Endocrine Disrupting Chemicals (EDCs) and Pathogens removal in an hybrid CW system for a tourist facility wastewater treatment and reuse

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Abstract
Within the frame of the "SWAMP" project (a demonstration project co-financed by the European Community) an hybrid constructed wetland (CW) system was realized for the treatment of wastewater produced by a medium-size hotel located in Florence, Italy. The system consisted of a 70 m^3 primary treatment by Imhoff tanks, a 160 m^2 HF and a 180 m^2 VF. The results obtained in the first year of operation regarding to the hygienic indicator bacteria (total coliforms, faecal coliforms, faecal streptococci and *Escherichia Coli*) and some selected organic compounds (Phthalates, PAHs and Estrogens) were reported. In terms of overall performances the following mean removal rates were obtained: COD 94%, BOD5 95%, TSS 90%. The CW system showed a satisfactory functioning of the treatment process, especially related to the sedimentation and filtration processes and to organic matter trapping and degradation inside the reed beds. As regard the EDCs a clear water quality increase during the purification process was observd. PAHs were found below 0.5 µg/l in the inlet and at significantly lower amounts in the outlet of the system. Total inlet phthalates concentration was included in the range 100 to 300 µg/l and this class of compounds showed a certain persistence even in the outlet (10-200 µg/l) due to HDPE liner release. Among the other compounds, 17-α-estradiol and ethynyl-estradiol were revealed in the inlet at concentration below 10 µg/l, while were absent in the outlet.

Keywords
Hybrid systems, Endocrine disruptors, Sustainable water management, Constructed wetlands, Reed beds, Wastewater reuse

INTRODUCTION

Chemical substances that can interfere with the normal functioning of the endocrine system have been termed Endocrine Disrupting Chemicals (EDCs) (Keith et al., 1997). The full list of EDCs includes a large range of anthropogenic and natural organic compounds, such as phthalates, pesticides, polychlorinated biphenyls (PCBs), dioxins, polyciclic aromatic hydrocarbons (PAHs), alkylphenols, bisphenols and steroid estrogens (Birkett and Lester, 2003). Several of these substances have been released in increasing amounts in the environment since decades, and, due to their low degradation rate, a significant increase of their background concentrations have been observed in the different environmental compartments (Skakkebaeck et al, 2000, Tyler et al 1998). In wastewater, EDCs, have a variety of source. A number of EDC classes (phthalates, pesticides, PCBs and bisphenols) are industrial products, worldwide used for several applications and are therefore ubiquitous pollutants (Staples et al, 1997, Kupfer, 1975, Safe S.H., 1994, Chen et al, 2002). Other kinds of EDCs compounds (dioxins and PAHs) are not commercial products, but are formed as by-products of various industrial and combustion processes; they are transported from atmosphere to soil and water bodies by the atmospheric runoff or deposited on the soil during the dry period and then go through the water cycle by land runoff (Birkett and Lester, 2003). Alkylphenols are metabolites of their ethoxylate precursors, which are non-ionic surfactants used in many industrial, commercial and household functions (Del Bubba and Lepri, 2002). The presence of steroid estrogens in wastewater mainly arises from direct women excretion, in particular from pregnant females and women using oral contraception or hormone replacement therapies (Arcand-Hoy et al, 1998; Andrews, 1995). Several studies have shown that EDCs are present in the effluents from domestic and industrial conventional sewage treatment plants (Birkett and Lester, 2003) and this evidence is of potential concern about their reuse for non industrial applications, such as irrigation of crops and aquaculture, since EDCs could represent a contamination source of food chain. The behaviour of some EDCs has been studied in pilot constructed wetlands pointing out that reed beds are effective in the removal of phthalates and alkylphenol ethoxylates (Del Bubba et al., 1998; Del Bubba and Lepri, 2002).

Another crucial parameter in order to assess the suitability of effluent wastewater to be reused is the occurrence of pathogens, which presence is usually evaluated by the analysis of the indicators total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS). Such microorganisms are commonly removed at high percentages in conventional sewage treatment plants and in constructed wetlands (Karpiscak et al., 2001; Arias et al., 2003), but the evaluation of residual concentrations is of strong importance for establishing the performances of the treatment plant and the final destination of the effluent wastewater.

For these reasons, the understanding of removal processes and fate of EDCs and pathogens during wastewater treatment, may facilitate controlling or limiting exposure of both humans and environment to these compounds and create more solid basis for the choose of different Sustainable Water Management approaches.

This paper presents the removal data of some EDCs and of TC, FC, FS and *Escherichia Coli* (which is required by the Italian national law about water reuse, D.Lgs. 185/2003) for an hybrid reed bed system located at an Hotel in Florence (Italy). This research was realized under the EC FP5 project SWAMP (for more details about the project see the website www.swamp-eu.org).

MATERIAL AND METHODS

Description of the CW system

The reed bed is composed by a first stage horizontal submerged flow (HF) and a second stage vertical flow (VF) (see Fig. 1). In Table 1 are reported the main features of the reed bed system, including the measured hydraulic loading rate (HLR) and organic loading rate (OLR) during the monitoring period. In fact, an important characteristic of this treatment plant is the tourist fluctuation, which involves a high variability of the daily flow and, consequently, of the loading rate. A pump station located after the Imhoff tanks regulates the loading inside the HF bed by a floating valve and so proportionally to the raw wastewater production.. The effluent from the HF bed is divided by a partition well into two independent siphons which feed the VF filter in an alternate way. The waterproofing of the bed has been realized by an HDPE geo-membrane.

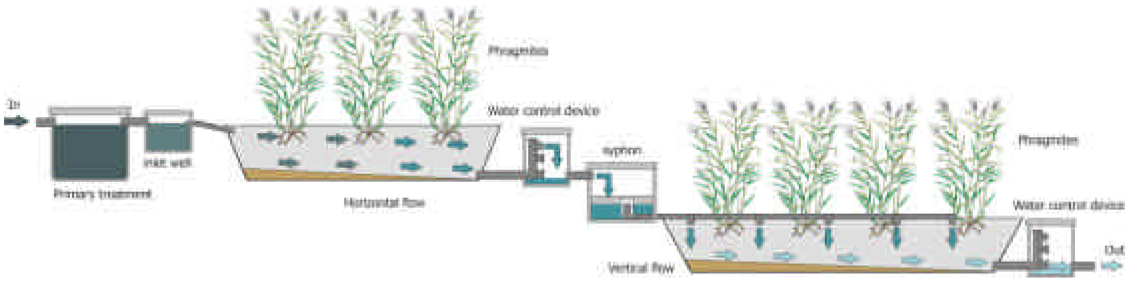

Fig. 1 - Schematic representation of the hybrid constructed wetland at the Relais Certosa Hotel.

Table 1 – Main features of the Relais Certosa Hotel wastewater treatment facility

Parameter	**Value**
Load (p.e.)	140
Inflow (m^3 d^{-1})	17-33
Surface Area HF (m^2)	160
Surface area VF (m^2)	180
HF depth (m)	0.7
HF Gravel size (mm)	5-10
VF filling media (sand+gravel)	Top 10 cm ∅ 6-12mm Middle 60 cm sand ∅ 0/4 Bottom 20 cm ∅ 30-40 mm
VF depth (m)	0.9
HRT – HF (theoretical) (d)	3
HLR (m^3 m^{-2} d^{-1})	HF: mean 0.17 min 0.11 max 0.23 VF: mean 0.15 min 0.10 max 0.21
OLR (g COD m^{-2} d^{-1})	HF: mean 23.5 min 6.8 max 38.1 VF: mean 2.0 min 0.9 max 5.7
Primary treatment	Imhoff – Total volume 70 m^3
Operating since	January 2003

Sampling and analyses

As regards the ECDs analyses, grab samples were collected in glass bottles previously washed with hot chromic mixture and repeatedly rinsed with ultra pure water; collection of samples for sanitary indicators was carried out in glass bottles previously sterilized by autoclave treatment.
The sampling took place monthly from September 2003 to February 2004 (six samples), at the inlet of the HF stage (after the Imhoff tank) and at the outlet of both the HF and VF stages. Inlet grab samples were collected three days before the outlet ones, according to the estimated hydraulic retention time (HRT) of the HF bed. Sampling campaigns were carried out when rain did not occur within three days before the collection of the inlet and the sampling of the outlet samples, so as to avoid dilution effects and HRT shortening, even if the rainwater is managed by a separated sewage system.

Analyses of ECDs were performed, separately for dissolved and particulate phases, after filtration of the samples. In order to determine the concentration of particulate matter, filtration of each sample was carried out on two aliquots of wastewater. One of the two filters was used for the extraction of EDCs, while the other was dried in oven at 105°C for one hour and finally weighed. Water phase, previously modified for pH and polarity by adding hydrochloric acid, sodium chloride and methanol, was extracted by a triple liquid/liquid treatment with methylene chloride, evaporated to 200 µl and analysed by a GC/MS Varian, model Saturn 4D in "micro selected ion storage (micro SIS)" way. For phenols and estrogens a derivatization with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) needed, in order to enhance the GC/MS response (Del Bubba et al., 2004).

Particulate samples were extracted according to the method of Desideri et al. (1987), significantly modified in order to obtain satisfactory recovery percentages (60-97%) for all the investigated compounds (Del Bubba et al., 2004). Instrumental analysis of the extracted compounds was performed as described above.

The bacteriological indicators TC, FC, FS and *Escherichia Coli* were evaluated according to the protocols for membrane filter procedures of Standard Methods for the Examination of Water and Wastewater (1995).

RESULTS AND DISCUSSION

Removal of indicator bacteria

The average removals of the four analysed hygienic indicators were in the range 99.93-99.99%, showing a very high efficiency of the system to remove pathogens (Tables 2-5). During the passage through the HF Reed Bed the bacteria were reduced 2.9-3.2 log units, whereas the reduction in the second stage VF bed was 0.7-1.2 log units.

Table 2 - Changes in Total Coliform density (cfu/100ml) through the CW system

	May03	Jun03	Jul03	Aug03	Sep03	Oct03	Nov03	Dec03	Jan04	Feb04	Mar04
HF IN	$1x10^7$	$3x10^7$	$2x10^7$	$1x10^7$	$4x10^6$	$7x10^6$	$7x10^5$	$4x10^6$	$2x10^6$	$8 x10^5$	$3x10^6$
HF OUT	19000	31000	42000	18000	16000	4700	2300	320	1900	6600	550
VF OUT	2700	29000	300	12000	20000	770	1600	77	130	330	60

Table 3 - Changes in Fecal Coliform density (cfu/100ml) through the CW system

	May03	Jun03	Jul03	Aug03	Sep03	Oct03	Nov03	Dec03	Jan04	Feb04	Mar04
HF IN	$8x10^6$	$3x10^7$	$7x10^6$	$8x10^6$	$2x10^6$	$3x10^6$	$5x10^5$	$7x10^5$	$1x10^6$	$4x10^5$	$9x10^5$
HF OUT	11200	14000	15000	11000	14000	3900	90	49	1300	2900	110
VF OUT	290	1200	420	270	19000	70	30	12	100	170	20

Table 4 - Changes in Faecal Streptococci density (cfu/100ml) through the CW system

	May03	Jun03	Jul03	Aug03	Sep03	Oct03	Nov03	Dec03	Jan04	Feb04	Mar04
HF IN	$1x10^6$	$1x10^6$	$4x10^6$	$1x10^6$	$2x10^5$	$4x10^5$	$4x10^5$	$3x10^5$	$4x10^5$	$4x10^5$	$3x10^5$
HF OUT	1200	3800	1200	1100	180	330	270	47	1400	2700	40
VF OUT	70	150	350	62	68	30	170	2	50	70	10

Table 5 – Changes in *Escherichia Coli* density (cfu/100ml) through the CW system

	May03	Jun03	Jul03	Aug03	Sep03	Oct03	Nov03	Dec03	Jan04	Feb04	Mar04
HF IN	$6x10^6$	$3x10^7$	$7x10^6$	$6x10^6$	$2x10^6$	$2x10^6$	$4x10^5$	$6x10^5$	$1x10^6$	$4 x10^5$	$5x10^5$
HF OUT	3900	11000	13000	4500	490	3900	70	33	1300	2600	90
VF OUT	210	1000	380	240	530	20	20	2	70	90	10

From such data, the following considerations can be highlighted:

- the reed population establishment inside HF and VF beds during July-August 2003 seems to have increased the pathogens removal in comparison to the first operating months; since October 2003 the removal rates increased, despite the different loading rates and the strong temperature changes during the seasons (water temp. range: 7-25 °C);
- from the same month the treated wastewater fulfilled the Italian regulation limits for reuse as regard the pathogens indicator *E. Coli* (80 percentile equal to 50 cfu/100ml and maximum admitted value equal to 200 cfu/100ml).

Removal of EDCs

The investigated organic compounds and their endocrine disrupting potencies (EDP), expressed as 17-ß-estradiol equivalent, are reported in Table 6.

Table 6 – Organic compounds investigated in the present work and their endocrine disrupting potencies (EDP); n.a. = quantitative data or magnitude order not available

Compound	EDA	Reference
Naphthalene	n.a., weak	Santodonato, 1997
Acenaphtylene	n.a., weak	Santodonato, 1997
Acenaphtene	n.a., weak	Santodonato, 1997
Fluorene	n.a., weak	Santodonato, 1997
Phenathrene	n.a., weak	Santodonato, 1997
Anthracene	n.a., weak	Santodonato, 1997
Fluoranthene	n.a., weak	Santodonato, 1997
Pyrene	n.a., weak	Santodonato, 1997
Benzo(a)anthracene	n.a., weak	Santodonato, 1997
Crisene	n.a., weak	Santodonato, 1997
Benzo(b)fluoranthene	n.a., weak	Santodonato, 1997
Benzo(k)fluoranthene	n.a., weak	Santodonato, 1997
Benzo(a)pyrene	n.a., weak	Santodonato, 1997
Indeno(1,2,3-c,d)pyrene	n.a., weak	Santodonato, 1997
Dibenzo(a,h)anthracene	n.a., weak	Santodonato, 1997
Benzo(g,h,i)perylene	n.a., weak	Santodonato, 1997
Diethylphthalate	$\sim 10^{-7}$	Harris et al., 1997
Di-n-butylphthalate	$\sim 10^{-6}$	Harris et al., 1997
Butylbenzylphthalate	$\sim 10^{-6}$	Harris et al., 1997
Bis-2-ethylhexylphthalate	n.a.	-
n-octylphenol	$\sim 10^{-5}$	White et al., 1994; Tabira et al., 1999
nonylphenol	$\sim 10^{-5}$	White et al., 1994; Tabira et al., 1999
Bisphenol F	n.a.	-
Bisphenol A	$\sim 10^{-4}$	Shafer et al., 1999
Estrone	0.19	Svenson et al., 2003
17-a-estradiol	n.a., high	Birkett and Lester, 2003
Mestranol	n.a., high	Birkett and Lester, 2003
17-ß-estradiol	1	-
Ethinylestradiol	2.4	Svenson et al., 2003
Estriol	0.0037	Svenson et al., 2003

Among these compounds, estriol, butylbenzylphthalate, phenols and the most part of PAHs were never detected in the analysed samples.

As regards PAHs, the compounds found in the inlet samples of the CW system were only Naphthalene, Phenanthrene, Fluoranthene and Pyrene, which concentrations for water and particulate phase ranged from 15 to 180 ng/L and from 2 to 7 mg/Kg, respectively. This clearly indicated a strong enrichment of PAHs in the particulate phase, according to their high value of log $K_{O/W}$ (Naphthalene 3.45; Phenanthrene 4.46; Fluoranthene 6,12; Pyrene 6.04). These compounds were below the detection limits (7-9 ng/L) in the outlet particulate matter, while the outlet concentrations for the dissolved phase ranged from 13 to 22 ng/L, giving rise to a removal efficiencies of about 60-70%.

Steroid estrogens were detected in two inlet samples in the range 164 - 259 ng/L and 2.3 - 2.9 mg/Kg for water and suspended matter, respectively. These data pointed out that also for estrogenic compounds the adsorption onto particulate material is high, according to the log $K_{O/W}$ values, included in the range 3.43 (estrone) – 4.67 (mestranol). All the estrogens were below detection

limits (15 ng/L) in dissolved and particulate phase of outlet samples of the HF stage, pointing out the high efficiency of this plant in the estrogen removal.
Phthalates, with the exception of the butylbenzyl ester, were determined in all the inlet samples and were, generally, the most abundant compounds. For the dissolved phase concentrations changed in the ranges 151-3788, 43-6134 and 40-2480 ng/L, for diethyl, di-n-butyl and bis-2-ethylhexylphthalate, respectively. The concentrations found in the particulate phase were affected by a less variation and were in the ranges 33-152, 49-194 and 209-4566 ng/L. According to the different values of log $K_{O/W}$ for the above-mentioned phthalates (ranging from 1,46 for diethyl to 13,1 for bis-2-ethylhexyl), the enrichment factors increased, going from diethylphthalate to bis-2-ethylhexylphthalate. The trend of phthalate along the CW system was different from those of the other classes of EDCs as shown in Figure 2 and 3 for dissolved and particulate phase, respectively. As shown by figures, diethyl and di-n-butyl-phthalate were highly removed during the treatment, while for bis-2-ethylhexylphthalate an opposite trend was observed. This trend was found in all the analysed samples suggesting that a source of this compound could be present inside the plant. In order to explain this unexpected behavior a little portion of the HDPE geo-membrane (50 cm^2) was placed in water (HPLC grade) under stirring for three days (the design HRT). The results of this test pointed out that the membrane released all the three phthalates. However the compound released at highest concentration was bis-2-ethylhexylphthalate, which accounted for more than 70% of the total phthalate release. These findings explain the different observed trend among phthalates.

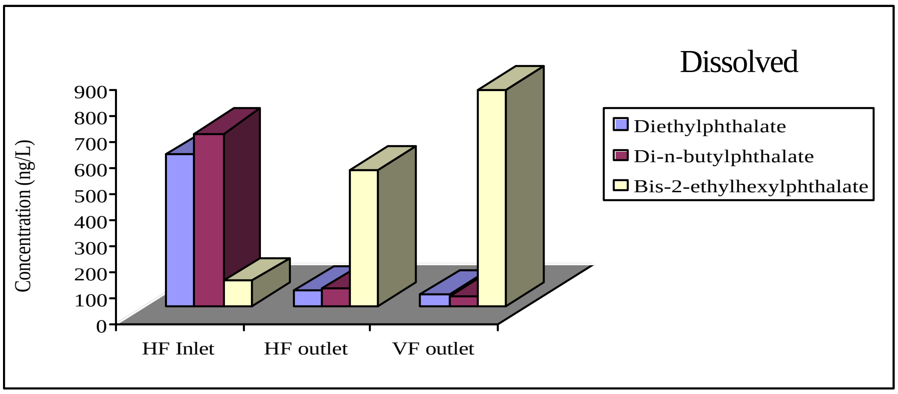

Fig. 2 – Concentration of diethyl, di-n-butyl and bis-2-ethylhexylphthalate in the dissolved phase of the sample collected on December 2004.

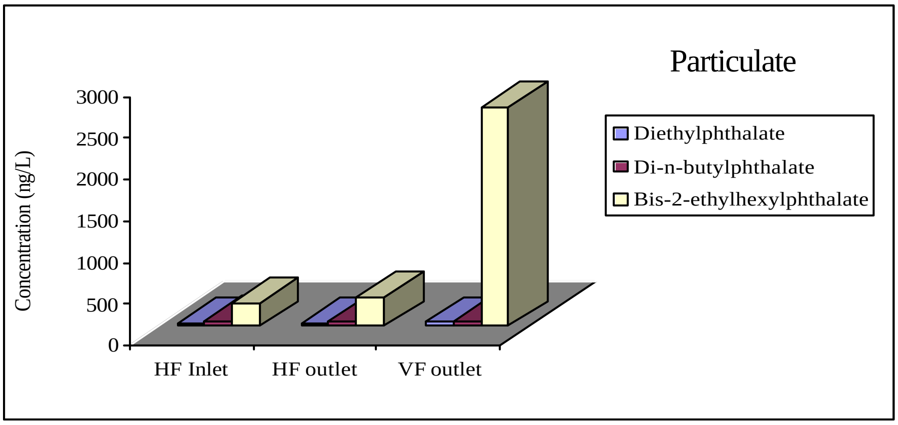

Fig. 3 - Concentration of diethyl, di-n-butyl and bis-2-ethylhexylphthalate in the particulate phase of the sample collected on December 2004.

CONCLUSIONS

The hybrid CW system, realized for the treatment of wastewater produced by a medium-size hotel, was suitable for the removal of pathogens and of ECDs since eight months from its conctruction, and the outlet water was able to be reused according to Italian regulations. Among ECDs trace amounts of estrogens (17-α-estradiol and ethynyl-estradiol), PAHs (Naphthalene, Phenanthrene, Fluoranthene and Pyrene) and phthalates (diethyl, di-n-butyl and bis-2-ethylhexylphthalate) were found in inlet wastewater. All of these compounds were removed at high percentages (up to 100% for estrogens), with the only exception of bis-2-ethylhexylphthalate, which was released by the HDPE liner. Therefore the use of geo-membrane with no ECDs release it is of paramount importance, especially for the reuse of the final effluent.

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