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Nematodes as a factor for consideration in the wastewater treatment and water reuse process

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ABSTRACT

Nematodes play an important role in wastewater treatment systems by contributing to removal the biochemical oxygen demand concentration or fecal bacteria. The nematodes removal can be used to evaluate the degree of efficiency of wastewater treatment systems. For water reuse, current regulations set the criteria for biological quality that pose a risk to public health (*E. coli*, intestinal nematodes eggs, *Legionella spp.*, etc.), regardless the control of certain organisms, such as plant-parasitic nematodes, that can cause diseases in crops and great economic losses in the agricultural sector. This study considers the effectiveness of the removal of total nematodes as method of measuring the efficiency of wastewater treatment systems. Additionally, the presence of plant-parasitic nematodes will be studied in the frame of water reuse. Finally, the biochemical oxygen demand, chemical oxygen demand, and total suspended solids were analyzed in order to evaluate the performance of treatment systems. It was detected the presence of plant-parasitic nematodes in the outputs of some systems, which demonstrate the need to include its control in the current regulations for water reuse in irrigation.

Keywords: Nematodes; Extended technologies; Indicators; Plant-parasitic; Wastewater treatment; Water reuse

1. Introduction

Nematological analysis has documented that this group of organisms is one that is very diverse and abundant. This was considered in the recommendations, guidelines and international legislation that was subsequently approved by the Committee on Standard Methods [1]. In wastewater, the removal of total nematodes can be used as an indicator of the efficiency of water treatments.

For this study, three groups of nematodes have been considered, according to Gadea classification [2]: (1) Rhabditoid type, including bacteriophagous nematodes that contribute to lowering both the biochemical oxygen demand (BOD_5) and the concentration of fecal bacteria [3], (2) Triloboid type, predaceous nematodes that contribute to keeping he populations of nematodes in check and (3) Tylenchoid type, which includes plant-parasitic nematodes that cause plant disease and reduce crop yield [3–6].

Tylenchoid type nematodes are characterized by the presence of a stylet that is often phytophagous. These nematodes are themselves detrimental to the plants but can also cause a predisposition in plants to other infestations. These plant parasites can reach

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wastewater treatment plants, survive the treatment process, be transmitted into the treated water and, in some cases, be successfully established in crops [3].

Plant-parasitic nematode genera have been found in irrigation water [7–10]. However, current regulations for water reuse set the criteria for biological quality that pose a risk to public health (*E. coli*, intestinal nematodes eggs, *Legionella spp*., etc.) do not address the control of organisms such as plant-parasitic nematodes when the reclaimed water is meant for irrigation purposes, despite the fact that these organisms can cause disease in crops, with subsequent economic loss.

This paper demonstrates the need to evaluate current regulations for water reuse to provide for the control of plant-parasitic nematodes when using treated wastewater for irrigation purposes, given the fact that nematodes are able to infest the host crops and become pathogenic.

2. Materials and methods

2.1. Experimental site description

The study was conducted at the Experimental Center of Carrión de los Céspedes, a R&D&I center for urban wastewater treatment and reuse, located in Seville, in the South of Spain, (http://www.centa.es).

Sampling conducted on a fortnightly basis of municipal raw wastewater (preliminary treated by a 3-cm barscreen, a 3-mm mesh sieve grit and grease chamber) and of final effluent from eight extended wastewater treatment technologies was carried out from March 2007 to May 2009. The wastewater treatment technologies consisted of: (1) one stabilization pond, (2) six constructed wetlands of different characteristics (water flow, substrate particle size, planting and working diagram) and (3) one peat filter. The Experimental site is described, with sampling points shown and a flow diagram in Table 1 and Fig. 1. Design and operational features of six constructed wetlands are outlined in Table 2.

2.2. Analytical procedure

Nematodes were isolated using a decanting-sieving technique, with 10 liter samples and then proceeding to direct filtration with 0.025-mm mesh sieves. With turbid samples, previous filtration was applied using inverted glass. Filtration is carried out by simply using the technique of decanting and sieving [11], in accordance with the washing, decanting and sieving technique outlined by Cobb [12].

Total nematodes were counted in order to determine the efficiency of removal. In each of the schemes, efficiency values were linked to the nematodes in the preliminary treatment. The presence and identification of plant-parasitic nematodes was used to assess the risk to crops irrigated with treated wastewater. The identification and counting of plant-parasitic nematodes was performed by optical microscopy.

Physico-chemical parameters related with wastewater treatment (BOD_5, COD, and TSS [13]) were evaluated in all samples. For the maturation pond samples, soluble BOD_5 and COD were analyzed, using filtered samples as indicated in Annex I of Spanish Royal Decree 509/1996 [14]. Efficiency of those values relates to the physico-chemical values in the preliminary treatment.

3. Results and discussion

Preliminary treated wastewater carries a high mean load of total nematodes (16/L) compared with the rest of the sampling points (Fig. 2). The high frequency (58%) of nematodes in the samples and the fact that the sampling was taken at very precise points between fortnightly intervals determines that the wastewater is an important vector of nematodes. Regarding the other treatment technologies studied, the highest abundance of nematodes was recorded both in the free flow constructed wetland (FFCW) and in the peat filter. The effluent with the lower abundance and frequency was the horizontal flow constructed wetland 2 (HFCW2) operating in combination with the vertical flow constructed wetland (VFCW1), both planted with *Phragmites australis* (Fig. 2).

Effective nematode removal necessitates high performance from both the maturation pond and the constructed wetlands operating in combination (Fig. 3). Other research that has been conducted claims that these technologies are highly efficient at removing all kinds of pathogens present in wastewater [15–19].

In constructed wetlands, the highest performance was reached with a combination of the vertical flow constructed wetland planted with *P. australis* (VFCW1) and the horizontal flow constructed wetland planted with *P. australis* (HFCW2). However, the performance of the vertical flow constructed wetland without plants (VFCW2) operating in combination with the free flow constructed wetland (FFCW) was lower towards the end of the process (68%). Birds inhabiting the free flow constructed wetland or using this as a transit point could stir the substrate. Bird species that may be carriers of nematodes inhabit the free flow constructed

Table 1
Details of wastewater treatment technologies

Treatment technology (outlets)	Population equivalent
Lagooning- second maturation pond	1,000
Peat filter	15
Vertical flow constructed wetland (1): VFCW1	100
Vertical flow constructed wetland (2): VFCW2	100
Horizontal flow constructed wetland (1): HFCW1	100
Horizontal flow constructed wetland (2): HFCW2	100
Horizontal flow constructed wetland (3): HFCW3	100
Free flow constructed wetland: FFCW	100

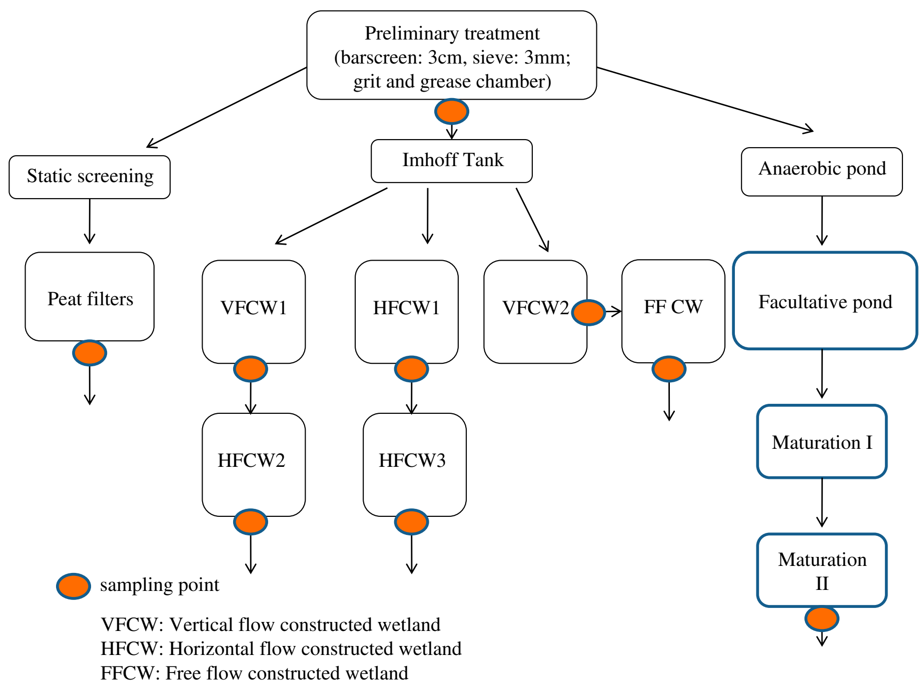

Fig. 1. Flow diagram and sampling points in wastewater treatment technologies studied.

wetland at the experimental centre [20–22]. Bird that are found in the area are: *Alauda arvensis*, *Ardea cinerea*, *Aythya ferina*, *Cuculus canorus*, *Fulica atra*, *Gallinula chloropus*, *Lanius excubitor*, *Motacilla alba*, *Passer montanus*, *Phylloscopus collybita*, *Turdus merula*, *Turdus viscivorus* and *Upupa epops*. Horizontal flow constructed wetland without plants (HFCW1) and horizontal flow constructed wetland planted with *P. australis* (HFCW3) show similar levels of removal effectiveness (94–90%).

Mean removal in the peat filter was lower than in other treatments. The reduced efficiency is due to the fact that there was a clogging of the filter during the sample period.

Among these were nematodes belonging to the Rhabditoid, Triloboid, and Tylenchoid types, a finding that was in keeping with other research [5]. The nematodes found were at all life stages, which indicates that the conditions are suitable for their growth (Fig. 4). Rhabditoid nematodes are often considered colonizers, and their populations can explode in order to take advantage of suitable conditions.

Two plant-parasitic nematode species that cause serious damages in host crops (*Pratylenchus pratensis* Filipjev, 1936 and *Ditylenchus dipsaci* Kühn, 1857) were present in the pretreated wastewater as well as in the outlet of some of the monitored technologies [23] (Fig. 5).

Table 2
Design, features and operating conditions of constructed wetlands (CW)

CW	Surface (m^2)	Substrate (mm)	Plants	Inlet[(1)]
VFCW1	317	10 cm of sand 1–2	*P. australis* (*Pa*)	Imhoff Tank (IT) (length: 4 m, width: 2 m, height: 3.3 m, dosing frequency)
		50 cm of gravel 4–12		
		15 cm of gravel 25–40		
VFCW2	288	30 cm of gravel 4–12	None planted	IT
		30 cm of calcareous gravel 3–8		
		10 cm of gravel 4–12		
		15 cm of gravel 25–40		
HFCW1	277	60 cm of siliceous gravel 12–20	None planted	IT
HFCW2	229	60 cm of siliceous gravel 4–12	*Pa*	VFCW1
HFCW3	211	60 cm of calcareous gravel 3–8	*Pa*	HFCW1
FFCW	240	Water column (10–50 cm)	*Typha, Iris, Cladium, Scirpus, Juncus*	VFCW2
		20 cm of gravel 4–12		

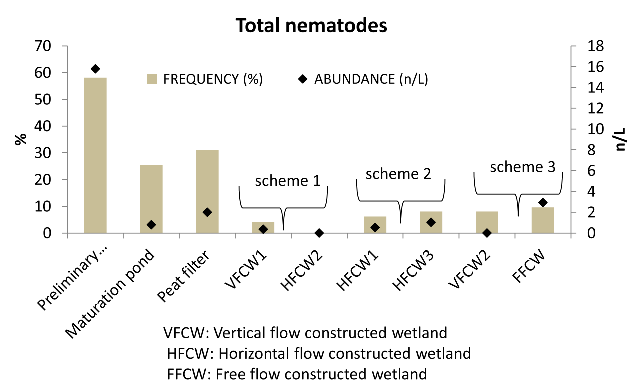

Fig. 2. Mean frequency (%) and abundance of total nematodes (n/L) in preliminary treated wastewater and in different treatment outlets.

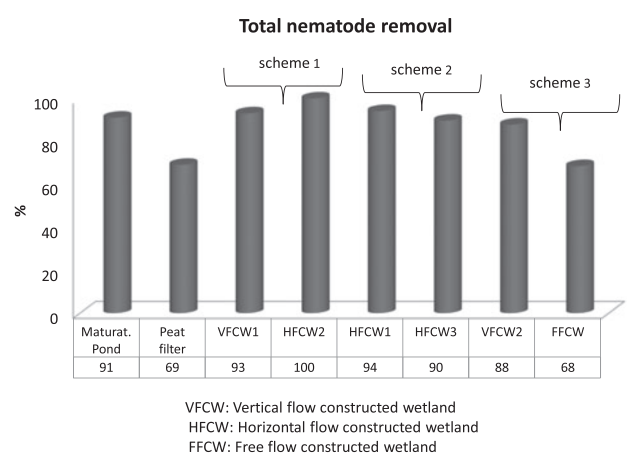

Fig. 3. Mean removal (%) of total nematodes within the outlet of wastewater treatment technologies studied.

Once these species invade crops, the damage can be considerable. *P. pratensis* may can reduce root growth or inhibit root development by forming local lesions on young roots. The lesions may also lead to secondary infection by fungi or bacteria, increasing the plant's susceptibility [24]. The overall root damage caused by this nematode can cause poor growth, reduce crop yield, or kill the plant off completely. These nematodes can cause serious economic hardship if the crop loss is drastic and profits are lost.

D. dipsaci, known as "stem and bulb nematode", is one of the most devastating plant-parasitic nematodes, especially in temperate regions. It is known to attack over 450 different plant species, including many weeds and, without some form of control, it can cause whole crops to fail (e.g. onions, garlic, cereals, legumes, strawberries, ornamental plants, especially flower bulbs) [25]. In most countries, regulatory measures are applied to minimize the spread of *D. dipsaci*. European and Mediterranean Plant Protection Organization lists *D. dipsaci* as an A2 quarantine pest [25].

The mean values of BOD_5, COD, and TSS in raw wastewater were: 400 mg/L O_2, 731 mg/L O_2 and 261 mg/L, respectively. Mean concentrations and performance of the final effluent are shown in Fig. 6, compared with the limits set by the European urban

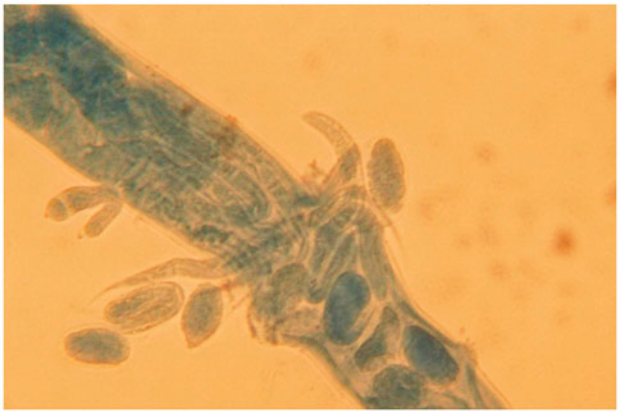

Fig. 4. Gravid female of Rhabditoid nematode, with eggs and juvenile stages found in treated effluent.

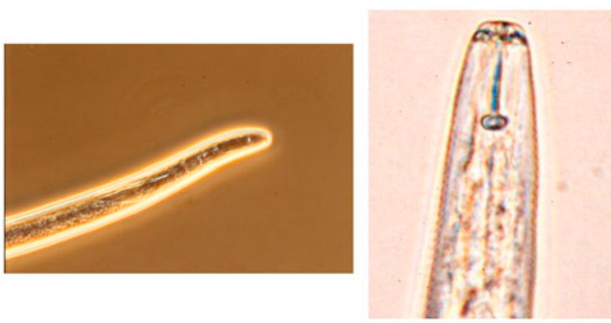

Fig. 5. *P. pratensis* (left) and *D. dipsaci* (right) in sewage treatment.

wastewater treatment directive (EUWTD) (91/271/EC) [26].

Both for BOD_5 and COD, the highest performance is found at the end of the process for the combination of constructed wetlands and for maturation pond: 96–98% for BOD_5 and 83–97% for COD, above values of the EUWTD (70 and 75%, respectively).

The highest average performance for TSS removal has been achieved with the outlet of the combination between constructed wetlands (94–98%), above values established by EUWTD (90%).

4. Conclusion

Based on this study, most of the wastewater treatment technologies achieve effective removal for BOD_5, COD, and TSS as outlined by the regulations for discharge into public waterways.

Final effluents of monitored systems show low values for total nematodes, so this can be used as a factor when establishing the degree of efficiency in

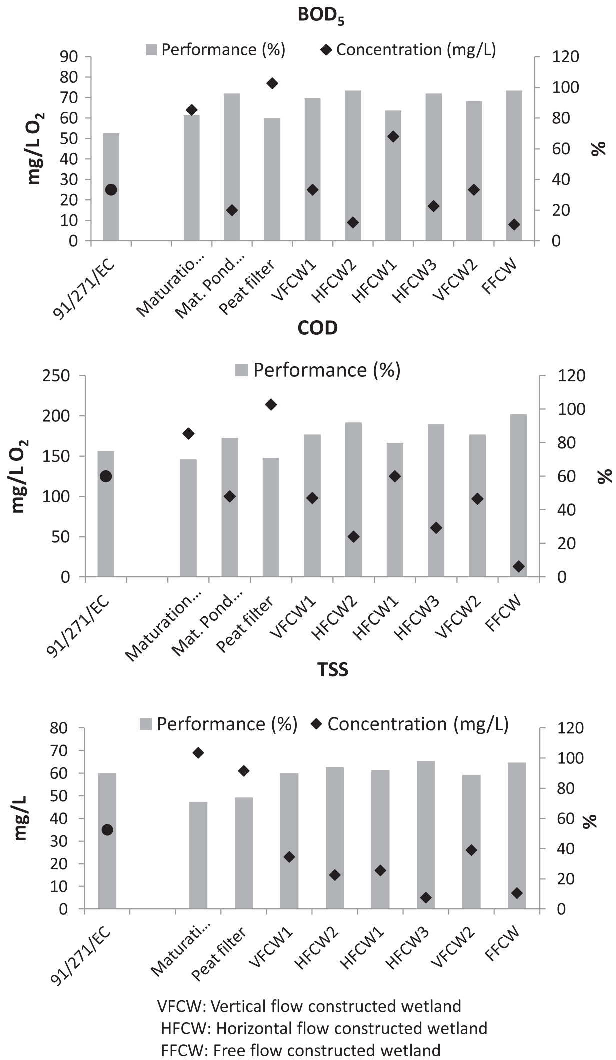

Fig. 6. Mean concentrations and performance of BOD_5, COD, and TSS in the outlet of different wastewater treatment technologies.

wastewater treatment systems. However, the systems allow nematodes to become established and let their populations increase. This is proved by the presence of gravid females and juvenile stages. In this sense, sludge and filters must be kept in mind when considering flow evacuation, the cleaning of systems and their maintenance in order to avoid contamination.

The presence of plant-parasitic nematodes in the outlet of some systems should be enough to require that they be controlled and reduced before the reclaimed water is used on agricultural crops. The current regulations for water reuse in irrigation must be altered to address the issue of plant-parasitic nematodes.

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