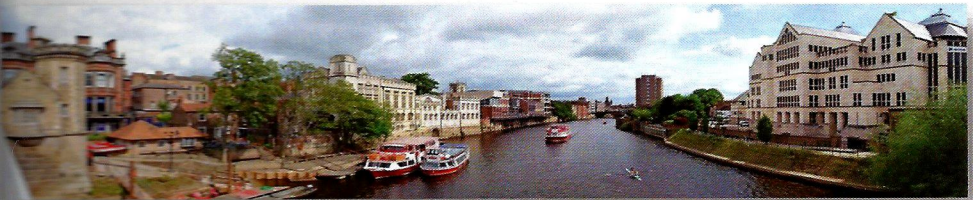


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Assessment of culturable endophytic community of *Canna flaccida* plants growing in a constructed wetland

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INTRODUCTION

Sustainable water management urges for the treatment of wastewater in an efficient way. With the latter purpose, ecotechnologies arise as promising solutions for rural and mountain areas lacking a sewage network. Constructed wetlands (CW) coupled with preliminary/primary systems foresee enhancement of the water quality and its possible reuse.

The challenge presented in this work reflects the application of a CW to domestic wastewater treatment coming from a tourism house, situated in a rural area. The use of different ornamental plants species to treat the wastewater was an important component of the system, increasing biodiversity and supporting the local ecosystem. Performance of the CW was assessed in terms of physic-chemical and microbial parameters. The endophytic culturable bacteria of a selected plant species, *Canna flaccida*, were evaluated.

METHODS

A CW was implemented at Paço de Calheiros, a guest house, to complement a previously installed septic tank (Figure 1). The area considered was 40.5 m². An expanded clay substrate was used where different species were planted: *Canna flaccida*, *Zantedeschia aethiopica*, *Canna indica*, *Agapanthus africanus* and *Watsonia borbonica*. The previous selection had in consideration their presence and occurrence at the site, ornamental value to the guest house, and biodiversity promotion (Calheiros et al, 2015).



Fig. 1. Constructed wetland at a rural site with the selected plant species, *Canna flaccida*, under study

Removal efficiency in the CW was assessed in terms of physic-chemical and microbial parameters. Endophytic culturable bacteria from *Canna flaccida* were evaluated through isolation and enumeration of pathogenic or indicators organisms, within its root and shoot, from inlet and outlet zones, as described in Pereira and Castro (2014). Endophytic isolates were

grouped by random amplified polymorphic DNA (RAPD) and identified by 16S rRNA gene sequencing. This plant was selected based on the fact that was one of the most abundant and well established plant.

RESULTS AND ON-GOING WORK

Plants established successfully, although *A. africanus* was outcompeted by the other species that were fast-growing. In average, the removal efficiency for organics was above 90%, with up to 99% removal for COD (inlet: 481 (20–1467) mg/L) and 98% removal for BOD (inlet: 182 (10–480) mg/L).

Endophytic bacteria are being identified through 16S rRNA sequencing analysis allowing to withdrawn correlations between sampling zones (inlet and outlet) and plant parts (shoot and root). Overall, 218 bacterial endophytes were isolated from *C. flaccida* tissues. RAPD-PCR analysis was used to group isolates and 117 different profiles were recognized. One isolate of each cluster was chosen for 16S rRNA partial gene sequence analysis in order to identify the bacterial endophytes. Eighty-three strains were originated from *C. flaccida* roots (CR) and 34 strains from shoots (CS).

Evaluation of the presence of pathogenic organisms within the plant will bring further insight and knowledge concerning the survival of such species.

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