles from 10 monitoring stations, was carried out. Sampling was done along ca. 8 km upstream of the stream mouth.

Samples were collected upstream (Stations 1A-1B) and downstream (Stations 3-9) of the Aljezur wastewater treatment plant (WWTP) and downstream of the fish farming plant (Stations 8-9) and wetlands (Station 9).

#### **METHODOLOGY**

Sampling was done close to synchronously at intervals of ca. 3 h, over 9 h periods. After collection samples were kept refrigerated and transported to the WWTP laboratory within 30 min. There they were analysed for pH, turbidity and membrane-filtered for microbiological analysis. The membranes were immediately laid on the plate counting-medium without delay, kept refrigerated and transported to LNEC laboratory in Lisbon, where their incubation was started ca. 5 h after the last sampling event. Except for temperature and dissolved oxygen (DO), which were measured in situ, the remaining parameters were determined within a few days at LNEC laboratory in samples that were kept at 4°C.

The Pearson's coefficient (r) was used as a measure of the correlation between parameters (p<0.05).

#### **RESULTS AND DISCUSSION**

#### General

Comparable trends were observed for the results obtained from two spring (May 2008) 2009) surveys.

Parameter	Analitycal method
Fecal coliforms (FC)	Membrane filtration / Colilert medium
Fecal enterococci (FE)	ISO 7899-2
Turbidity	Nephelometry
SST	Membrane filtration / dry weight
NH <sup>4+</sup>	Spectrophotometry
NO <sub>3</sub> <sup>-</sup>	Spectrophotometry
SiO <sup>4-</sup>	Spectrophotometry
CBO <sub>5</sub> (20°C)	Manometric
Chlorophyll a	Spectrophotometry - Lorenzen
Dissolved oxygen	Specific probe
UVA extinction [Kd(360 nm)]	UV spectrophotometry - 5 cm light path
UVB extinction [Kd(305 nm)]	UV spectrophotometry - 5 cm light path

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Except for S7, stream CBO was regularly around or lower than the detection limit (1 mg O<sub>2</sub>/L). Accordingly, no indicator regrowth was detected.

In the two surveys S7-CBO reached values around 10 mg O<sub>2</sub>/L. However, such raises were clearly due to algal biomass development, as they were locally accompanied with chlorophyll a increments in the water.

#### **Microbial decay**

After a raise due to the WWTP discharge, that was more perceptible for FC, the numbers of both fecal indicators decayed along the stream.

However, such behaviour can no be ascribed to UV-driven die-off, as the observed typical UVB water-Kd values (/cm) indicated that >90% of UV radiation were extinct while penetrating ca. 20 cm of the water column.

#### **Correlation between parameters**

Strong correlations were observed between the concentration of FC and N<sub>total</sub> (r > 0.96) or PO<sub>4</sub><sup>3-</sup> (r >  $^{3-}$ 0.82). While these parameters are not directly associable to bacterial die-off, their decay accompanied that of FC. Such analogy in behaviour was possibly due similar rates of dilution by tide water or transfer to bed sediments.

Less significant (r < 0.70) correlations were found for FE.

In S5, S6 and particularly in S7 chlorophyll a was found at relatively high concentrations (up to 100 mg/L, which correlated well with those observed for  $PO_4^{3-}$  (r = 0.86) and  $NH^{4+}$  (r = 0.92), but not with SiO<sub>4</sub><sup>4-</sup> levels. These results show the promoting influence of nutrient compounds on the development of algal blooms. Furthermore, since no increased  $SiO_4^{4-}$  concentrations were found in bloom water samples, diatoms were not predominant in the developed algal mass. Therefore, the occurrence of toxin producing microorganisms (e.g., cyanobacteria) is probable instead and needs to be investigated.





### CONCLUSIONS

- . As expected the number of fecal indicators decays along the Aljezur stream, although not apparently due to solar irradiation;
- . At least for spring conditions, P and N behaviour approaches that of fecal coliforms in the Aljezur stream;
- . There, the occurrence of cyanotoxin producing microorganisms is likely to happen and should, therefore, be investigated

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