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Comparative study on removal of pathogenic and parasitic organisms using extended wastewaters treatment technologies

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ABSTRACT

Although there is an absence of common guidelines or regulations about wastewater reuse at European Community level, there are several countries or federal regions that have published their own standards or regulations. In Spain, the current Royal Decree 1620/2007 regulates the legal regime for reuse of treated wastewaters for different uses. The aim of present study is to evaluate the removal performance of certain parasitic and pathogenic organisms by means of the use of different extensive wastewater treatment technologies in small communities. The study results were assessed based on both the EU Urban Wastewater Directive (91/271/EEC) and Spanish Royal Decree. The selected extended technologies are installed in the Experimental Plant of Urban Wastewater Treatment of Carrión de los Céspedes, PECC, (Sevilla, Andalusia, Spain) property of Andalusian Water Agency (Andalusian Department of the Environment). These extensive technologies are: 1) stabilization ponds, 2) constructed wetlands, and 3) peat filters. The following parasitic and pathogenic organisms have been considered: 1) Helminths ova, 2) total and phytoparasitic nematodes, and 3) *Escherichia coli.* The following physical-chemicals parameters have been analysed: 1) COD, 2) BOD_5 3) TSS, and (4) turbidity. Samples were collected and analysed fortnightly from March, 2007 to May 2008 using PECC's monitoring protocol. We consider that the evaluation of removal efficiency of studied organisms, as well as on reduction of organic matter, could be used as tool to assess the suitability of treated wastewaters reuse in small communities according to the uses specified in the Spanish Royal Decree 1620/2007.

Keywords: Reuse; Wastewater; Extended technologies; Pathogenic and parasitic organisms

1. Introduction

The total volume of renewable freshwater in the global hydrologic cycle is several times more that is needed to sustain the current world population. However, only about 31% of the annual renewable water is accessible for human uses due to geographical and seasonal variations associated with the renewable water [1].

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Europe has a long history in water management in general and more specifically in the "small water cycle" that the treatment and distribution of drinking water and the collection and treatment of wastewater are well developed practices. However, the recycling of treated wastewater has not been widely applied in most European countries. But due to the increasing need for protection of water resources, the growing environmental awareness and the public inclination to sustainability the pressure on water recycling is gradually increasing and some European countries have developed water reuse criteria (such as in Belgium, Cyprus, France, Italy, Greece, and Spain) [2].

In Spain, the current Royal Decree 1620/2007 regulates the legal regime for reuse of treated wastewaters according to different uses: urban, agricultural, industrial, recreational and environmental [3].

2. Public health concerns

When we consider the reuse of treated domestic wastewaters we should start from the premise that these waters typically contain a range of parasitic and pathogenic organisms which, depending on the species and concentrations, pose a potential risk to human health and whose presence must therefore be reduced in the course of wastewater treatment [4–6].

The 1989 World Health Organization (WHO) agricultural water reuse guidelines [7] draws attention to potential risks caused by helminths when wastewater was used for irrigation. In the 2006 guidelines [8], helminth ova (HO) are pointed out as one of the major concerns, particularly in developing countries, to reuse not only in wastewater in agriculture but also in aquaculture. For these reasons, WHO establishes efficiencies of several log removal for parasites to reuse wastewater safely.

Helminthiasis is common diseases in developing countries with an uneven distribution around the world. The affected population in developing countries is 25–33% [9], whereas in developed countries it is less than 1.5% [8]. Thus, it is a problem that mostly concerns developing countries, especially in regions where poverty and poor sanitary conditions are dominant; under these conditions helminthiasis incidence rates reach 90% [9]. There are several kinds of helminthiasis; ascariasis is the most common and is endemic in Africa, Latin America and the Far East. Helminthiasis is transmitted through: consumption of polluted crops, direct contact with polluted faeces or polluted wastewater, and ingestion of polluted meat [10].

The WHO has performed research to establish recommended limits. For agricultural irrigation of crops that are eaten uncooked, it recommends a value of ≤1 HO/L [7] and recent epidemiological research work shows that a limit < 0.1 HO/L is needed if children under 15 years are exposed [11].

Other parameter of control in the field of wastewaters reuse is faecal coliforms which are the bacterial indicator organisms most extensively used, and it is assumed that they are indicators of faecal pollution in general. Furthermore, even though faecal coliforms might be a useful indicator of faecal pollution in developed countries, this is not always the case in developing countries due to the presence of a wide variety and greater numbers of microorganisms. That is not to say that faecal coliforms are not useful pollution indicators in developing countries, but rather that care must be taken to select an additional indicator for specific purposes [10].

The Spanish Royal Decree 1620/2007 on reuse of treated wastewaters [3] established several limits for intestinal nematodes ova and *E. coli* according to different uses: urban, agricultural, industrial, recreational, and environmental. These microbiological limits are shown in Table 1, as well as the limits for TSS and turbidity.

Nematological analysis, considered in recommendations, guidelines and international legislations, approved by the Commitee on Standard Methods (1959), shows a group with great abundance and diversity. In wastewaters the removal of total nematodes can be used as a factor of efficiency degree in wastewater systems and in framework of reuse of treated wastewaters should be a parameter to take in account, principally for the potential ova transmission by pregnant female.

For this study three biological types of nematodes have been considered, according the Gadea classification [12]: (1) Bacteriofagous nematodes (Rabditoide type) that contribute to reduce both BOD_5 and faecal bacteria [13], (2) Predators (Triloboide type) that contribute to the balance in the nematodes population in these means besides feeding other microorganims, and (3) Phytoparastics (Tilencoide type), considered that remain in the studied systems and would be passed on later.

The main aims of this study are to analyse the removal efficiency of helminth eggs, and *E. coli*, according to the different uses established in the Spanish Royal Decree on reuse of treated wastewaters [3], to analyse the efficiency of different non-conventional or extensive technologies studied, according the EU Urban Wastewater Treatment Directive (91/271/EEC): UWWTD [14], and suggest the inclusion of other parasitic and pathogen organisms, non issued in regulations of treated wastewaters reuse, as for example the protozoan *Cryptosporidium* and *Giardia*, and total and Phytoparasitic nematodes.

3. Methodology

The study has been carried out at the treatment systems located in the Experimental Plant of urban wastewater treatment of Carrión de los Céspedes (PECC) [15], in Seville, Spain. The studied systems are the extended wastewaters treatment technologies including: (1) waste

Table 1
Uses and control parameters of Spanish Royal Decree [3]

Quality	Use	Description	Intestinal nematodes eggs/10l	*E. coli* (CFU/100 ml)	TSS (mg/l)	Turbidity (TNU)
1.1	Urban	Residential: irrigation, sanitation	1	0	10	2
1.2		Urban soils: irrigation, fountains, fire-preventions, etc.	1	200	20	10
2.1	Agricultural	Irrigation for fresh food	1	200	20	10
2.2		Irrigation for not fresh food with a posterior industrial treatment, pasture and aquiculture	1	1,000	35	No limit
2.3		Irrigation without contact with fruit, products, cereals, etc.	1	10,000	35	No limit
3.1	Industrial	Process and cleaning except food industry	No limit	10,000	35	15
		Process and cleaning in food industry	1	1,000	35	No limit
3.2		Cooling tower and evaporative condensers	1	No limit	5	1
4.1	Recreational	Irrigation of golf fields	1	200	20	10
4.2						
4.3		Hold backs and run-off without public access	No limit	10,000	35	No limit
5.1	Environmental	Aquifer recharge by percolating	No limit	1,000	35	No limit
5.2		Aquifer recharge by injection	1	0	10	2
5.3		Forestry, without public contact	No limit	No limit	35	No limit
5.4		Wetlands and minimal flow	To study in each case			

stabilisation ponds, (2) constructed wetlands, and (3) peat filters.

3.1. The stabilisation pond system

The stabilisation pond system installed in PECC consists of two anaerobic ponds laid out in parallel, with an unit volume of 200 m^3 and a depth of 4 m, one facultative pond with a capacity of 3,500 m^3 which receives the effluent of the anaerobic ponds and finally there are two maturation ponds which are fed by the effluent from the facultative pond, with capacities 400 and 600 m^3. These ponds can be used in serial or in parallel distribution. The water level in both the facultative pond and the maturation ponds can be varied to modify the volume, surface area and retention times of the ponds. In present study the effluent of second maduration pond has been assessed. During the monitoring, the stabilisation ponds have been working in parallel.

3.2. Constructed wetlands

Given the widespread development and implementation of this technology worldwide [16], the PECC has been decided to include all of the different existing systems to which end it has laid out a plot of 1,500 m^2, with six different types of constructed wetlands (cw): one free flow, two vertical flow and two horizontal flow, which in turn have different substrates and plants densities, and which can be combined in different combinations. During the monitoring, six constructed wetlands working in different combinations have been studied:

- Vertical flow constructed wetland 1 in combination with horizontal flow cw 4, both planted with *Phragmites australis.*
- Horizontal flow cw 2, without plants, in combination with horizontal flow cw 6, planted with *P. australis.*
- Vertical flow cw 3 without plants, in combination with free flow cw 4 planted with different plants (*Typha sp.*, *Iris sp.*, *Cladium mariscus* and *Cyperus sp.*)

The working diagram of monitored constructed wetlands is shown in Fig. 1.

3.3. The peat filters

The peat filters installed at the PECC has six 25 m^2 filtering units, grouped in three beds, each with two modules. The filters consist of a series of layers made of (in ascending order): coarse gravel (30 cm), fine gravel (10 cm), sand (10 cm) and peat (40 cm). For this study

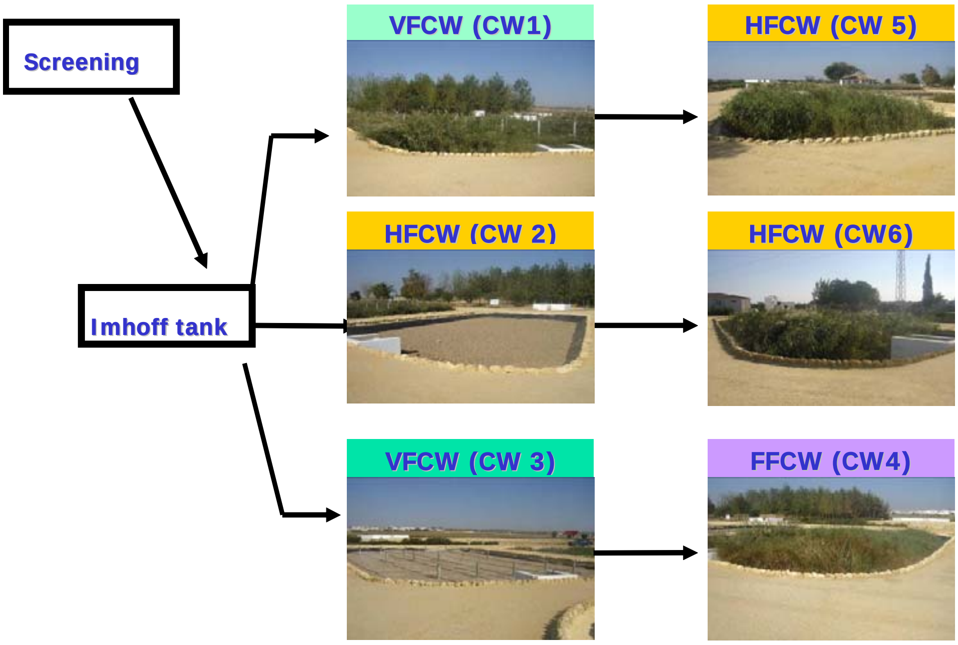

Fig. 1. Working diagram of constructed wetland allocated in PECC.

samples were taken at filtering units 5 and 6 which are working as primary settling tank before a trickling filter.

From those extensive treatments, fortnightly sampling of influent and effluent was carried out from March 2007 to May 2008. All technologies installed in the PECC, extensive and intensive wastewater technologies, are presented in Fig. 2.

Samples were analysed for a range of biological (Hel-

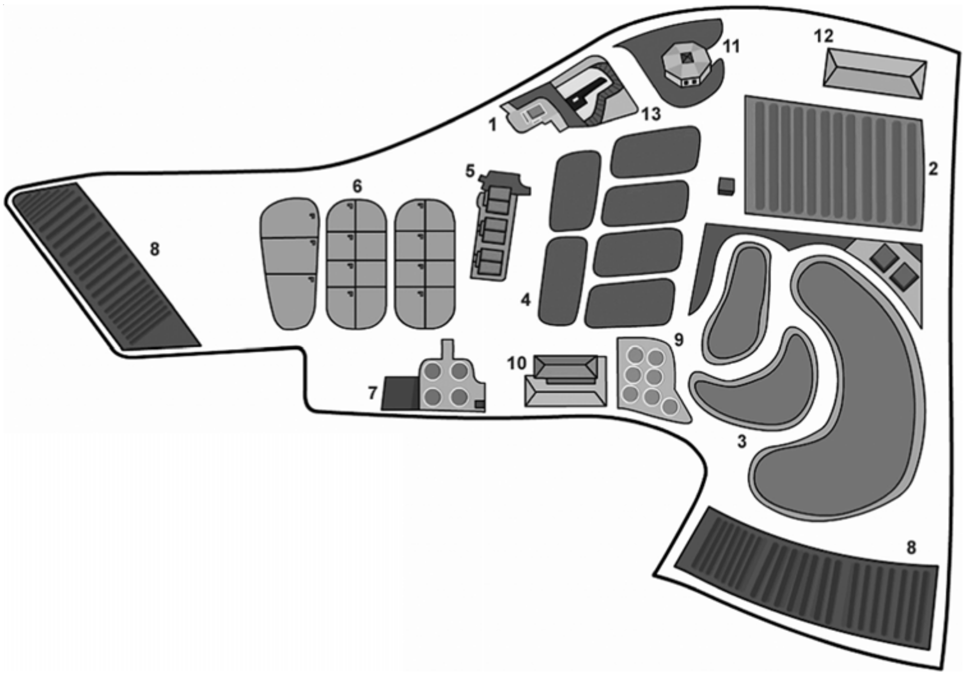

Fig. 2. Experimental plant of Carrión de los Céspedes, PECC [22]. 1. Preliminary treatment, pumping and distribution; 2. Green filter; 3. Stabilisation ponds; 4. Constructed wetlands; 5. Peat filters; 6. Plots for prototypes (extended aeration, SBR, biocatalysis, MBR, etc.); 7. Sludge treatment area; 8. Reuse area; 9. Aquatic crops; 10. Laboratories; 11. Main building; 12. Training building; 13. Meteorological station.

minths ova, total and Phytoparasitic nematodes and *E. coli*) and physicochemical parameters: COD, BOD_5, TSS, and turbidity (Table 2).

Table 2
Physicochemical and microbiological parameters measured

Parameter	Method/Technique
COD, mg/L	S.M. 5220 C
BOD_5, mg/L	S.M. 5210 B
TSS, mg/L	S.M. 2540 E
Turbidity, TNU	Nephelometry
Helminth, eggs/10L	Modified Bailinger method (Bouhoum and Schwartzbrod, 1989)
E. coli, ufc/100mL	Membrane filtration method, ount of *E. coli* positive β-glucuronidase
Total and Phytoparasitic nematodes, N/10L	Decantation and filtration, in dense or very dense samples: decantation and filtration method of Cobb, according to Flegg

SM: Standard Methods [23]

4. Results and discussion

The results of monitoring of physicochemical parameters are shown in Table 3. These reveal that the studied technologies met a BOD_5 and COD reduction values in according with those established in the UWWTD [14]. Regarding the TSS, the values of peat filters are close to those established in the regulation, but the effluents of maturation pond present less efficiency, due principally to high microalgae concentration existing in this system. The high removal efficiency of BOD_5, COD and TSS have been obtained in the combination between constructed wetlands, similar to values obtained in other wastewater treatments plants that work with same monitored technologies in Andalusia, Spain [17,18].

Table 4 shows the removal values of turbidity and *E.coli*, expressed in terms of percentage and log removal, respectively. The highest *E. coli* removal was obtained in maturation pond (5 log removal), followed by combinations between constructed wetlands (4–3 log removal) and finally peat filters (1 log removal).

In analyzing the fulfilment of monitored wastewater technologies according to some parameters included in Spanish Royal Decree [3] (Table 5), it is concluded that none of their treated wastewater can be reused in an specific use. Analyzing each system, we can conclude that maturation pond is a good technology to removal *E.coli*

Table 3
Removal efficiencies for physicochemical parametres and comparision with recommendations of Urban Wastewater European Directive [14]

Technology	Mean influent – effluent value (mg/L)			Efficiency (%) (91/271/EEC)			Efficiency (%)		
	BOD_5	COD	TSS	BOD_5	COD	TSS	BOD_5	COD	TSS
Peat filters	356–78	744–194	297–42	70–90	75	90	80	75	83
Maturation pond	385–56	821–165	297–60				83	77	77
cw 1 + cw 5(1)	370–8	813–48	297–13				98	94	96
cw 2+ cw 6(2)	380–25	812–73	297–5				94	90	98
cw 3 + cw4(3)	370–10	816–57	297–8				97	92	97

(1): Vertical flow cw 1 in combination with horizontal flow cw 4, both planted with *Pragmites australis.*
(2): Horizontal flow cw 2, without plants, in combination with horizontal flow cw 6, planted with *P. australis.*
(3): Vertical flow cw 3 without plants, in combination with free flow cw 4 planted with different plants.

Table 4
Removal efficiencies for turbidity and *E. coli*

Technology	Mean influent – effluent value			Turbidity (efficiency%)	*E. coli* (log removal)
	Turbidity (NTU)	*E. coli* ($\log_{10}$)	*E. coli* (CFU/100ml)		
Peat filters	294–41	8.3–7.4	6.4E+08–4.6E+07	84	1.1
Maturation pond	294–67	8.3–3.7	6.4E+08–7.8E+04	76	4.6
cw 1 + cw 5(1)	294–12	8.3–4.9	6.4E+08–4.8E+05	96	3.3
cw 2 + cw 6(2)	294–10	8.3–4.3	6.4E+08–6.4E+06	95	4.0
cw 3 + cw4(3)	294–16	8.3–4.1	6.4E+08–1.3E+05	94	4.2

Table 5
Comparison of monitored wastewater treatment systems to the established values in Spanish Royal Decree [3]

Use	Specific use	*E.coli* (CFU/100 ml)	TSS (mg/l)	Turbidity (TNU)
Urban	Residential: irrigation, sanitation	No	cw2+cw6 cw3+cw4	No
	Urban soils: irrigation, fountains, fire-preventions, etc.	No	Combination between cw (Ccw)	cw2+cw6
Agricultural	Irrigation for fresh food	No	Ccw	cw2+cw6
	Irrigation for not fresh food with a posterior industrial treatment, pasture and aquiculture	No	Ccw	All systems
	Irrigation without contact with fruit, products, cereals, etc.	Maturation pond	Ccw	All systems
Industrial	Process and cleaning except food industry	Maturation pond	Ccw	cw1+cw5 cw2+cw6
	Process and cleaning in food industry	No	Ccw	All systems
	Cooling tower and evaporative condensers	All systems	cw2+cw6	No
Recreational	Irrigation of golf fields	No	Ccw	cw2+cw6
	Hold backs and run-off without public access	Maturation pond	Ccw	All systems
Environmental	Aquifer recharge by percolating	No	Ccw	All systems
	Aquifer recharge by injection	No	cw2+cw6 cw3+cw4	No
	Forestry, without public contact	All systems	Ccw	All systems
	Wetlands and minimal flow	To study in each case		

in specific use of irrigation without contact with fruit, products, cereals, etc; as well as in uses of process and cleaning in industry, except food industry; and hold backs and run-off without public access. Combinations in various constructed wetlands are good options to achieve the values both TSS and turbidity established in different uses. For the specific use for forestry, without public contact, all systems are adequate, but only constructed wetlands exceed in TSS.

Regarding the parasitic microorganisms, only five species, *Toxocara canis*, *Syphacia* sp., *Giardia* sp., *Entamoeba coli* and *Iodamoeba butschilii*, interesting in human and animal parasitology have been identified. Furthermore, different stages (eggs, larvae and adults) of nematodes whose morphological characters are compatibles with the order Strongylida and Rabditiform have been located (Table 6).

Analyzing total nematodes, according the efficiency of studied treatments, the obtained results show that the effluents present minimum values of these. We can consider that the system where there is the biggest efficiency in nematode removal is maturation pond, following of combinations between constructed wetlands and finally peat filters.

The results obtained with organism removal are according with those obtained in other works. According to Feachem et al. [19], stabilisation pond can removes up to 6 log units of bacteria and 100% of protozoa and hel-

Table 6
Parasitic microorganisms in the influent and studied systems

Technology	Microorganism
Influent	*Toxocara canis* (egg) *Giardia sp.*(cyst) *Entamoeba coli* (cyst) *Iodamoeba butschilii* (cyst) Rabditiform (larvae) Rabditiform (adults)
Stabilisation pond (maturation pond)	Strongylida (egg)
cw1	Strongylida (egg) *Siphacia* sp. (egg) *Entamoeba coli* (cyst) *Giardia* sp. (cyst)
cw1 + cw 5	—
cw2	—
cw 2 + cw 6	—
cw3	*Toxocara canis* Strongylida (egg) Rabditiform (larvae)
cw 3 + cw 4	—
Peat filters	—

minths ova. Several factors contribute to this high removal but concerning helminth ova, sedimentation is the most effective. On the other hand, constructed wetlands are often highly efficient in the removal of microorganisms such as bacteria from wastewater [16,20].

5. Conclusions

Regarding efficiency of wastewater treatments studied (stabilization pond-maturation pond, combinations between constructed wetlands and peat filters) we can conclude that most of the systems achieve efficiencies according to the values established in the UWWTD [14], although maturation pond presents values of TSS below those established in this normative, due principally to high microalgae concentration in effuents. One solution to reduce the TSS final concentration could be the installation of sand filters following this process.

The efficiency of *E. coli* and helminths ova removal is highest in maturation pond followed by combinations of constructed wetlands, and finally peat filters. These results are according with other works where it is mentioned that stabilization ponds and constructed wetlands are very efficient process for removing all kinds of pathogens.

About parasitic microorganims we can conclude that no one of intestinal helminths parasites in human was found. The discovery of *Toxocara canis* eggs is remarkable, because this species causes visceral larva migrans in humans. Giardiasis (*Giardia* sp.) is the major protozoosis that affect humans in Andalusia, Spain. The absence of identifiables parasites both in maduration pond and combination between different constructed wetlands shows the efficiency on removal of these kinds of microorganisms using these systems of wastewater treatment.

Although more studies to determine the infectivity and the species/genotypes of the parasites are needed the presence of cysts of *Giardia* in water samples proved the need that this protozoan is included in the current regulations of treated wastewater reuse, as well as both total and phytoparasitic nematodes, principally female with eggs.

The prevision of International Water Management Institute (IWMI) that estimates that for 2025 will be over 66% of world population that will suffer water shortage and 52 countries will suffer droughts, is necessary use possible alternatives to increase the hydraulic resources and in this field, the reuse of treated wastewaters can be a valuable alternative. Respect to the use of extensive wastewater technologies, in framework of water reclamation and reuse, there is an inherent advantage of these, the impact on microbiological parameters through natural attenuation effects such as filtration and degradation in the soil-root matrix or the desactivation effect through sunlight (UV). These benefits make extensive systems attractive for small communities, helping them to meet the objectives of UWWTD [5] and, in Spanish context, of National Plan of Water Quality: Sanitation and Wastewater Treatment (2007–2015) [21].

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