Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2020.26009

198 (2020) 126–139
September

Treatment of olive mill and municipal wastewater mixture by pilot scale vertical flow constructed wetland

Ayoub El Ghadraoui[a,b], Naaila Ouazzani[a,b], Abdelaali Ahmali[a,b], Tawfiq El Hakim El Mansour[a,b], Faissal Aziz[b], Abdessamed Hejjaj[b], Massimo Del Bubba[c], Laila Mandi[a,b,*]

[a]*Laboratory of Water, Biodiversity and Climate change, Faculty of Sciences Semlalia, University Cadi Ayyad, Marrakech, Morocco, emails: ayoub.elghadraoui@unifi.it (A. El Ghadraoui), ouazzani@uca.ma (N. Ouazzani), abdelaali.ahmali@edu.uca.ac.ma (A. Ahmali), taoufiqelhakimelmansour@gmail.com (T.E.H. El Mansour)*
[b]*National Center for Studies and Research on Water and Energy (CNEREE), University Cadi Ayyad, P.O. Box: 511, 40000 Marrakech, Morocco, Tel./Fax: +212524434813; emails: mandi@uca.ma (L. Mandi), faissalaziz@gmail.com (F. Aziz), dashaouz@yahoo.fr (A. Hejjaj)*
[c]*Department of Chemistry, University of Florence, Sesto Fiorentino, Florence, Italy, email: massimo.delbubba@unifi.it (M. Del Bubba)*

Received 26 December 2019; Accepted 18 April 2020

ABSTRACT

The aim of the present study is to examine the feasibility of a pilot scale constructed wetland (PS-VFCW) in which pozzolan is adopted as new filling media to enhance the treatment efficiency of a mixture of olive mill wastewater (OMWW) and municipal wastewater (MWW). Special attention was devoted to the removal of specific toxic phenolic compounds. The PS-VFCW consisted on a polyvinyl chloride tank (height: 0.60 m, diameter: 0.56 m, and surface area: 0.24 m^2). The pilot plant was planted with *Phragmites australis* and filled from the bottom with 10 cm of gravel, 10 cm of pozzolan, and 30 cm of sand. The applied hydraulic loading rate (HLR) was 60 L/m^2/d with a mean influent concentration of 6,100; 131; 9.45; 10.19; 232; 2.04; and 12.40 mg/L for chemical oxygen demand (COD), polyphenols, orthophosphates (PO_4^{3-}), total phosphorus (P), sulfate (SO_4^{2-}), nitrite (NO_2^-), and ammonium (NH_4^+), respectively. The PS-VFCW was monitored for a period of 1 y. Obtained results show that the PS-VFCW achieved high removal rates: 91%, 89%, 94%, 94%, 58%, 92%, and 95%, respectively for COD, polyphenols, PO_4^{3-}, P, SO_4^{2-}, NO_2^-, and NH_4^+. High pressure liquid chromatography (HPLC) analysis of phenolic fractions in studied effluents highlights the removal of a variety of these compounds, especially toxic one's such as tyrosol and hydroxytyrosol after treatment by the PS-VFCW. Moreover, analysis on the CW filling medium demonstrates the absence of phenolic compounds which is probably due to their biodegradation by microorganisms or adsorption by pozzolan.

Keywords: Vertical flow constructed wetland; Pozzolan; Olive mill wastewater; Organic load; Polyphenol; Hydroxytyrosol

1. Introduction

Olive oil is a liquid obtained by pressing of olive fruit *Olea europaea*. It is mainly composed of mixed triglyceride esters (~99%) and free fatty acids, mono-, and diacylglycerols, and an array of lipids such as hydrocarbons, sterols, aliphatic alcohols, tocopherols, and pigments [1]. Five types of oil are distinguished as extra virgin olive oil, virgin olive oil, refined olive oil, lampante oil, and olive oil [2]. The olive oil production is an industrial activity of crucial importance for the economy of Mediterranean countries. About 3.1 million tons of olive oil are produced in 2018/2019 worldwide [3]. According to the international olive council, the fourth first olive oil producers are Spain (51%), Italy (9%), Greece (7%), and Morocco (6%) [3].

* Corresponding author.

Olive mill wastewater (OMWW) is an industrial liquid waste produced during the olive-oil extraction. Olive oil can be extracted from the fruit by mechanical pressing, as well as three-phase or two-phases centrifugation [4]. Centrifugation methods offer the advantages of complete automation compared to pressing and have been therefore widely adopted since some decades in the olive-oil production industry [5]. However, centrifuge-based methods have some disadvantages, including the production of large OMWW volumes, which accounted recently for many million m^3 per year in the Mediterranean region alone [6]. In fact, the production of 100 kg of olive oil generates from 100 to 800 L of OMWW, depending on the centrifugation approach adopted [4].

OMWW is an aqueous, dark, foul-smelling, and turbid suspension, showing different chemical compositions, depending on several parameters such as the genotype of olives, their ripening level, the climatic and soil conditions, and the extraction method [7]. Accordingly, for commonly reported parameters, such as pH, electrical conductivity (EC), chemical oxygen demand (COD), and total polyphenols, very wide ranges of values can be found in literature [6]. In any case, an acidic pH (4.8–5.7), very high EC (5–81 mS/cm) and organic load (COD = 16.5−156 g/L), as well as a significant content of phytotoxic and antibacterial phenolic substances (total polyphenols = 0.8–8.9 g/L) are common findings [6]. Such a composition makes the treatment and/or the disposal of OMWW a critical environmental problem in the Mediterranean area, especially considering the relatively short period of time in which OMWW are produced.

Several methods have been tested for OMWW treatment such as physical treatment (dilution, filtration, evaporation, sedimentation, and centrifugation) and physicochemical treatment (flocculation, precipitation, adsorption, chemical oxidation, ion exchange, and coagulation). However, these treatments were either not able to reduce organic loads and toxicity to acceptable limits or relatively expensive as large quantities of chemicals are required [6]. Some authors tried adsorption/desorption techniques [8,9] or biological treatments such as activated sludge [10] and membrane bioreactors [11,12]. However, these systems exhibited non-uniform performances, due to the toxicity of OMWW. Achak et al. [13] tested a combination of sand filter with macrophytes system composed of soil as substrate and a mixture of three type of aquatic plants *Phragmites australis*, *Typha latifolia*, and *Arundo donax*. Beside studies carried out by Achak et al. [13,14] demonstrating that CW system could resist to clogging while treating OMWW for over 6 months with good removal efficiencies, most of the studies above-mentioned didn't succeed to overcome the clogging after less than half year of system operating. Moreover, the removal of phenolic compounds was still not satisfying.

Constructed wetland (CW) is considered one of the cheapest municipal wastewater (MWW) treatment systems which is more adapted to low socio-economic conditions in developing countries. The involvement of biological (biodegradation, assimilation, and plant uptake), chemical (precipitation, adsorption), and physical (sedimentation, filtration) phenomena in CW treatment allows these systems to have the capacity to treat several types of wastewaters, including the most polluted ones [15]. CWs are commonly used to treat domestic wastewater. Lately they have been also applied to the treatment of a variety of wastewaters [6]. However, only few studies have been published on the treatment of OMWW by CWs [6]. Most of these researches highlighted the importance of OMWW pre-treatment, including its dilution, in order to reduce the OMWW toxicity for plants and microorganisms responsible of degradation processes. In this context, dilution by fresh water [16] or by MWW, the latter performed after pre-treatment with trickling filter [17], addition of calcium hydroxide, lime putty, and hydraulic lime [7] and electrochemical oxidation [18] were tested. Despite the good removal performances obtained by these authors, the effluent quality did not completely meet the standards requested for water reuse and/or discharge in surface water.

The filling medium is a very important component of CWs as it contributes to the chemical, physical, and biological treatment processes. Hence, the selection of the material to be used as filling medium of CWs is crucial to enhance the CW efficiency. This choice usually depends on the cost and the availability of the material [19]. Additional criteria, related to physicochemical characteristics of the material, as well as to the kind of pollutants to be removed, could support the choice of the filling medium [20]. Different materials, such as gravel and sand have been commonly used as CW filling media. However, many other materials have been tested occasionally as owing to their specific characteristics. Among them, naturally occurring materials (e.g., natural zeolites), waste products (e.g., tire chips, rice straw), or man-made products (e.g., Filtralite® and biochar) [20,21] have been evaluated.

However, to the best of our knowledge, there is currently no published work using pozzolan as filling material of CWs. Pozzolan is a naturally available rock of volcanic origin, which contain aluminum and iron oxides able to interact with many kinds of pollutants, thus increasing their removal through sorption. In this regard, pozzolan was used as sorption material for the removal of some heavy metals like arsenic [22], but also as coagulant agent for the removal of dissolved organic matter [23].

Based on the aforementioned considerations, the aim of this work was to study the performances of a novel designed PS-VFCW, incorporating a pozzolan-layer inside the system, for the treatment of OMWW properly diluted with MWW. A special focus was attributed to the removal of phenolic compounds and their fate inside the PS-VFCW, especially those considered more toxic (e.g., hydroxytyrosol and tyrosol) to the environment when OMWW is discharged without being treated.

2. Material and methods

2.1. Pilot-scale constructed wetland description

The PS-VFCW (Fig. 1) is built from a polyvinyl chloride circular tank (height 0.60 m and surface area0.24 m^2). The PS-VFCW is filled by 30 cm of sand (0.25/0.40 mm) as infiltration layer followed by 10 cm of pozzolan (5/20 mm) as transition layer and 10 cm of gravel (20/40 mm) as drainage layer (Fig. 1). The selection of such material was oriented

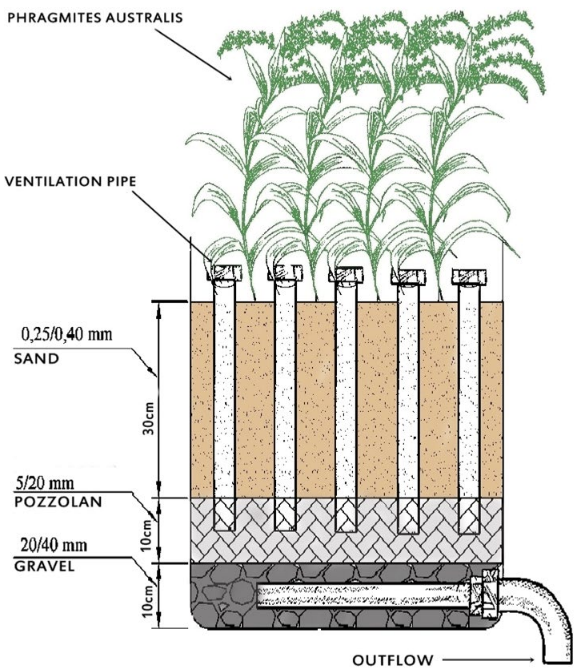

Fig. 1. Diagram of the pilot scale constructed wetland with vertical flow.

based on the utility and the availability. Sand is an inexpensive alternative to soil and recommended to use in CW receiving agricultural wastewater [24]. Sand was often used in several studies involving CW and OMWW [17]. Numerous studies have demonstrated the ability of pozzolan for the removal of different pollutants such as heavy metals and organic matter due to its chemical composition and structural porosity [22,23]. In order to collect the treated water, a drain is installed in the bottom of the PS-VFCW. Five ventilation pipes are also installed to provide the necessary oxygen during the functioning of the system. The PS-VFCW is planted with young shoots of *P. australis* at a density of 4 plants/m^2. *P. australis* is the most widely used plant species in CW system. Different studies demonstrated its high phytoremediation ability, high resistance to toxicity and high propagation rate [25–27]. The applied hydraulic loading rate (HLR) was 60 L/m^2/d. The feeding of the system was performed using a peristaltic pump which uploads to the pilot a total volume of 15 L/d of the mixture. In this work, the used feeding frequency is one-day feeding and two days' rest. This frequency is often mentioned in literature and CW guidelines [28,29]. Resting period will allow the CW to charge with oxygen in order to maintain aerobic conditions necessary for biodegradation process and will also give time to microorganism to degrade organic matter remained inside the system [14].

The system was feed with a mixture of 1% of OMWW/99% of MWW (corresponding to 90% organic load of OMWW and 10% of MWW). This mixture allows us to dilute organic load of OMWW and to optimize the operation of the PS-VFCW by adding further microorganisms available in MWW. The dilution rate was chosen according to two main reasons, the first is the fact that OMWW is produced in a short period of the year which enables to dose a small HLR inside the CW during the year, the second one is to respect the nominal organic load to be received by PS-VFCW. The organic load rate in COD applied in this study was 366 g/m^2/d.

2.2. Sampling

The OMWW was taken from an olive mill factory working with a traditional extraction system (i.e., mechanical pressing). It is located in Rass El Ain 50 Km on the N8 of the city of Marrakech, Morocco. The sampling was performed during the month of February 2016 and stored in adequate conditions of temperature and obscurity.

The MWW used for OMWW dilution was weekly collected from the inlet of the Marrakech (Morocco) wastewater treatment plant (activated sludge).

In order to monitor the performance of PS-VFCW, samples were collected at the inlet and the outlet of the system every week to analyze the physicochemical parameters and every 2 weeks in sterile bottles to analyze bacteriological parameters. An aliquot of samples is stored in the fridge at –16°C for further use in case of need. For all analysis, three replicates were carried out.

2.3. Analytical methods

2.3.1. Pozzolan characterization

Pozzolan substrate was imported from the city of Timahdite in the Fes-Meknes Region (Morocco) 33°14′13″N, 5°03′36″W, this area is rich in karstic volcanic rocks derived from two dormant volcanoes present in the area.

Pozzolan mineral composition was determined by X-ray fluorescence using a portable XRF analyzer (Olympus NDT, Waltham, USA). Scanning electron microscopy (SEM) was used in this case to highlight morphological shape of pozzolan. The analysis was carried out in the Center of Analysis and Characterization at the Faculty of Sciences Semlalia of Marrakech, using the VEGA3 TESCAN device.

2.3.2. Wastewater analysis

2.3.2.1. Physicochemical parameters

EC and pH were measured using a multi parameter probe model HI 9829 (HANNA, Woonsocket, RI, USA). Total suspended solids (TSS) were determined after filtering a sample through a Millipore filter (0.45 μm) and drying the retained residue at 105°C for 120 min (AFNOR-T90-105) [30]; COD was determined by a digestion followed by colorimetric dichromate method (AFNOR-T90-101) [30]; P was performed by molybdate and ascorbic acid method after potassium peroxodisulfate digestion (AFNOR-T90-023) [30]; PO_4^{3-} was performed by molybdate and ascorbic acid method (AFNOR-T90-022); $NH4^+$ was determined by indophenol method (AFNOR-T90-015) [30]; NO_2^- were determined by colorimetric method after diazotization (AFNOR T 90-013) [30]. NO_3^- were reduced to nitrites after their passage over a copper cadmium column [31]. The measurement of sulfates was carried by nephelometric method [31].

2.3.2.2. Bacteriological parameters

Total coliform (TC) and fecal coliforms (FC) count was performed according to the AFNOR Standard NF EN ISO 9308-1 (September, 2000) [30] in TTC Tergitol medium. The dishes were incubated at 37°C for TC and 44.5°C for FC for a period of 24 h and then the number of forming colony units was calculated. The fecal streptococci (FS) count was performed according to the Standard AFNOR NF ISO 7899-2 (August, 2000) [30] in BEA medium. The dishes are incubated at 44.5°C for 24 h and then the number of colonies was calculated. Three repetitions were carried out in order to obtain the best results and to avoid errors.

2.3.2.3. Phenolic compounds

Phenolic compounds have been evaluated using two approaches; the first one was the analysis of the total phenol in water using the [32] protocol which consists of an ethyl acetate extraction followed by the spectrophotometric Folin–Ciocalteu method, using Gallic acid as the reference standard. The second approach was the investigation of selected individual phenolic compound in extracts from OMWW–MWW mixed wastewater and CW medium, using high performance liquid chromatography (HPLC) with the following characteristics. Column: Eurospher II 100-5 C18, 250 mm × 4.6 mm + pre-column of the same stationary phase (Knauer, Berlin, Germany). Detection: photodiode array PDA, Mobile Phase: 5 ACN/95 water (o-phosphate pH = 2.6), temperature: 25°C, flow rate: 1 mL/min. For solid substrate, Macheix method was adapted to determine phenolic compounds. Ten grams of each soil sample was shaken in 20 mL cold methanol (80% v/v) during 15 min and the mixture was centrifuged for 3 min at 5,000 rpm at 4°C. This step was repeated three times before the supernatants were evaporated to remove methanol. A solution of ammonium sulphate (40% v/v) was added to the extract followed by meta-phosphoric acid solution 20% (1/10 v/v). This phase was followed by depigmentation and defatting of with petroleum ether (v/2). The extract was purified by ethylene acetate (v/v) and evaporated to dryness at 35°C with a rotary evaporator and the residue was recovered in 2 mL of Grade HPLC pure methanol before being analyzed with HPLC.

2.4. Statistical analysis

Statistical analyses were performed using STATISTICA 9 (StatSoft, Inc., Tulsa, Oklahoma, USA) software by analysis of variance (ANOVA), all differences were considered significant at 5%.

3. Results and discussion

3.1. Pozzolan morphological and chemical characteristics

The pozzolan SEM images obtained at different zooms show the amorphous nature of the natural material and the presence of multiple pores of variable sizes (Fig. 2). Chemical characteristics of the natural pozzolan used as new substrate in the PS-VFCW are presented in Table 1. XRF analysis showed that silica SiO_2 (36.58%), iron oxide Fe_2O_3 (15.87%), and alumina Al_2O_3 (12.58%) are the main components of the investigated pozzolan; these characteristics are similar to those described by the literature [22,23,33].

3.2. Influent characteristics

Results of different analyzes carried out on the various effluents used in this study are presented in Table 2.

The studied OMWW presented an acidic pH (5.01) and high content in EC, TDS, COD, TSS, and phenolic compounds. Calheiros et al. [25] have found a similar pH value ranged between 4.5 and 5 for press and 3-phase extraction. However, measured COD concentration in OMWW (264.05 g/L) was higher than the values quoted in literature which are between 120 and 130 g/L for press extraction, 40 g/L for 3-phase extraction and between 5

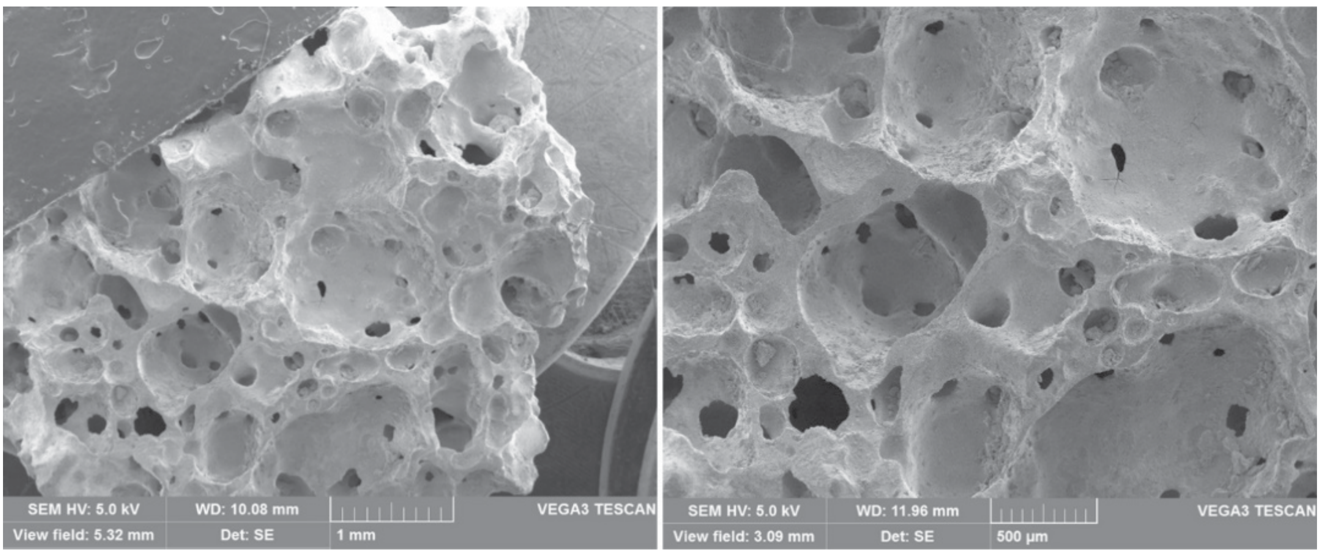

Fig. 2. Scanning electron microscopy (SEM) images of natural pozzolan at different zoom.

Table 1
Chemical composition of pozzolan

Constituent	Current study	[22]	[23]	[34]
Major components (%)				
SiO_2	36.58	39.56	46.08	69.2
Al_2O_3	12.58	13.12	16.43	13.2
Fe_2O_3	15.87	21.19	12.65	1.7
CaO	8.97	9.50	9.62	2.7
MgO	5.21	5.89	5.51	0.8
K_2O	0.87	1.27	1.51	3.0
Na_2O	3.12	3.24	3.90	3.9
TiO_2	2.54	4.14	2.88	0.2
Mn_2O_3	0.11	0.28	0.18	–
SO_3	0.21	0.51	–	0.1
P_2O_5	0.33	0.52	–	0.1
Trace elements (ppm)				
S	754	–	–	–
Sr	437	–	689.8	–
V	239	–	–	–
Zr	203	–	273.8	–
Cr	134	–	145.5	–
Zn	103	–	–	–
Ni	66	–	49.1	–
Cu	49	–	–	–
Th	34	–	–	–
Y	22	–	26.1	–
Rb	10	–	33.1	–
As	8	–	–	–

and 25 g/L for 2-phase extraction [35,36]. Total polyphenol concentration in OMWW was about 8.73 g of Gallic acid equivalent/L. Niaounakis and Halvadakis [37] reported a polyphenol concentration ranged between 0.5 and 24 g/L for press extraction. Regarding microbiological content, investigated OMWW were exempt from total coliform, fecal coliform, and streptococcus which are key factor in order to decide the fate of the treated effluent (Table 2).

In order to test the treatment feasibility of the harsh and toxic OMWW in PS-VFCW, OMWW were diluted by MWW. Thus, their COD content go from 264.05 to 6.10 g/L. The mixture also allowed to decrease EC which was rather high in OMWW and could harm macrophytes and microorganisms intervening in the treatment. EC decreased from 28.23 to 4.44 mS/cm. In addition, total polyphenols content decreased significantly from 8.73 g/L in OMWW to 131 mg/L in the mixture. The dilution with MWW provides also a large fraction of microorganisms supposed to play an important role in the treatment by PS-VFCW.

3.3. Purifying performances of the PS-VFCW

Table 3 summarizes the evolution of parameters during the treatment period and the removal efficiency obtained by the PS-VFCW. Results show that the PS-VFCW influent has a neutral pH with an average value of 7.26 ± 0.095 (Table 3, Fig. 3a). At the PS-VFCW effluent, pH rises to 8.21 ± 0.08, this alkalinization phenomenon can be attributed to biological oxidation [17]. The obtained pH values after treatment are in accordance with the limit of Moroccan standard for water quality for irrigation which are between 6.5 and 8.4 [38]. EC increased from 4.61 ± 0.06 mS/cm (Fig. 3b) at the inflow to 7.48 ± 0.012 mS/cm at the outflow. The same phenomenon was reported by Achak et al. [13] and Yalcuk et al. [39]. This increase is mainly due to the evapotranspiration inside the PS-VFCW [24], especially in

Table 2
Characterization of various influents (mean of three replicates ± standard deviation)

Parameters	MWW	OMWW	(OMWW + MWW)
pH	7.07	5.01	7.26
Dissolved oxygen, mg/L	0.91	0.70	1.18
EC, mS/cm	2.21	28.23	4.44
TDS, g/L	0.32	22.10	2.22
TSS, mg/L	228.33 ± 13.50	2,066.00 ± 11.27	577.78 ± 13.87
Total polyphenol, g/L	0.005 ± 0.001	8.73 ± 0.43	0.13 ± 3.27
COD, g/L	0.519 ± 0.41	264.05 ± 11.49	6.10 ± 0.54
PO_4^{3-}, mg/L	0.82 ± 0.06	31.14 ± 0.65	9.45 ± 0.46
SO4²–, mg/L	136.67 ± 12.58	1,320 ± 0.05	232.61 ± 33.99
NH_4^+, mg/L	12.95 ± 0.52	6.33 ± 0.30	12.40 ± 0.94
NO_3^-, mg/L	0.04 ± 0.01	1.32 ± 0.05	0.22 ± 0.04
NO_2^-, mg/L	1.251 ± 0.08	96.23 ± 9.41	2.04 ± 0.08
P, g/L	0.95 ± 0.06	41.61 ± 4.37	10.19 ± 0.48
TC, UFC/100 mL	2.13 × 107	0	$5.30.10^6$
FC, UFC/100 mL	8.67 × 106	0	$3.70.10^6$
FS, UFC/100 mL	1.00 × 105	0	$1.27.10^5$

Table 3
Mean inlet, outlet, and removal efficiency of the CW (mean of three replicates ± standard deviation)

	Mean inlet	Mean outlet	Mean removal
pH	7.26 ± 0.09	8.21 ± 0.08	–
EC, mS/cm	4.61 ± 0.06	7.48 ± 0.01	–
TSS, mg/L	288 ± 42	3.51 ± 2.52	99%
COD, g/L	6.1 ± 0.2	0.57 ± 0.04	91%
P, mg/L	10.2 ± 0.3	0.58 ± 0.08	94%
PO_4^{3-}, mg/L	9.5 ± 0.9	0.49 ± 0.12	94%
NH_4^+, mg/L	12.4 ± 0,2	0.53 ± 0.02	95%
NO_3^-, mg/L	0.22 ± 0.01	2.32 ± 0.15	–
NO_2^-, mg/L	2.0 ± 0.3	0.16 ± 0.06	92%
SO_4^{2-}, mg/L	232 ± 18	97.6 ± 10.24	58%
Total polyphenol, mg/L	132 ± 4	15.53 ± 2.06	89%

hot season. However, despite that, the effluent EC value still under the limit of the Moroccan standards for water quality for irrigation which is 12 mS/cm [38].

Obtained results indicated high removal percentage of TSS, organic matter and nutrients by the PS-VFCW (Table 3). The removal of TSS was very high (99%) (Fig. 3c). This result is higher than those reported by Achak et al. [13] when treating diluted OMWW by combining sand filter and CW (70%). This high efficiency of PS-VFCW could be attributed to the degradation by bacteria located in the root zone of the macrophytes [40]. It could be also related to the use of fine sand in filtration layer, as TSS can be eliminated also by sedimentation through the media [41]. With a mean value of 3.51 ± 2.52 mg/L of TSS, the treated mixture is highly below the Moroccan standards of water quality intended for the irrigation which is between 100 and 200 mg/L depending on the irrigation techniques.

In the present study, PS-VFCW receives an organic load around 366 g of COD/m^2/d with a mean concentration of 6.1 ± 0.2 g/L at the inlet. After treatment, the concentration drops to a mean of 0.57 ± 0.04 g/L at the outlet with a mean removal efficiency of 91% (Fig. 4a). This removal rate is considered higher than what's reported in the literature. Jemmal et al. [33] reported a removal rate of 73.46% for COD while treating in average 29 g COD/m^2/d using a trickling filter as a pre-treatment system. Herouvim et al. [17] achieved an abatement rate of 73% as a mean removal for CW in series treating 217 g COD/m^2/d. Organic matter is generally removed from the medium through adsorption and retention or through biological degradation under oxygenated conditions in the porosity of the PS-VFCW. The average COD concentration at the outlet is very low 0.57 ± 0.04 g/L showing a good biodegradation inside the pilot regardless of the high organic load applied.

For NH_4^+ the removal efficiency reached 95% (Table 3, Fig. 4b). This result is higher than those obtained by Achak et al. [13] and Yalcuk et al. [39] which reported a removal rate of 75% and 49%, respectively, in their studies. Obtained results show good nitrification of NH_4^+. The removal of NO_2^- reached a rate of 92% (Table 3, Fig. 4c). The abundance of oxygen provided by the root of *P. australis* and the presence of ventilation pipes allow nitrifying bacteria to complete transformation of NO_2^- into NO_3^- which is in accordance with literature [42].

NO_3^- content increases to a mean concentration of 2.32 ± 0.15 mg/L at the outlet (Table 3, Fig. 5a). This NO_3^- value is very low in comparison to NH_4^+ eliminated inside the pilot (an important part of nitrogen has been lost by denitrification).

The lack of anoxic area in the bottom of PS-VFCW especially in pozzolan layer could explain the slight increasing of NO_3^- at the outlet since several authors demonstrate that in CWs, NO_3^- is totally removed by denitrification which occurs in anoxic zones of the layers inside the CW or in anoxic microsites of the attached biofilm to the plant tissue or the substrate [43], or direct plant uptake [44]. Even though, the mean NO_3^- content at the outlet remains below the limits of the Moroccan standard for irrigation water (less than 30 mg/L).

PS-VFCW has shown a high removal efficiency regarding P (94%) and PO_4^{3-} (94%) while receiving 764.25 and 708.75 mg/m^2/d, respectively (Table 3, Figs. 5b and c). Similar results were found by other authors: 95% and 87% removal efficiency were respectively found by Herouvim et al. [17] and Yalcuk et al. [39]. This high performance regarding P and PO_4^{3-} removal could be explained by the implication of multiple phenomena such as sedimentation, adsorption, biological transformation, and uptake by the plant [36].

Prochaska and Zouboulis [28] studied the removal of P in CW and concluded that the main mechanism for its removal is through precipitation with calcium or aluminum, complexation with some hydroxides, and/or adsorption by the substrate. These phenomena seem to be optimized in this study by using natural pozzolan as new material in the transition layer of PS-VFCW. The high percentage of iron oxide and aluminum oxide in the pozzolan could probably increase their role in phosphorus complexation/precipitation inside the planted filter. However, these processes are valid only as long as the media is not saturated with P and there are free available adsorption sites.

As shown on Table 3 and Fig. 6, the mean concentration of SO_4^{2-} was reduced to an average concentration of 97.6 ± 10.24 mg/L. Despite the moderate removal efficiency of SO_4^{2-} (58%), the outlet concentration is below the Moroccan water quality standard for irrigation which is 250 mg/L. Sulfate can cause diarrhea in some case [45]. The mean concentrations of TC, FC, and FS in influent and effluent of the PS-VFCW are presented in Table 4. The average load of TC, FC, and FS at the influent was 5.48 ± 0.16, 5.31 ± 0.11, and 5.11 ± 0.19 Log unit/100 mL, respectively.

In the PS-VFCW, removal efficiencies were 2.76 ± 0.11 Log units for TC, 2.56 ± 0.53 Log units for FC and 3.87 ± 0.30 Log units for FS.

These results are in accordance with those found in the literature regarding the removal of TC and FC in CW. Sleytr et al. [46] investigated the removal of bacteria in vertical flow CW and showed a removal efficiency of 1.6 Log units regarding the elimination of FC; 1.9 Log units was found by Torrens et al. [47] and Barrett et al. [48] reported a removal efficiency of 2.9 Log units for TC in

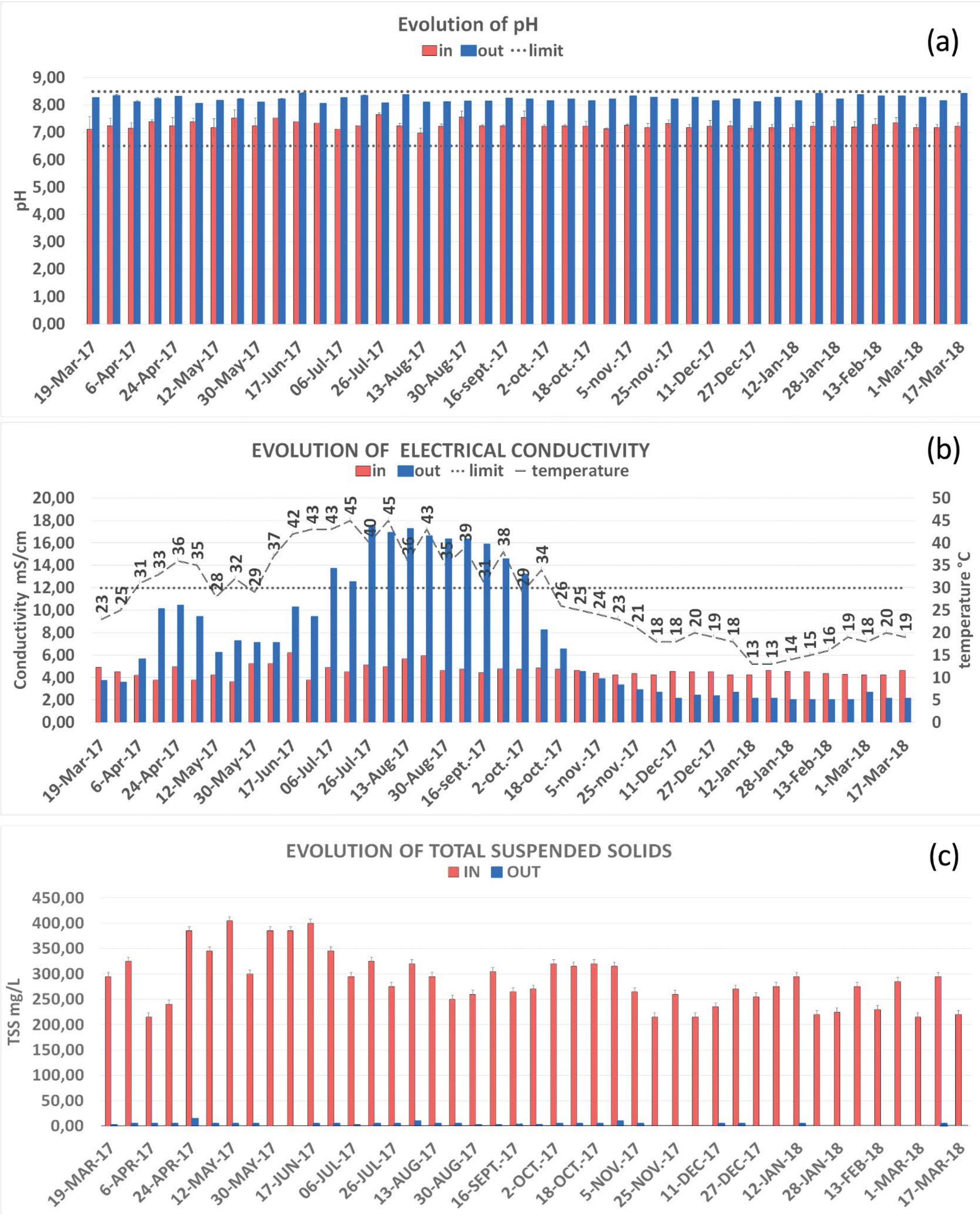

Fig. 3. Evolution of pH (a), electrical conductivity (b), and total suspended solids (c) during the treatment period.

CW. Nerveless, the average concentration of FC obtained at the outlet in this study was higher than 1,000 CFU/100 mL, the limit value of the B category in Moroccan standards of water quality for irrigation; therefore, the effluent could be reused for the irrigation of cereal, industrial and fodder crops, pastures, and trees plantations. However, if CW is followed by a disinfection system, the effluent could comply with the A category and could be used for the irrigation of crops intended for raw consumption, sports fields, and landscapes.

Several physical, chemical, and biological mechanisms interfere in the removal of bacteria in CW [46]. Physical factors include adsorption, sedimentation, and filtration [41]. Chemical factors include oxidation, solar radiation, biocide

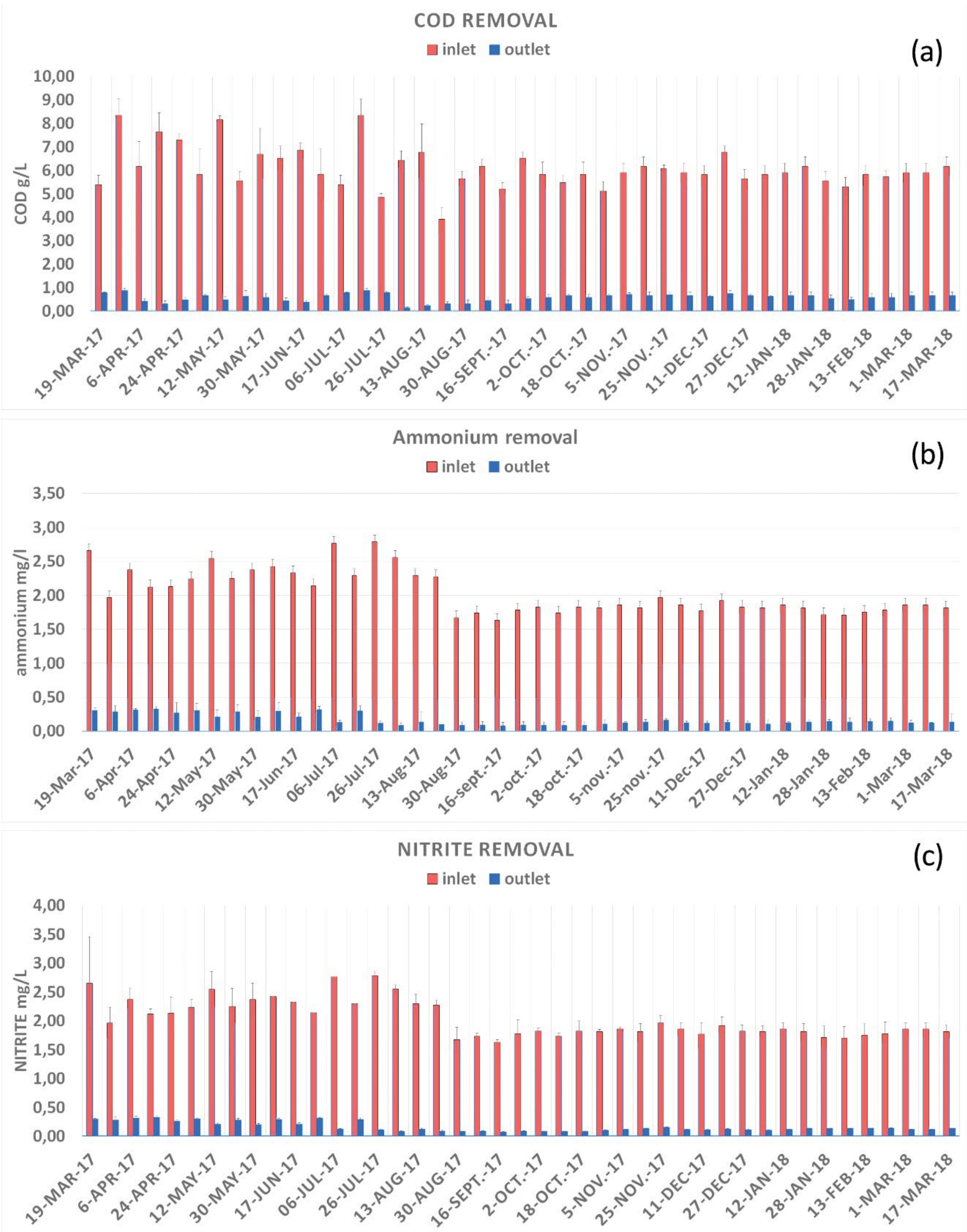

Fig. 4. Evolution of COD (a), ammonium (b), and nitrite (c) during the treatment period.

excreted by roots [49], and biological factors include predation by nematodes/protists/zooplankton, competition, inactivation, and natural die off [50]. However, the literature shows many controversial results regarding the role of the macrophytes in the removal of bacteria in CW, some authors have observed that the plant exhibits a little to no effect on the removal of bacteria [51], at the opposite side, Brix [52] shows the great importance of the macrophytes in the bacteria elimination by CW. The role of pozzolan in fecal bacteria removal couldn't be seen in the studied PS-VFCW. More investigations have to be conducted to elucidate the role of this substrate in bacteria removal.

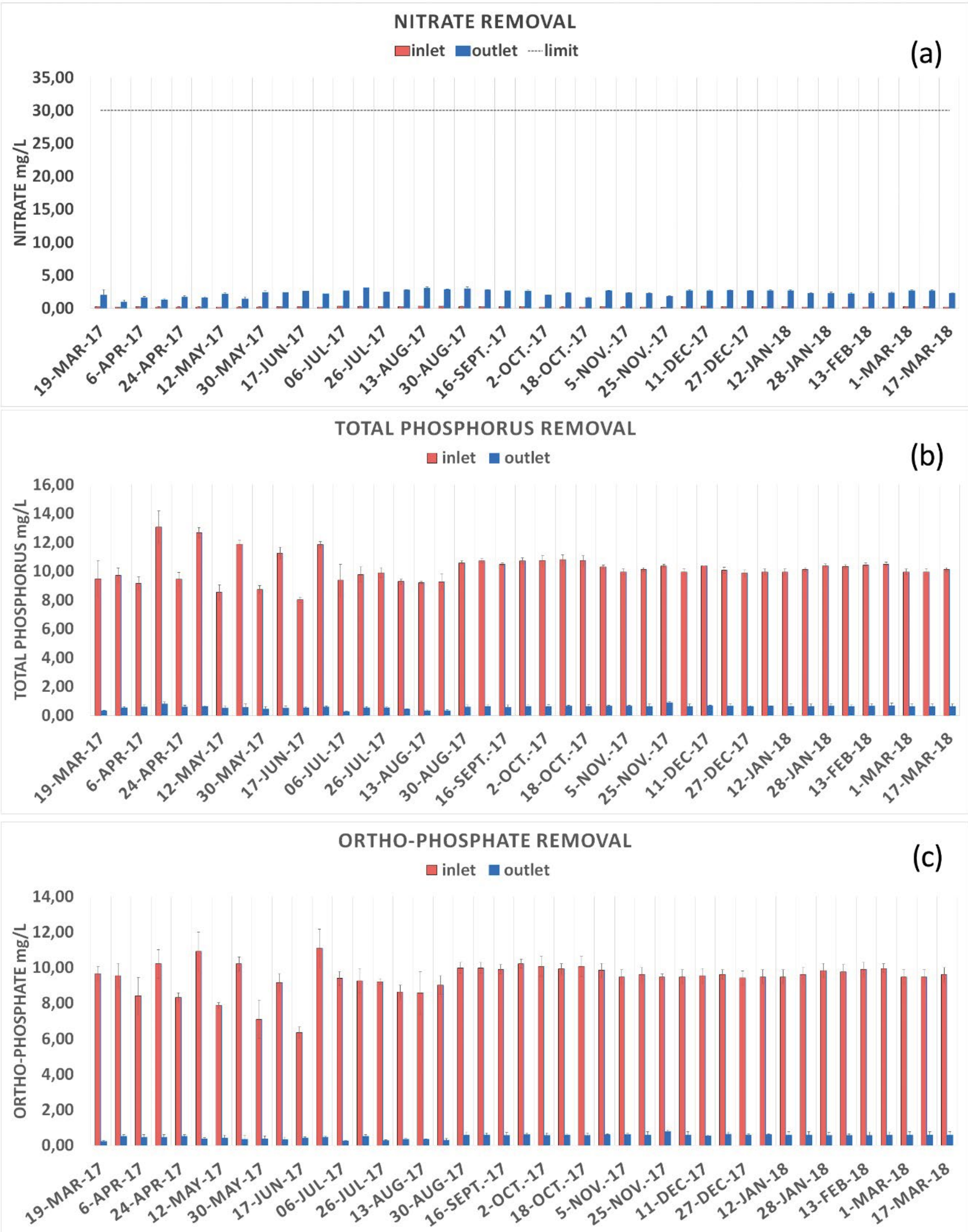

Fig. 5. Evolution of nitrate (a), phosphorus (b), and ortho-phosphate (c) during the treatment period.

3.4 Polyphenols removal and behavior in the PS-VFCW

The removal rate of polyphenols by PS-VFCW reached 89% (Table 3, Fig. 7). Specific phenolic compounds investigation was conducted on liquid (crude OMWW, crude mixture, and treated mixture) and solid (sand and pozzolan) samples in order to highlight their fate after the treatment. As shown in Fig. 8a, HPLC analysis detected the presence of several phenolic compounds in OMWW such as 3,4-dihydroxyphényléthanol (hydroxytyrosol), 4-hydroxyphényléthanol (tyrosol), acid

Table 4
Average concentration and log unit removal (mean of three replicates ± standard deviation) of total coliform, fecal coliform, and fecal streptococci

Parameters	Influent (Log unit)	Effluent (Log unit)	Removal (Log unit)
TC	5.48 ± 0.16	2.74 ± 0.11	2.76 ± 0.11
FC	5.31 ± 0.11	2.81 ± 0.2	2.56 ± 0.53
FS	5.11 ± 0.19	1.23 ± 0.22	3.87 ± 0.30

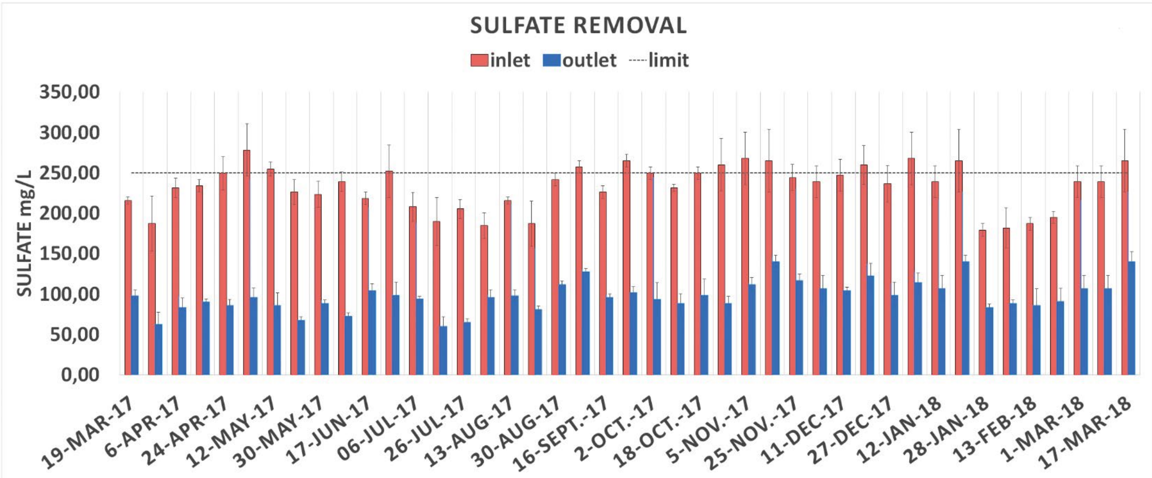

Fig. 6. Evolution of sulfate during the treatment period.

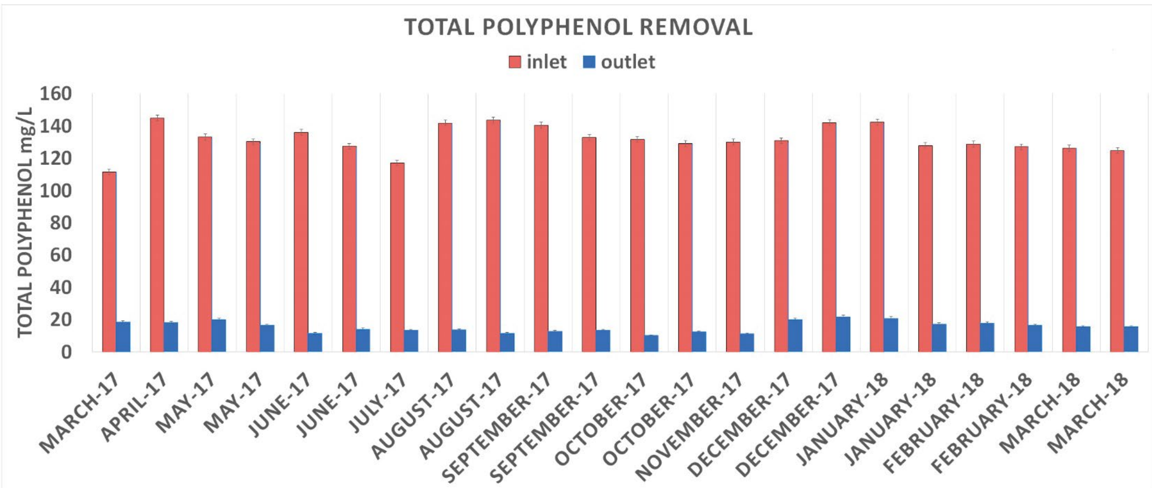

Fig. 7. Evolution of polyphenol during the treatment period.

3,4-dihydroxycinnamique trans-caffeate (caffeic acid), acid para-4-hydroxycinnamique (p-caumaric acid), and acide (E)-3-phényl-prop-2-enoïc (cinnamic acid) with a dominance of hydroxytyrosol.

These results converge with those reported by Tsagaraki et al. [53] which discloses that hydroxytyrosol, tyrosol, cinnamic acid, cafeic acid, and p-caumaric acid are the main phenolic compounds well represented in OMWW. Fig. 8b shows that mixing OMWW with MWW allowed the complete elimination of tyrosol, caffeic acid, p-caumaric acid and the reduction of both hydroxytyrosol and cinnamic acid concentrations by dilution effect. The treatment of the mixture (OMWW + MWW) by the new design of PS-VFCW allowed the complete elimination of hydroxytyrosol and other unidentified compounds (Fig. 8c). However, cinnamic acid still presents in the treated mixture. In the literature, only few papers [16,17] studied the polyphenol removal in CW and obtained a removal rate of around 70%. Achak et al. [13] managed to obtain a removal of 95% when combining sand filter to CW for OMWW treatment.

Phenolic compounds analysis inside the system PS-VFCW were carried out on sand (infiltration layer) which we divided on 3 layers 0–10, 10–20, and 20–30 cm, and on pozzolan (transition layer). The result in Fig. 9 shows that phenolic compounds such as hydroxytyrosol and cinnamic acid identified in the influent were not detected inside the system for both sand and pozzolan substrates.

The elimination of polyphenolic compounds in the substrate is probably due to biodegradation by fungi (e.g., Ascomycetes and Basidiomycetes) and bacteria (e.g., Pseudomonas) [54]. On the other hand, new unidentified phenolic compounds were detected in the sand and pozzolan that could be high molecule mass compounds resulting from the polymerization of monocyclic aromatic molecules such as hydroxytyrosol or cinnamic acid found in the mixture [55]. According to Tziotzios et al. [56] monocyclic aromatic molecules are recalcitrant to biodegradation.

The efficiency of PS-VFCW to remove phenolic compounds is probably due to the high oxygenation of the system by roots and ventilation pipes. According to Herouvim et al. [17], planted CW show higher efficiency than unplanted filter regarding the degradation of polyphenols; microorganisms responsible for this degradation are favored by the supply of oxygen carried out by plants roots. A planted soil which is characterized by an acid pH, high amount of organic matter, and high polyphenol concentration could facilitate the development of fungi, main microorganisms responsible of polyphenol degradation [57]. Moreover, the dilution of OMWW with MWW which add high amount of microorganisms in the influent and therefore increasing biological degradation contributes probably to increase the PS-VFCW efficiency in removing polyphenol compounds. Pozzolan substrate could have also participated to the elimination of total polyphenol by adsorption, as its adsorption capacity regarding organic matter was demonstrated by Sieliechi et al. [23]. The increasing of the pH after mixture application on the PS-VFCW could allow transformation of polyphenol to phenates [26]. The phenates have high retention attraction to cations such as aluminum and iron oxides and silicates.

4. Conclusion

This study demonstrates the feasibility of a new design of pilot scale vertical flow constructed wetland (PS-VFCW) for the treatment of a mixture of OMWW and MWW. In the CW a layer of pozzolan was added in order to enhance the removal mechanism. The obtained mixture was characterized by high content of polyphenol (132 mg/L) and organic matter (6.1 g/L). Monitoring the removal efficiencies during the treatment period have shown the ability of PS-VFCW to adapt to the mixture and to provide high removal performances of 91%, 89%, 94%, 94%, 58%, 92%, and 95% respectively for COD, polyphenols, PO_4^{3-}, P, SO_4^{2-}, NO_2^-, and NH_4^+. The combination of dilution and PS-VFCW treatment was also efficient to remove all existing toxic

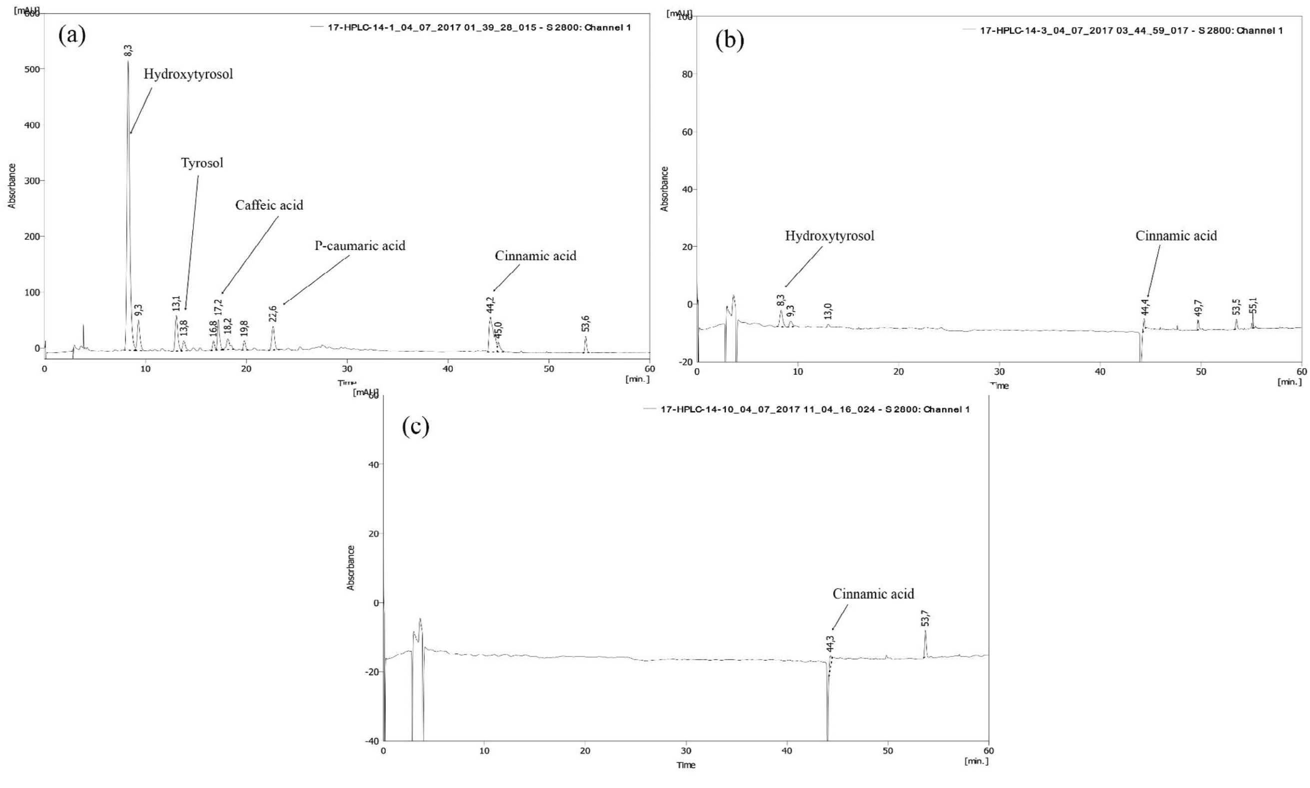

Fig. 8. High performance liquid chromatography chromatogram of phenolic compounds for (a) crude OMWW, (b) crude mixture OMWW + MWW, and (c) treated mixture.

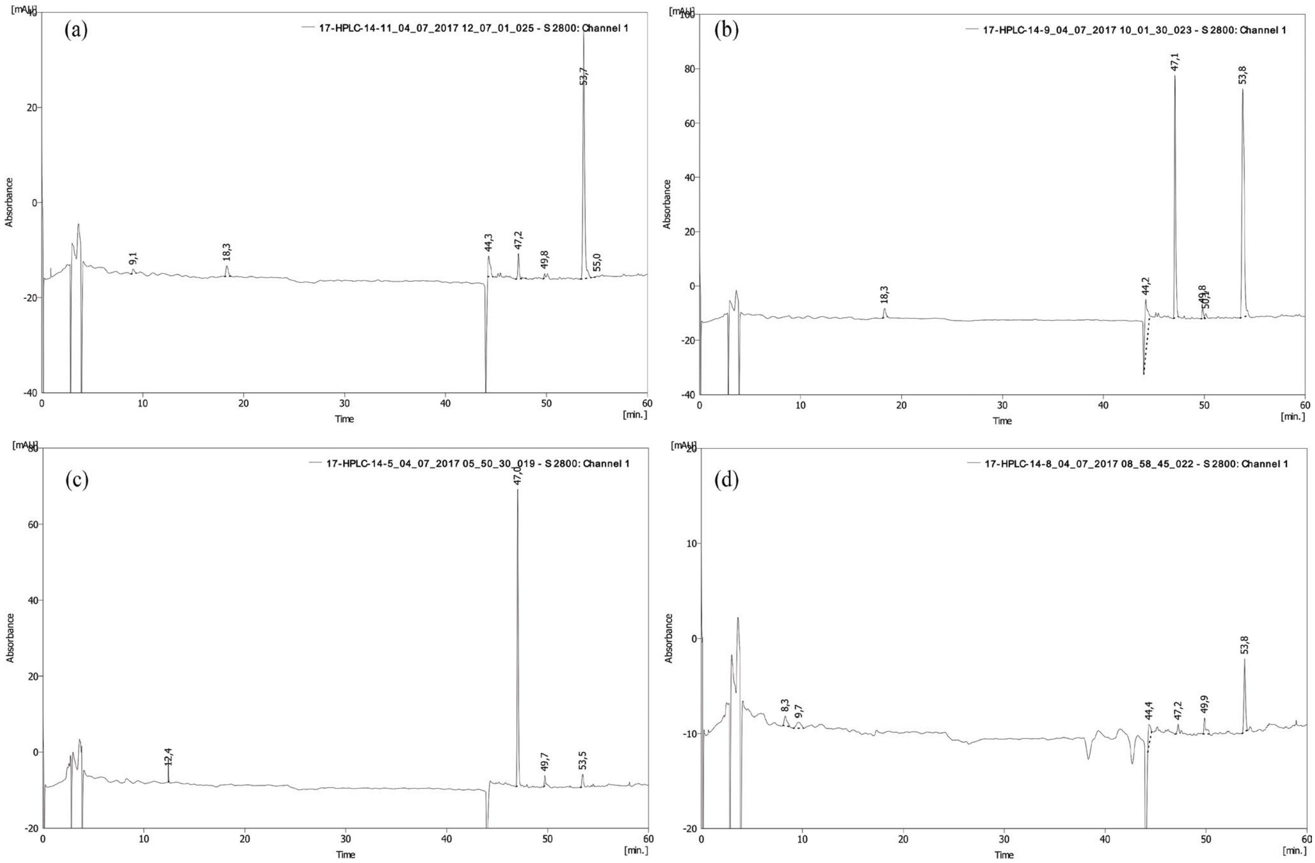

Fig. 9. High performance liquid chromatography chromatogram of phenolic compounds for (a) sand 0–10 cm, (b) sand 10–20 cm, (c) sand 20–30 cm, and (d) pozzolan.

phenolic compounds (hydroxytyrosol, tyrosol, caffeic acid, P-caumaric acid, and cinnamic acid) present in the mixture and no trace were detected in the CW substrate. In addition, the quality of treated mixture by the PS-VFCW met the Moroccan irrigation standards; the treated water could be reused for irrigation of cereal, industrial and fodder crops, pastures, and plantations trees. Therefore, the proposed treatment strategy could be a good option for the treatment of OMWW by injecting it in full scale CW respecting the proportion used in the current study.

Acknowledgments

This work was financed by the IRRIGATIO Project (ERANETMED-13-069, 2014 call, seventh framework program). A big thanks go to the National Center for Studies and Research on Water and Energy (Cadi Ayyad University) and the Pole of competences on Water and Environment (PC2E) for their technical and financial support to this work.

References

[1] D. Boskou, Olive Oil - Chemistry and Technology, Academic Press and AOCS Press, Greece, 2006.
[2] J.L. Quiles, Olive Oil and Health, CABI Publishing, United Kingdom, 2006.
[3] International Olive Council, 2019. Available at: http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-Figures
[4] J.A. Alburquerque, J. Gonzálvez, D. García, J. Cegarra, Agrochemical characterisation of "alperujo", a solid by-product of the two-phase centrifugation method for olive oil extraction, Bioresour. Technol., 91 (2004) 195–200.
[5] A. Roig, M.L. Cayuela, M.A. Sánchez-Monedero, An overview on olive mill wastes and their valorisation methods, Waste Manage., 26 (2006) 960–969.
[6] C.M. Galanakis, Olive Mill Waste: Recent Advances for Sustainable Management, Academic Press, Greece, 2017.
[7] M. Del Bubba, L. Checchini, C. Pifferi, L. Zanieri, L. Lepri, Olive mill wastewater treatment by a pilot-scale subsurface horizontal flow (SSF-h) constructed wetland, Ann. Chim., 94 (2004) 875–887.
[8] M. Achak, A. Hafidi, N. Ouazzani, S. Sayadi, L. Mandi, Low cost biosorbent "banana peel" for the removal of phenolic compounds from olive mill wastewater: kinetic and equilibrium studies, J. Hazard. Mater., 166 (2009a) 117–125.
[9] M. Achak, L. Mandi, N. Ouazzani, Removal of organic pollutants and nutrients from olive mill wastewater by a sand filter, J. Environ. Manage., 90 (2009b) 2771–2779.
[10] T. El Moussaoui, A. Kessraoui, N. Ouazzani, M. Seffen, L. Mandi, Synthetic urban wastewater treatment by an activated sludge reactor: evolution of bacterial biomass and purifying efficiency, J. Mater. Environ. Sci., 9 (2018) 817–827.
[11] Y. Jaouad, M. Villain, N. Ouazzani, L. Mandi, B. Marrot, Biodegradation of olive mill wastewater in a membrane bioreactor: acclimation of the biomass and constraints, Desal. Water Treat., 57 (2016) 8109–8118.

[12] Y. Jaouad, M. Villain-Gambier, L. Mandi, B. Marrot, N. Ouazzani, Key process parameters involved in the treatment of olive mill wastewater by membrane bioreactor, Environ. Technol., 40 (2019) 3162–3175.

[13] M. Achak, N. Ouazzani, L. Mandi, Élimination des polluants organiques des effluents de l'industrie oléicole par combinaison d'un filtre à sable et un lit planté, Rev. Des Sci., 24 (2011) 35–51.

[14] M. Achak, W. Boumya, N. Ouazzani, L. Mandi, Preliminary evaluation of constructed wetlands for nutrients removal from olive mill wastewater (OMW) after passing through a sand filter, Ecol. Eng., 136 (2019) 141–151.

[15] H. Brix, T. Koottatep, C.H. Laugesen, Wastewater treatment in tsunami affected areas of Thailand by constructed wetlands, Water Sci. Technol., 56 (2007) 69–74.

[16] I.E. Kapellakis, N.V. Paranychianakis, K.P. Tsagarakis, A.N. Angelakis, Treatment of olive mill wastewater with constructed wetlands, Water, 4 (2012) 260–271.

[17] E. Herouvim, C.S. Akratos, A. Tekerlekopoulou, D.V. Vayenas, Treatment of olive mill wastewater in pilot-scale vertical flow constructed wetlands, Ecol. Eng., 37 (2011) 931–939.

[18] P. Grafias, N.P. Xekoukoulotakis, D. Mantzavinos, E. Diamadopoulos, Pilot treatment of olive pomace leachate by vertical-flow constructed wetland and electrochemical oxidation: an efficient hybrid process, Water Res., 44 (2010) 2773–2780.

[19] A.V. Dordio, A.J.P. Carvalho, Organic xenobiotics removal in constructed wetlands, with emphasis on the importance of the support matrix, J. Hazard. Mater., 252–253 (2013) 272–292.

[20] Y. Yang, Y. Zhao, R. Liu, D. Morgan, Global development of various emerged substrates utilized in constructed wetlands, Bioresour. Technol., 261 (2018) 441–452.

[21] J.A. Alburquerque, J. Gonzálvez, D. García, J. Cegarra, Agrochemical characterization of "alperujo", a solid by-product of the two-phase centrifugation method for olive oil extraction, Bioresour. Technol., 91 (2004) 195–200.

[22] G.P. Kofa, S. NdiKoungou, G.J. Kayem, R. Kamga, Adsorption of arsenic by natural pozzolan in a fixed bed: determination of operating conditions and modeling, J. Water Process Eng., 6 (2015) 166–173.

[23] J.M. Sieliechi, B.S. Lartiges, S.K. Ndi, R.K. amga, G.J. Kayem, Mobilization of heavy metals from natural pozzolan by humic acid: implications for water and environment, Int. J. Environ., 2 (2012) 11–15.

[24] C. DuPoldt, R. Edwards, L. Garber, B. Isaacs, J. Lapp, T. Murphy, G. Rider, M. Sayers, C. Takita, H. Webster, A Handbook of Constructed Wetlands. A Guide to Creating Wetlands for: Agricultural Wastewater, Domestic Wastewater, Coal Mine Drainage, Stormwater in the Mid-Atlantic region, US Government Publishing Office, Washington, DC, 2000.

[25] C.S.C. Calheiros, A.O.S.S. Rangel, P.M.L. Castro, Treatment of industrial wastewater with two-stage constructed wetlands planted with *Typha latifolia* and *Phragmites australis*, Bioresour. Technol., 100 (2009) 3205–3213.

[26] A.C. Barbera, G.L. Cirelli, V. Cavallaro, I. Di Silvestro, P. Pacifici, V. Castiglione, A. Toscano, M. Milani, Growth and biomass production of different plant species in two different constructed wetland systems in Sicily, Desalination, 246 (2009) 129–136.

[27] A. Toscano, A. Marzo, M. Milani, G.L. Cirelli, S. Barbagallo, Comparison of removal efficiencies in Mediterranean pilot constructed wetlands vegetated with different plant species, Ecol. Eng., 75 (2015) 155–160.

[28] C.A. Prochaska, A.I. Zouboulis, Removal of phosphates by pilot vertical-flow constructed wetlands using a mixture of sand and dolomite as substrate, Ecol. Eng., 26 (2006) 293–303.

[29] A. Stefanakis, C.S. Akratos, V.A. Tsihrintzis, Vertical Flow Constructed Wetlands: Eco-engineering Systems for Wastewater and Sludge Treatment, Elsevier, Amsterdam, 2014.

[30] AFNOR, Recueil de Norme Française: Eau, Méthodes D'essai, 2nd ed., Paris Édition, Paris, 1997.

[31] J. Rodier, L'analyse de l'eau, Eaux Naturelles, Eaux Résiduaires, Eau de Mer, 8ème édition, Dunod, Ed., Collectif, Jean Rodier, Livres, Fr. 8ème Édition, Paris, 1996.

[32] J.J. Macheix, A. Fleuriet, J. Billot, Fruit Phenolics, CRC Press, Boca Raton, FL, 1990.

[33] Y. Jemmal, N. Zari, M. Maaroufi, Experimental characterization of siliceous rocks to be used as filler materials for air-rock packed beds thermal energy storage systems in concentrated solar power plants, Sol. Energy Mater. Sol. Cells, 171 (2017) 33–42.

[34] G. Espinoza-Hijazin, Á. Paul, M. Lopez, Concrete containing natural pozzolans: new challenges for internal curing, J. Mater. Civ. Eng., 24 (2012) 981–988.

[35] A.C. Caputo, F. Scacchia, P.M. Pelagagge, Disposal of by-products in olive oil industry: waste-to-energy solutions, Appl. Therm. Eng., 23 (2003) 197–214.

[36] N. Azbar, A. Bayram, A. Filibeli, A. Muezzinoglu, F. Sengul, A. Ozer, A review of waste management options in olive oil production, Crit. Rev. Environ. Sci. Technol., 34 (2004) 209–247.

[37] M. Niaounakis, C.P. Halvadakis, Olive Processing Waste Management, Literature Review and Patent Survey, 2nd ed., Pergamon, European Patent Office, Rijswijk, Netherlands, 2006.

[38] S.E.E.E, State Secretariat at the Ministry of Energy, Mines, Water and Environment, Charge of Water and Environment, Kingdom of Morocco, Water Quality Standards for Irrigation, 2007. Available at: http://www.eau-tensift.net/fileadmin/user_files/pdf/publications/3_Irrigation.pdf

[39] A. Yalcuk, N.B. Pakdil, S.Y. Turan, Performance evaluation on the treatment of olive mill waste water in vertical subsurface flow constructed wetlands, Desalination, 262 (2010) 209–214.

[40] J. Vymazal, L. Kropfelová, Growth of *Phragmites australis* and *Phalaris arundinacea* in constructed wetlands for wastewater treatment in the Czech Republic, Ecol. Eng., 25 (2005) 606–621.

[41] J. Vymazal, H. Brix, P.F. Cooper, R. Haberl, R. Perfler, J. Laber, Constructed Wetlands for Wastewater Treatment in Europe, J. Vymazal, H. Brix, P.F. Coope, M.B. Green, R. Haberl, Eds., Removal Mechanisms and Types of Constructed Wetlands, Backhuys Publishers, Leiden, 1998.

[42] L. Mandi, K. Bouhoum, N. Ouazzani, Application of constructed wetlands for domestic wastewater treatment in an arid climate, Water Sci. Technol., 31 (1998) 379–387.

[43] R.H. Kadlec, R. Knight, J. Vymazal, H. Brix, P. Cooper, R. Haberl, Constructed Wetlands for Pollution Control: Processes, Performance, Design and Operation, International Water Association, London, 2000.

[44] J.P. Robins, J. Rock, D.F. Hayes, F.C. Laquer, Nitrate removal for platte valley, nebraska synthetic groundwater using a constructed wetland model, Environ. Technol., 21 (2000) 653–659.

[45] Y. Chen, Y. Wen, Q. Zhou, J. Huang, J. Vymazal, P. Kuschk, Sulfate removal and sulfur transformation in constructed wetlands: the roles of filling material and plant biomass, Water Res., 102 (2016) 572–581.

[46] K. Sleytr, A. Tietz, G. Langergraber, R. Haberl, Investigation of bacterial removal during the filtration process in constructed wetlands, Sci. Total Environ., 380 (2007) 173–180.

[47] A. Torrens, P. Molle, C. Boutin, M. Salgot, Removal of bacterial and viral indicator in vertical flow constructed wetlands and intermittent sand filters, Desalination, 246 (2009) 169–178.

[48] E.C. Barrett, M.D. Sobsey, C.H. House, K.D. White, Microbial indicator removal in onsite constructed wetlands for wastewater treatment in the southeastern US, Water Sci. Technol., 44 (2001) 177–182.

[49] L. Kong, Y. Bin Wang, L.N. Zhao, Z.H. Chen, Enzyme and root activities in surface-flow constructed wetlands, Chemosphere, 76 (2009) 601–608.

[50] H. Wand, G. Vacca, P. Kuschk, M. Krüger, M. Kästner, Removal of bacteria by filtration in planted and non-planted sand columns, Water Res., 41 (2007) 159–167.

[51] C.C. Tanner, J.S. Clayton, M.P. Upsdell, Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands-II. Removal of nitrogen and phosphorus, Water Res., 29 (1995) 27–34.

[52] H. Brix, Do macrophytes play a role in constructed treatment wetlands?, Water Sci. Technol., 35 (1997) 11–17.

[53] E. Tsagaraki, H.N. Lazarides, K.B. Petrotos, Olive Mill Wastewater Treatment, V. Oreopoulou, W. Russ, Utilization of By-Products and Treatment of Waste in the Food Industry, Springer, Boston, MA, 2007.

[54] R.L. Sinsabaugh, Phenol oxidase, peroxidase and organic matter dynamics of soil, Soil Biol. Biochem., 42 (2010) 391–404.

[55] N. Asses, L. Ayed, H. Bouallagui, S. Sayadi, M. Hamdi, Biodegradation of different molecular-mass polyphenols derived from olive mill wastewaters by *Geotrichum candidum*, Int. Biodeterior. Biodegrad., 63 (2009) 407–413.

[56] G. Tziotzios, S. Michailakis, D.V. Vayenas, Aerobic biological treatment of olive mill wastewater by olive pulp bacteria, Int. Biodeterior. Biodegrad., 60 (2007) 209–214.

[57] R. Mutabaruka, K. Hairiah, G. Cadisch, Microbial degradation of hydrolysable and condensed tannin polyphenol-protein complexes in soils from different land-use histories, Soil Biol. Biochem., 39 (2007) 1479–1492.