Design and start-up of a constructed wetland as tertiary treatment for landfill leachates

Ester Coppini, Laura Palli, Alexandra Antal, Massimo Del Bubba, Elisangela Miceli, Renato Fani and Donatella Fibbi

ABSTRACT

In this paper, the design and start-up of a constructed wetland (CW) for the tertiary treatment of landfill leachates is presented. The flux is characterized by high salinity, high concentration of nitrogen (almost completely in the form of nitrate) and a biochemical oxygen demand (BOD)/chemical oxygen demand (COD) ratio close to zero. The CW pilot plant suffered from mechanical and hydraulic malfunctions which led to an uneven growth of plants inside the tanks. Despite this, COD has been reduced in the range of 0–30%, reduced forms of N (ammonia and nitrite) are also oxidized and removed by 50–80% and 20–26% on average. Considering the low number of plants and the loading rate, CW pilot plant allowed to remove more than 16 kg of COD, leading to a specific removal of 10 gCOD/d · m^2. Moreover, bacterial communities associated to plants were isolated and analyzed in order to evaluate the influence of such communities on phytoremediation. Bulk soil registered the lowest bacterial titers, while plant compartments and rhizospheric soil showed to be more suitable for bacterial colonization. Twenty-five different bacterial genera were observed among the analyzed isolates, with the predominance of *Pseudomonas* genus.

Key words | constructed wetland, landfill leachates, *Phragmites australis*, phytoremediation, pilot plant, tertiary treatment

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INTRODUCTION

Landfill leachate (LFL), especially old LFL, is very difficult to treat using conventional biological processes (Ahmed & Lan 2012). Parameters such as pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), BOD/COD ratio, ammonia and micropollutants vary widely, especially with the age of the landfill (Ahmed & Lan 2012). Among the different LFL properties, the BOD/COD ratio is commonly recognized as being the most representative of LFL age, going from over 0.6 for young landfills to less than 0.1 for old ones (Alvarez-Vazquez et al. 2004). As a consequence, while nitrification is generally readily achievable, COD removal is considerably more challenging. Moreover, the presence of humic substance in LFL might boost the transportation of heavy metals, resulting in an increase of the heavy metal and salt concentrations in the leachates (Al-Wabel et al. 2011).

Among the treatments presented in literature for these specific streams, constructed wetlands (CWs) seems to be very promising (Kumarathilaka et al. 2017). In fact, they seem to be very effective for the treatment of wastewaters rich in heavy metals (Vymazal & Březinová 2016) and with high salinity (Liang et al. 2017). Unfortunately, very often these systems cannot be used as only treatment for LFLs, but they can be incorporated with other treatment processes for complete removal of contaminants (Liu et al. 2015).

This paper presents the design and the start-up of a CW pilot plant for the tertiary treatment of LFL. The CW is located downstream of a membrane bio-reactor (MBR) designed to pre-treat a mixture of LFLs prior to being discharged in the main line of a full-scale wastewater treatment plant (WWTP) treating urban and industrial

wastewater (Calice WWTP in Prato, managed by GIDA SpA). The main aim of the MBR plant is to intensively treat the mixture of leachates in a separate plant, since they represent less than 2% of the total volume treated by the main WWTP but they possess more of the 50% of the total COD entering the plant. The CW pilot plant has been constructed in order to reduce the impact of the WWTP in the receiving river, after a successful application of a similar CW as tertiary treatment in another WWTP managed by the same company in 2011 (Fibbi *et al.* 2012, 2011).

MATERIALS AND METHODS

Description of the study site and the constructed wetland

The experimental plant studied is a hybrid multistage system located at Calice WWTP (Prato, Italy), managed by GIDA spa. CW plant was designed with two parallel lines, line A and line B. Each line is a two-stage subsurface flow system (SFS), a vertical one (SFS-v) followed by an horizontal one (SFS-v). Line A SFS-v owns a surface of 480 m^2, equally subdivided into four parallel separated tanks (SFS-v 1, SFS-v 2, SFS-v 3 e SFS-v 4), having each a total height of 1.2 m. Line B SFS-v owns a surface of

420 m^2, equally subdivided into two tanks (SFS-v 5 e SFS-v 6), having each the same height of 1.2 m. Furthermore, both SFS-h lines (A and B) owns a height of 0.7 m and total surface of 360 m^2 each, divided into three tanks, each one receiving the same hydraulic load. The SFS-v has been fed intermittently through the following program: 24 min of feeding and 96 min of stop, for a total of 12 cycles per day. On the contrary, the SFS-h have been fed continuously. The maximum hydraulic load supplied to the entire system was 95 m^3/day that corresponds to a theoretical hydraulic retention time (HRT) of 13 h for the vertical stage (during the feeding stage) and 1.9 d for the horizontal stage. During the start-up of the system, after an hydraulic test (lasted 15 days) a gradual increase in wastewater flow have been used in order to allow the system to adjust to the new water chemistry. In particular, the wastewater flow varied from 0 to maximum load (95 m^3/d) in about 30 days.

Each tank was planted in March 2017 with macrophyte *Phragmites australis* (common reed) for a total of 6,480 plants. As substrate in the SFS-v medium have been used, from top to bottom: 20 cm fine gravel (5–10 mm), 60 cm sand (0.02–0.4 mm), 10 cm fine gravel (5–10 mm) and 10 cm coarse gravel (40–70 mm). In the SFS-h, the medium is entirely of fine gravel (8–12 mm). Moreover, a system for the pre-oxidation with peracetic acid (in order to break down humic substances and enhance the
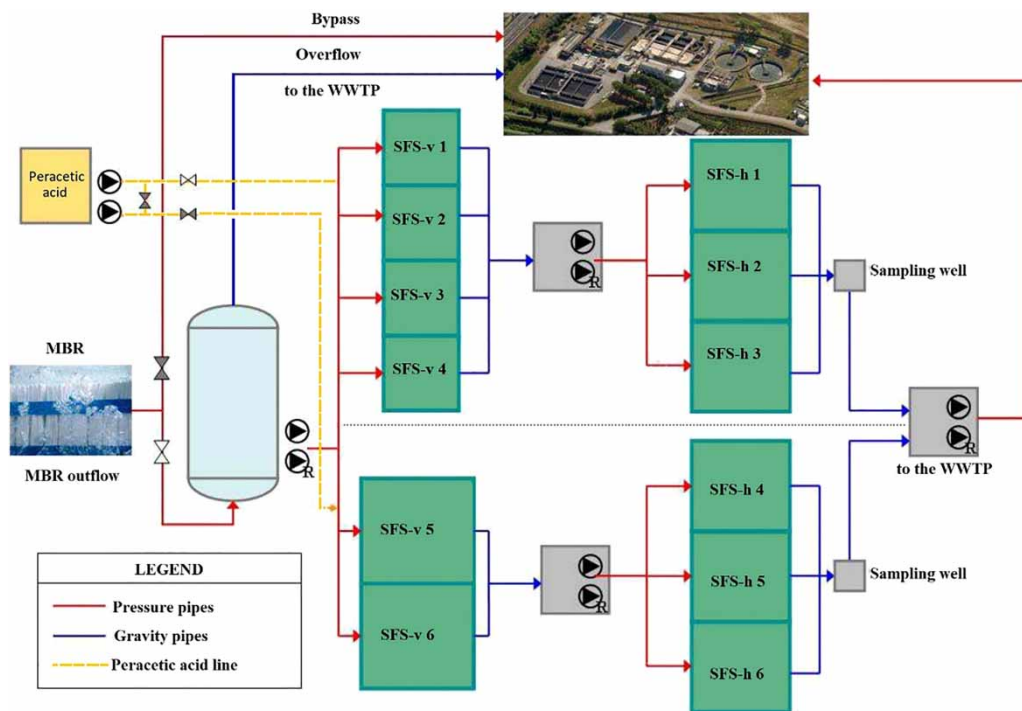

Figure 1 │ Schematic overview of the subsurface flow constructed wetland system.

biodegradability of the wastewater) is provided but has not been used until now.

A schematic presentation of the subsurface flow constructed wetland system is shown in Figure 1, while Figure 2 depicts different realization steps of the pilot plant.

Sampling

Although no significant changes in inlet and outlet flows during the day are expected, composite 24 h samples were collected daily at: (a) the inlet of the CW, (b) the outlet of the SFS-v and at (c) at the outlet of the SFS-h (outlet of the CW), using automatic samplers, model Liqui-port 2000 RPT20 (Endress + Hauser, Reinach, Switzerland) during the period September–December 2017 and May–July 2018.

Analytical methods

Macroparameters

Turbidity was determined with a WTW Turb 555 IR Turbidity Measuring Instrument. COD was determined with

LCK514/LCK014 Hach-Lange cuvette test kits. Sample digestion was performed with a Hach–Lange LT200 Thermostat and the concentrations were evaluated with a Hach–Lange DR 3800 Visible Spectrophotometer. pH was determined with a XS pH70 portable pH-meter.

Inorganic compounds analysis

All inorganic compounds were determined with a ThermoFisher Scientific Gallery™ Automated Discrete Photometry Analyzer. Chlorides react with mercury (II) thiocyanate to form a soluble non-ionic compound. The thiocyanate ions released reacts in acid solution with iron (III) nitrate to form a red/brown iron (III) thiocyanate complex and the intensity of the stable color produced is measured spectrophotometrically at a wavelength of 480 nm (EPA Method 9251). Sulphate ion is precipitated in an acid medium with barium chloride and the resulting turbidity is measured spectrophotometrically at 405 nm (EPA Method 9038). Nitrites were determined by diazonizing with sulfanilamide and coupling with N(1-naphthyl) ethylenediamine to form an azo-dye that is measured spectrophotometrically at 540 nm

Figure 2 │ Different realization steps of the pilot plant. In clockwise order: excavation and forming, laying of geotextile membrane; laying of fine gravel; inflow distribution pipes.

(EPA Method 353.2). Nitrates were reduced to nitrite by hydrazine under alkaline conditions and analyzed as previously described. Ammonia (APAT IRSA-CNR 4030) reacts with hypochlorite ions generated by the alkaline hydrolysis of sodium dichloroisocyanurate to form monochloramine. This reacts with salicylate ions in the presence of sodium nitroprusside at pH around 12.6 to form a blue compound, and the absorbance is measured spectrophotometrically at 660 nm. The water sample undergoes digestion to convert all the insoluble organic and insoluble hydrolysable P and N species into orthophosphate and nitrate, respectively, for the determination of total oxidized nitrogen as nitrate and total phosphorus as orthophosphate. The orthophosphate ion is derivatized with ammonium molybdate and antimony potassium tartrate (catalyst) to form a 12-molybdophosphoric acid complex. The complex is then reduced with ascorbic acid to form a blue heteropoly-compound and the absorbance is measured at 880 nm.

Metals analysis

Samples were treated with HNO_3 69% and underwent a microwave-assisted acid extraction/dissolution of available metals using a CEM MARS6 Microwave Reaction System (APAT IRSA-CNR 3010B, US EPA 3015A). Metals concentrations were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a Thermo-Fischer Scientific iCAP 7000 Series Spectrometer (APAT IRSA-CNR 3020). Quantification was performed with external standard. The selected analytical wavelengths are showed in Table 1.

Chemicals

The reagents HNO_3 69% and Chromasolv quality H_2O were purchased from Honeywell Fluka, and were all Suprapur® grade. ICP multi-element analytical standards were purchased from AccuTrace. Ultrapure water was obtained from a Thermo Scientific Barnstead MicroPure Water Purification System. LCA 700/703/709 Hach–Addista standards were used as total phosphorus and nitrogen standard and respectively as multi-element electrolytes calibration control. For nitrites, nitrates, chlorides, phosphates and ammonia, Gallery™ automated discrete photometry analyzer reagent kits for water and soil testing (Thermo-Fisher Scientific) were used. Also 2.5–10,000 NTU turbidity standard were purchased from ThermoFisher Scientific. For biological analyses, HClO was purchased

Table 1 | Selected wavelengths

Element	Wavelength (nm)
Al	396.132
Fe	259.940
Zn	206.060
Pb	206.353
Cd	214.438
Ni	231.622
Cu	224.700
As	189.222
Mn	257.610
Se	196.270
Sn	189.989
Ba	230.424
B	249.678
Cr	206.560
Sb	217.581

from SAI S.p.A., NaCl and Mg_2SO_4 from Sigma Aldrich, USA and tryptic soy agar medium (TSA) from Biolife Italiana Srl.

Associated microbial community isolation and characterization

In order to investigate the influence of bacterial communities from *P. australis* on the phytoremediation process, anatomical compartments of the plants were divided, grouped and pooled, then surface-sterilized with 1% HClO solution and finally washed with sterile water to remove the epiphytic bacteria. Samples were pottered in a sterile mortar with the addition of 2 mL of 0.9% NaCl. 100 μL samples of tissue extracts and their different dilutions were plated in tryptic soy agar. 5 g of rhizospheric soil were treated at room temperature for 1 h with 20 mL of 10 mM Mg_2SO_4, which allowed detachment of the bacteria from soil particles. 100 μL of the suspension and different dilutions were plated in tryptic soy agar. The total number of aerobic heterotrophic fast-growing bacteria was expressed as colony-forming units (CFU), which were determined for each sample. Cell lysates of each bacterial isolate were then obtained by thermal lysis by incubating an isolated bacterial colony for each isolate at 95 °C for 10 min, and cooling on ice for 5 min. In order to taxonomically affiliate each

bacterial isolate, 16S rRNA genes were amplified and sequenced. Polymerase chain reaction (PCR) amplification of 16S rRNA genes was carried out in 20-μL reactions using DreamTaq DNA Polymerase reagents (ThermoFisher Scientific) at the concentrations suggested by the company, and 0.5 μM of primers P0 (5′-GAGAGTTTGATCCTGGCTCAG) and P6 (5′-CTACGGC-TACCTTGTTACGA) (Di Cello & Fani 1996); 1 μL of cell lysate was used as template. Amplification conditions were the following: 90-s denaturation at 95 °C, 30 cycles of 30 s at 95 °C, 30 s at 50 °C, and 1 min at 72 °C, followed by a final extension of 10 min at 72 °C. Direct sequencing of the amplified 16S rRNA genes was performed with primer P0 by an external company (IGA Technology Services, Udine, Italy). Taxonomic affiliation of the 16S rRNA gene sequences was performed using the 'classifier' tool of the Ribosomal Database Project (RDP) (Cole *et al.* 2014).

Respirometric tests

CW inflow was characterized through respirometric tests in order to assess its inhibition potential toward nitrifying bacteria and in order to evaluate its biodegradability. Such tests were carried out using activated sludge samples taken from the oxidation tank of the WWTP of Calice. The sludge used for the tests was previously aerated for 24 h without exogenous substrate in order to be brought to endogenous conditions. Oxygen consumed for substrate degradation was calculated as the difference between the total oxygen consumption and the oxygen consumed for endogenous respiration as the area between the total oxygen uptake rates (OURs) and the OURs related to decay.

The fraction of biodegradable COD (bCOD) was then evaluated according to Equation (1).

$$bCOD = \frac{\Delta O_2}{1 - Y_H} \frac{V_R + V_S}{V_S} \tag{1}$$

where ΔO_2 is the oxygen consumption for COD degradation (total oxygen consumption minus oxygen consumed for endogenous respiration minus oxygen consumed for ammonia oxidation), Y_H is the yield coefficient for heterotrophic biomass (assumed 0.68 mgCOD/mgCOD), V_R is the volume of the sludge before spiking and V_S is the volume of the spiked sample.

In order to evaluate possible inhibitory effect of the inflow towards nitrifying bacteria, samples of NH_4Cl solution were spiked before and after sample spiking, making

sure that the previous exogenous substrate have completely been degraded and endogenous conditions have been reached again. From the observation of the two obtained peaks (OUR_{max}) presence of toxic and/or inhibitory substances for nitrifiers could be assessed.

RESULTS AND DISCUSSION

Start-up of the plant and plant growth

As described previously, *P. australis* was planted in March 2017. The plant was operated ever since with a stop between January and May 2018. Two sampling campaigns have been conducted: one between September and December 2017 and the other between May and July 2018, after the re-start of the plant. During the first period of operation some mechanical and hydraulics malfunctions have been encountered, such as pumps breaking and issues with the programmable logic controller (PLC) controlling timing and flow rates of the inflows. For these reasons, the plant was stopped at the end of 2017 and re-started after four months. These problems led to an uneven distribution of the inflow inside the two lines and inside each tank. As a consequence, growth of plants has not been even inside the whole plant, with line A having almost a double number of grown plants with respect to line B. Moreover, even inside each tank, the growth of *P. australis* have not been even, as a sign that only parts of the surface of the CW has been affected by the flow, as can be seen in Figure 3.

After a survey of the CW, it is estimated that the following percentages of planted *P. australis* can be considered grown and active for the phytoremediation purposes: Line A-Vertical stage: 100%; Line A-Horizontal stage: 100%, Line B-Vertical stage: 50%; Line B-Horizontal stage: 40%.

Influent characterization

Influent of the CW pilot plant is a mixture of landfill leachates pre-treated in an MBR tank. The permeate of the plant act as inflows for the CW. Quality characteristics of the flux are presented in Table 2.

As it can be seen, the flux is characterized by high salinity and high concentration of nitrogen which is almost completely in the form of nitrate. Another important point to be addressed is the COD of the inflow which is very high and almost completely not biodegradable; in fact, after the respirometric test, the results of which are presented in Figure 4, the bCOD value resulted equal to 0.

Figure 3 | Example of uneven growth of *P. australis* in horizontal tank, line B of the CW: in red circle good growth of plants due to good irrigation with the influent and in blue circle poor/absence of growth. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2019.030.

Table 2 | Quality characteristics of the influent of the CW

Parameter	Measure unit	2017		2018	
		Mean value	Standard deviation	Mean value	Standard deviation
pH	–	8.6	0.3	8.1	0.3
Turbidity	NTU	2	1.8	1.9	2.8
COD	mg/L	2,480	492	2,188	515
bCOD/COD*	–	–	–	0	–
TN	mg/L	415	196	417	158
$N\_NH_4^+$	mg/L	20	45	38	87
$N\_NO_2^-$	mg/L	1.3	2.8	7.9	11.6
$N\_NO_3^-$	mg/L	263	208	317	214
TP	mg/L	18	6	20	6
Cl^-	mg/L	4,083	503	3,803	401
Al	mg/L	1.05	0.48	0.25	0.08
Fe	mg/L	4.71	1.37	2.91	0.34
B	mg/L	12.04	3.42	10.83	2.47
Cr	mg/L	0.86	0.29	0.96	0.16

Data are obtained from daily evaluation from September and December 2017 and from May and July 2018, with the exception of metals, whose data are obtained from weekly evaluation in the same periods.
*Value obtained from a single respirometric test.

The peak that can be seen in the figure, in fact, is due only to the aerobic respiration of the ammonia. The non-biodegradability of the COD is due to the fact that this flux is the effluent of an MBR treatment working at high sludge retention time and high mixed liquor suspended solids (Coppini *et al.* 2018). Within the test, no inhibition of the nitrifying biomass has been seen (data not shown).
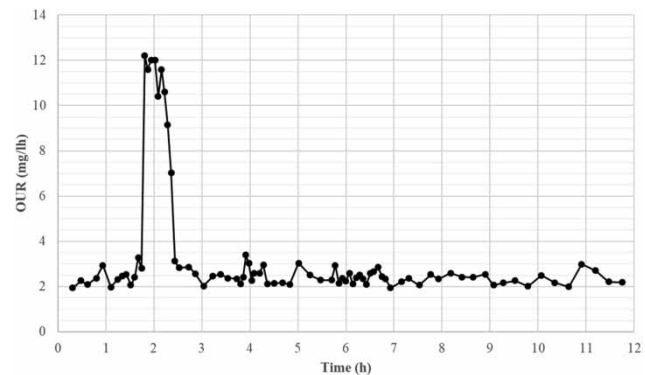

Figure 4 | Respirogram of the inflow of the CW pilot plant.

Performance of the plant

Performance of the plant in the two different sampling periods have been evaluated in terms of COD, $N\_NH_4^+$, $N\_NO_2^-$, P and metals (Al, Fe, B, Cr) removal. In particular, during the first period, only the macroparameters were evaluated while in the second period also metals were analyzed. Figures 5 and 6 depict the concentrations of COD, $N\_NH_4^+$, $N\_NO_2^-$ and P in the influent and in the effluent of the CW pilot plant in the two periods of sampling. As can be seen, COD is reduced in the range of 0–30% (with a mean removal of 7% in the first period and 3% in the second period). It is important to underline that the COD of the effluent is caused mainly by compounds recalcitrant to biological degradation, as reported previously, for this reason this reduction is to be considered satisfactory.

Reduced forms of N (ammonia and nitrite) are also oxidized and removed by 51% and 26% on average in the first
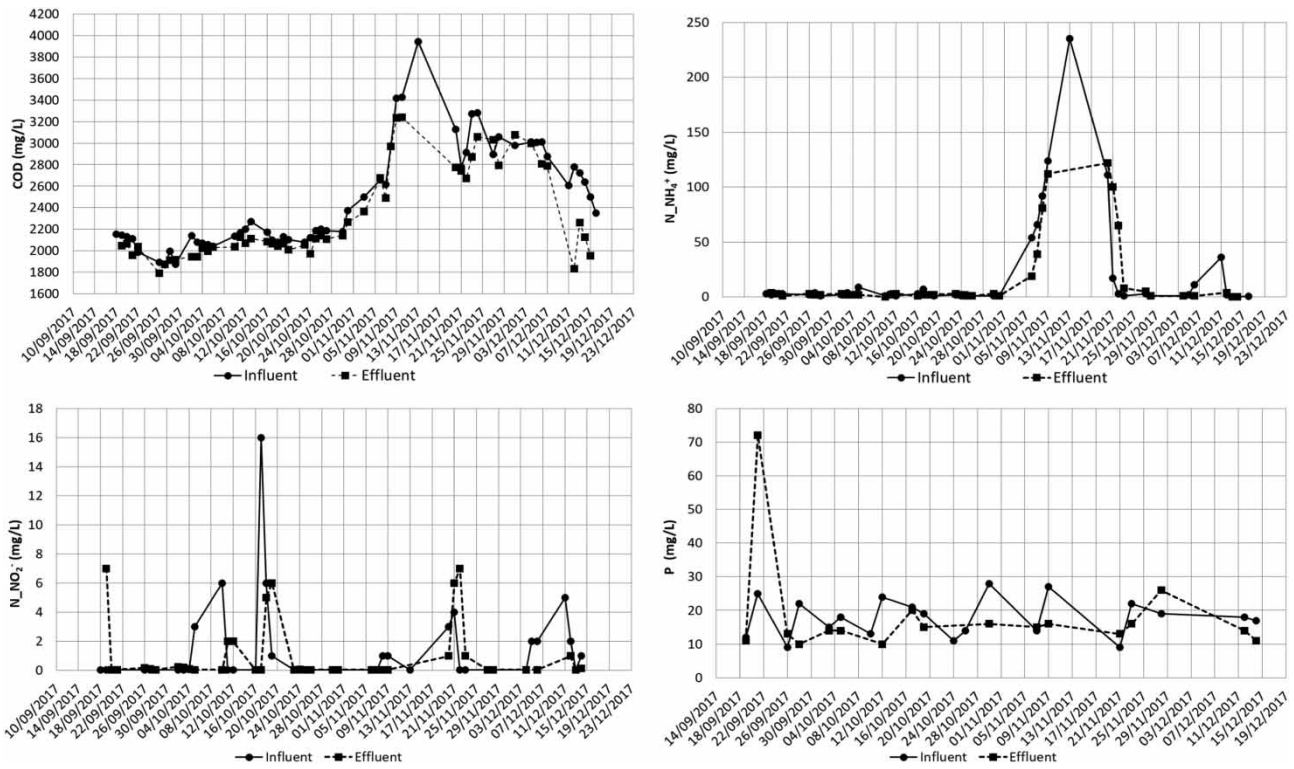
Figure 5 | Concentration of COD, N_NH$_4^+$, N_NO$_2^-$ and P in the influent and in the effluent of the CW pilot plant in the first period of sampling.
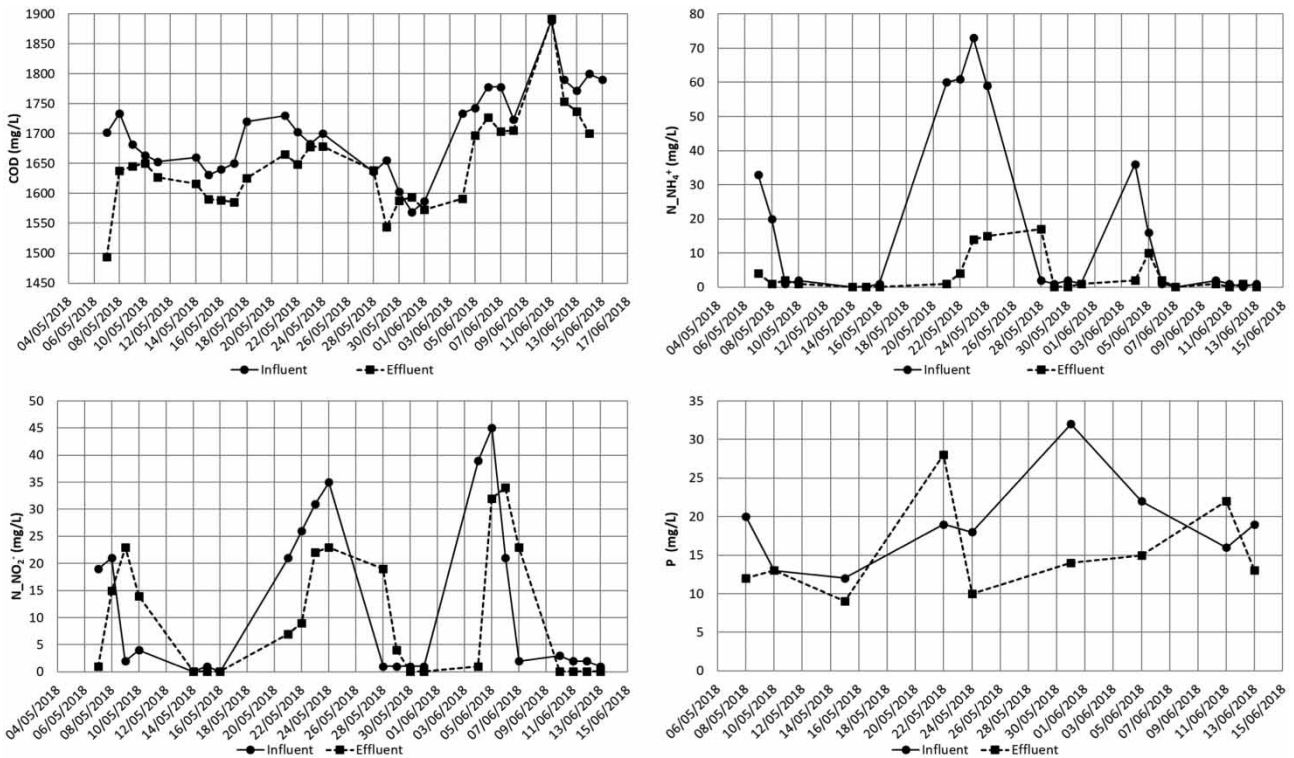

Figure 6 | Concentration of COD, N_NH$_4^+$, N_NO$_2^-$ and P in the influent and in the effluent of the CW pilot plant in the second period of sampling.

Table 3 | Mean concentration of metals in influent and effluent and removal percentages

	Al (mg/L)	Fe (mg/L)	B (mg/L)	Cr (mg/L)
Influent	0,25	2,91	10,83	0,96
Effluent	0,22	2,60	9,93	0,89
*Mean removal (%)**	$-11\% \pm 15\%$	$-11\% \pm 8\%$	$-8\% \pm 6\%$	$-7\% \pm 10\%$

*Mean of 13 samples ± standard deviation.

period and by 80% and 19% in the second period, respectively. This is in accordance to the absence of inhibitory effect towards nitrifying biomass reported in the section 'Influent characterization'. As far as nitrate is concerned, a slight increase in its concentration has been observed, probably due to nitrification of ammonia, on average by 14% in the first period and by 2% in the second period.

Also, P is removed from the plant, with a mean removal of 18% in the first period and 20% in the second period. As far as pH is concerned, while the inflow pH varies among 8.1 and 9.1, the pH of the effluent is always very stable around 8.4. Moreover, also Cl^- are removed by the pilot plant by 3% in the first period and 13% in the second period, on average.

These removals are the overall removal obtained within the CW as a whole (SFS-v and SFS-h). Regarding the removal stage-by-stage, the vertical stage showed average removal of 6%, 21%, 0% and 11% in the first period and of 15%, 89%, 14% and 12% in the second period for COD, $N\_NH_4^+$, $N\_NO_2^-$ and P, respectively. On the other hand, the SFS-h showed average removal, with respect to the outlet of SFS-v of 1%, 0%, 22% and in the first period for COD, $N\_NH_4^+$ and $N\_NO_2^-$, respectively, and negative removal (increase) for P. In the second period, average removals were of 11% and 1% per $N\_NO_2^-$ and P, with negative removals for COD and $N\_NH_4^+$. Observing these data, it is possible to conclude that most of the work have been done by the first step of the plant, the vertical CW. This is not surprising because only the more recalcitrant compounds reach the SFS-h, while the more degradable compounds are easily removed in the first step.

As far as metals are concerned, they have been analyzed on average once per week; mean concentrations and percentage removals are reported in Table 3. Even though the plants are still growing and the pilot plant is still not at its full potential, metals are still being adsorbed, leading to a reduction of their concentrations in CW effluent.

Specific removals

Comparing the obtained results with literature is not easy, given the many differences that the studied CW pilot plant presents with published studies: (a) the very high salinity of the influent; (b) the feeding flow which is a pre-treated LFL with very low BOD/COD ratio (almost equal to zero); (c) the number of plants which is less than 3 plants/m^2 while most articles present values ranging from 5 to 12 plants/m^2 (Fountoulakis *et al.* 2017); (d) the HRT of the system, which is calculated to be 6 d for the whole system, while most published results have been obtained with HRTs ranging from one to three weeks (Bulc 2006; Akinbile *et al.* 2012; Grisey *et al.* 2012); (e) the organic loading rate (OLR) of the system which is calculated to be 147 gCOD/$m^2 \cdot$ d for the whole system, while published results varied from 20 to 40 gCOD/$m^2 \cdot$ d (Wojciechowska *et al.* 2010; Fountoulakis *et al.* 2017).

In order to properly compare the obtained results with published ones, it therefore important to evaluate the specific removal per m^2. Obtained results are presented in Table 4. Taking as example the removal of COD, which is often considered to be a good indicator of performances of CWs, it is interesting to see that, in 2017, the studied CW was able to remove more than 16 kg of COD per day, leading to a specific removal of 10 gCOD/d $\cdot m^2$. Bulc (2006), for example, obtained only 7.9 gCOD/d $\cdot m^2$, while Fountoulakis *et al.* (2017) and Wojciechowska (2017) obtained higher removal for m^2 (17 and 18 gCOD/d $\cdot m^2$, respectively), but were treating domestic wastewater and untreated LFL. These values are easily explained taking into account the very low amount of plants together with the very high value of OLR of our CW.

Table 4 | COD, $N\_NH_4^+$, NO_2^-, P, Al, Fe, B and Cr removed by the pilot plant: total loads and specific removal per m^2

	COD		$N\_NH_4^+$		$N\_NO_2^-$		P		Al	Fe	B	Cr
	2017	2018	2017	2018	2017	2018	2017	2018	2018	2018	2018	2018
Total removal (g/d)	16,720	5,130	285	1,235	24	190	95	380	2.85	29.45	85.5	6.65
Removal per m^2 (mg/d $\cdot m^2$)	10,321	3,167	176	762	15	117	59	235	1.76	18.18	52.78	4.10

Table 5 | Bacterial titers obtained for aerobic heterotrophic bacterial communities associated to different compartments of *P. australis* plants and to soil samples

Compartment	Cultivable bacterial titers (CFU/g)	
	Vertical CW	Horizontal CW
Aerial part	$4,77 \times 10^5$	$1,25 \times 10^5$
Stem	$7,78 \times 10^5$	$5,16 \times 10^6$
Roots	$3,52 \times 10^6$	$4,7 \times 10^6$
Rhizosphere	$2,82 \times 10^5$	$2,11 \times 10^5$
Bulk soil	$1,75 \times 10^4$	$1,95 \times 10^4$

Bacterial titers are expressed in CFU per gram of sample.

Bacterial communities associated to *P. australis* plants

In the phytoremediation process, vegetation offers organic matter and additional oxygen for the development of a plant-associated microbiota, which is the main protagonist of pollutants removal (Zhou *et al.* 2013). Microorganisms can colonize soil and/or the internal plant tissues. Bacterial communities associated to *P. australis* plants from both vertical and horizontal CWs were analyzed in order to evaluate the influence of such communities on phytoremediation. As described before, bacterial isolates were obtained from different tissues of the plants and from rhizospheric and bulk soil. Table 5 shows the total number of aerobic

heterotrophic bacteria obtained for each plant compartment and soil sample, expressed in CFU per gram of fresh plant tissue or soil. Bulk soil registered the lowest bacterial titers, while plant compartments and rhizospheric soil seemed to be more suitable for bacterial colonization, exhibiting higher bacterial loads, ranging from 10^5 to 10^6 per gram of plant fresh tissue or soil. Plant root secretions in the rhizosphere may offer a nutrient-rich region for bacteria, which can then enter plant roots and colonize plant internal tissues (Kobayashi & Palumbo 2000).

The composition of cultivable bacterial communities associated to the different plant compartments and soils was determined by amplification and sequencing of 16S rRNA gene of 454 bacterial isolates, as described before. As shown in Figure 7, 25 different bacterial genera were observed among the analyzed isolates, with the predominance of *Pseudomonas* genus. Interestingly, *Pseudomonas* genus was found also as one of the most abundant strain in roots of a pilot-scale horizontal-flow reed bed treating wastewater containing ppm concentrations of alkylphenols, which are well-known for their bacterial toxicity (Sacco *et al.* 2006). Furthermore, each compartment showed a specific bacterial community, consisting of specific percentages of the different bacterial genera. Since the plant-associated microbiota has been described as capable of degrading many toxic compounds (Germaine *et al.* 2006;
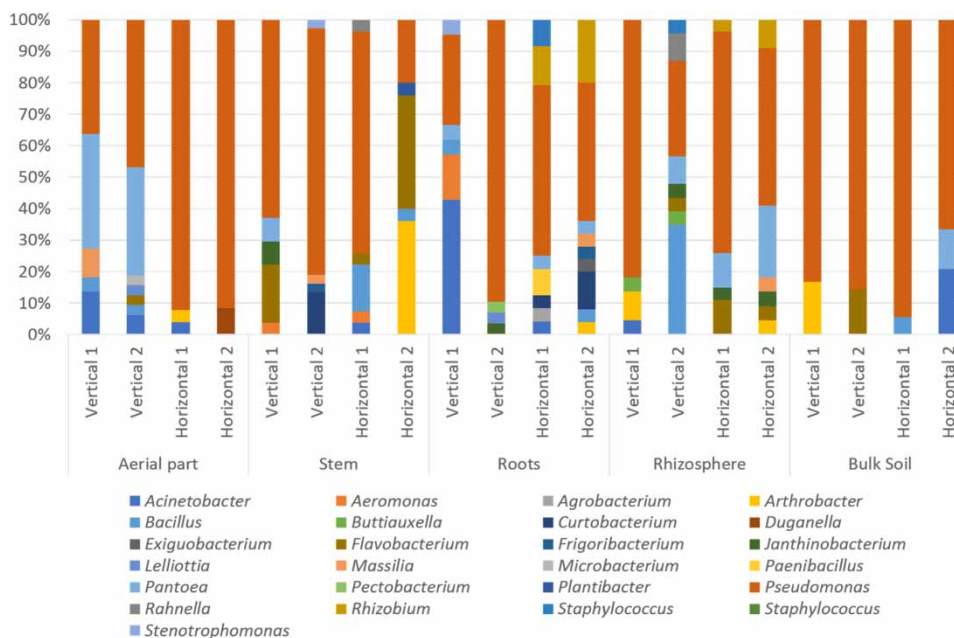

Figure 7 | Cultivable bacterial taxa, expressed in percentages, isolated from *P. australis* plants from CW pilot plant. Bacterial communities were analyzed in both vertical and horizontal flow systems, in duplicate. 'Vertical 1' and 'Vertical 2' indicate the two replicates for vertical flow system, while 'Horizontal 1' and 'Horizontal 2' indicate the two replicates for horizontal flow system.

Ho *et al.* 2012) the ongoing characterization of *P. australis* bacterial communities may pave the way to a better understanding of the phytoremediation process and to the improvement of CW effluents.

CONCLUSIONS

The CW pilot plant worked from March 2017 until now, with a stop between May and July 2018, fed with a mixture of LFL pre-treated in an MBR plant. Several factors affected the obtained results: (a) the very high salinity of the influent; (b) the very low BOD/COD ratio of the feeding flow; (c) the mechanical and hydraulics malfunctions which led to an uneven growth of plants. Nevertheless, the CW pilot plant allowed removal of more than 16 kg of COD, leading to a specific removal of $10 \ gCOD/d \cdot m^2$. In order to increase the removal of pollutants, it would be interesting in the future to act as follows: (a) evaluate a different flow management such as decreasing the inflow (and consequently the OLR), increasing the HRT of systems and (b) pre-oxidizing the inflow with the peracetic acid system (in order to break down humic substances and enhance the biodegradability of the wastewater).

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