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# Occurrence and removal of parasites, enteric bacteria and faecal contamination indicators in wastewater natural reclamation systems in Tenerife-Canary Islands, Spain

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# ABSTRACT

Two wastewater natural reclamation systems (WWNRS) have been compared regarding their efficiencies on faecal bacteria removal and the persistence of enteric pathogens. These WWNRS are constituted of a combination of anaerobic treatment, small sub-surface flow constructed wetland refilled of volcanic ashes and a final pond as water reservoir. Faecal coliforms, enterococci, *Escherichia coli, Clostridium perfringens*, somatic coliphages, *Salmonella* sp., *Campylobacter* sp., *Cryptosporidium* sp., *Giardia* sp. and helminth eggs were analyzed in constructed wetlands inlet and outlet and storage pond effluent. Low numbers of protozoan positive samples (4.54% in Albergue de Bolico for both protozoa, and 19.05% in Carrizal Alto for *Giardia* sp.) and absence of helminth eggs were found. Both systems demonstrated efficient reduction of faecal contamination indicators in the wastewaters (removal rates values of 2 log<sub>10</sub>). The natural systems for wastewater treatment used to be efficient in *Salmonella* abatement, this fact was confirmed in the reported systems, since enterobacteriaceae were found in only one of the effluents. *Campylobacter* species associated with the access of animals to storage ponds were detected in the reclaimed water.

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### 1. Introduction

Wastewater natural reclamation systems (WWNRS) imply an important economical saving and abatement of environmental contamination associated with intestinal pathogens in locations where centralized wastewater treatment systems are unfeasible. Several studies have shown that these systems, included in lowcost systems, allow a substantial improvement of microbiological quality wastewater, similar to that obtained by conventional technologies (Green et al., 1997; Hill and Sobsey, 2001).

To determine the microbiological quality of wastewater treatment effluents, faecal contamination indicators such as coliforms and enterococci are usually employed. The choice of these microorganisms is based on the simplicity and cost-efficiency in determining their presence and quantifying them, and this determination provides information related to the presence and behavior of the principal pathogens present in wastewaters (Vera et al., 2008; Sleytr et al., 2007). For some decades, the faecal coliform group of bacteria has been used as indicators of water quality with respect to the presence of human pathogens. However, commonly used bacterial indicators of faecal contamination are believed to have limited predictive value for various pathogens, especially human viruses and protozoa (Berg et al., 1987; Lucena et al., 2004). These microorganisms are now recognized as being more resistant to natural inactivation and to water treatment processes than the current bacterial indicators of water quality (Keswick et al., 1984; Payment et al., 1985). Bacteriophages and spores of sulphite-reducing bacteria have been proposed as potential indicators of viruses and parasite protozoa, respectively (IAWPRC, 1991; Payment and Franco, 1993). The bacteriophages considered to date as potential surrogate indicators are somatic coliphages (SOMCPH), F-specific RNA bacteriophages (FRNAPH) and bacteriophages infecting *Bacteroides fragilis* (IAWPRC, 1991).

Some works have been focused on a small group of faecal contamination indicators and pathogens in similar systems to the WWNRS described in this paper (Thurston et al., 2001; Karim et al., 2004). However, it is recommendable to study a wide number of indicators and pathogens with the aim of increase actual knowledge on behaviors and relationships between microorganisms in these systems in order to establish design and operation criteria to assess the desired level of treatment regarding a certain quality





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criteria. Therefore, the objectives of this study have been to determine the presence and the abatement of enteric pathogens and faecal contamination indicators, establishing the relationships between these populations in two WWNRS located in Tenerife (Canary Islands, Spain). The removal efficiencies of pathogens and faecal indicators in septic tank devices were not evaluated in this study supported by the Depuranat project" Sustainable residual water management in rural areas, "co-financed by the European Interregional Cooperation Programme (Interreg IIIB Atlantic Arc) (Vera et al., 2008). This is a direct consequence of the main objective of this applied research project: to evaluate several natural reclamation system as a feasible wastewater treatments for conditioning effluents quality to required standard for wastewater reuse. The aim was to demonstrate that natural reclamation system could contribute to put in value reclaimed wastewater in rural and isolated settlements taking as reference septic tank effluents.

#### 2. Materials and methods

#### 2.1. Systems description

Both studied WWNRS are located in Teno Rural Park (Tenerife, Canary Islands, Spain), a landscape of difficult access and complicated orography. These WWNRS are constituted of a combination of anaerobic treatment (a septic tank with high hydraulic retention time (HRT)) and a small sub-surface flow constructed wetland refilled of volcanic ashes as substrate and with a diverse number of macrophytes.

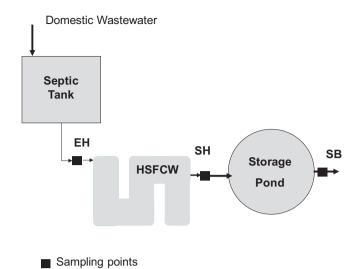
#### 2.1.1. Carrizal Alto

Carrizal Alto is a very small village (12 inhabitants) exclusively dedicated to agriculture. The WWNRS were constructed and set in operation in 2000 in order to assessment its capacity for wastewater treatment according to quality requirements for wastewater discharge and/or reuse. The global system is composed by a septic tank as primary treatment dimensioned for 125 inhabitants with a volume of 180 m<sup>3</sup>. This element is clearly over-dimensioned and therefore, operates with high HRT (estimated about 90 d). Effluent from septic tank feeds a subsurface horizontal flow constructed wetland with a total surface of 100 m<sup>2</sup>, internally divided in five independent parallel channels with the same substrate (volcanic ashes 6-20 mm) and different vegetal species growing up in each other (Phragmites australis, Zantedeschia aethopica, Canna generalis, Alocasia sp. and Dioscorea alata). Finally, reclaimed wastewater is stored in a reservoir (200  $m^2$  and a maximum depth of 3 m) for its reuse in crop irrigation and ecosystems recovery.

## 2.1.2. Albergue de Bolico

Albergue de Bolico is a housing centre with an ambitious environmental education program focused to promote domestic wastes recycling and energy efficiency. As a part of this training philosophy, a WWNRS was built for treating wastewater from this building following the same basical design of Carrizal Alto WWNRS. In this case, the system is composed by a septic tank for 80 population equivalent with a capacity of  $100 \text{ m}^3$  (HRT has been estimated on 8.5 d) and a horizontal subsurface flow constructed wetland of  $50 \text{ m}^2$  in just one unique wetland in snake-shape in order to optimize the available space. Constructed wetland substrate is also volcanic ashes (6–20 mm) with the same macrophytes than Carrizal Alto's CW and colonizing plants from the environment. Regard to the final pond, it is smaller than Carrizal Alto one ( $50 \text{ m}^2$  and maximum depth of 2 m).

In general, both constructed wetlands have achieved, during the study period, effluents of similar physic-chemical qualities. In function of season and occupancy level, these have oscillated



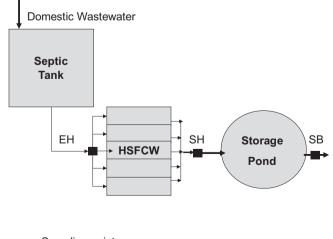
**Fig. 1.** Albergue de Bolico Wastewater Natural Reclamation System. EH: constructed wetland inlet, SH: constructed wetland outlet, SB: storage pond outlet. HSFCW: horizontal subsurface flow constructed wetland.

from 15 to 20 mg/l of SST, 10–18 mg/l of BOD and over 60–70 mg/l of COD. However, it is important to remark that certain worsening of Carrizal Alto CW performance has been noted in spring, possibly due to the degraded material drag for torrential rainfalls and physical degradation of volcanic substrate after 10 years of operation.

## 2.2. Analytical method

Water samples were collected between June 2005 and October 2006 at three samplings points in both systems: constructed wetland inlet (EH); constructed wetland outlet (SH); storage pond outlet (SB) (Figs. 1 and 2). Samples were taken every 15 days depending on weather conditions and operating conditions of the systems.

Samples 1 l were collected from each point in sterile borosilicate bottles and transported to the laboratory at  $4 \,^{\circ}$ C. Microbiological analyses were carried out within the following 6 h. Faecal coliforms (FC) were enumerated by membrane filter procedures according to



Sampling points

**Fig. 2.** Carrizal Alto Wastewater Natural Reclamation System. EH: constructed wetland inlet, SH: constructed wetland outlet, SB: storage pond outlet. HSFCW: horizontal subsurface flow constructed wetland.

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