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A.-C. Chevremont, J.-L. Boudenne, B. Coulomb, Anne Marie Farnet da Silva. Impact of watering with UV-LED-treated wastewater on microbial and physico-chemical parameters of soil. *Water Research*, 2013, 47 (6), pp.1971-1982. 10.1016/j.watres.2013.01.006 . hal-02069427

HAL Id: hal-02069427

<https://hal.science/hal-02069427>

Submitted on 15 Mar 2019

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Impact of watering with UV-LED-treated wastewater on microbial and physico-chemical parameters of soil

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A B S T R A C T

Advanced oxidation processes based on UV radiations have been shown to be a promising wastewater disinfection technology. The UV-LED system involves innovative materials and could be an advantageous alternative to mercury-vapor lamps. The use of the UV-LED system results in good water quality meeting the legislative requirements relating to wastewater reuse for irrigation. The aim of this study was to investigate the impact of watering with UV-LED treated wastewaters (UV-LED WW) on soil parameters. Solid-state ¹³C NMR shows that watering with UV-LED WW do not change the chemical composition of soil organic matter compared to soil watered with potable water. Regarding microbiological parameters, laccase, cellulase, protease and urease activities increase in soils watered with UV-LED WW which means that organic matter brought by the effluent is actively degraded by soil microorganisms. The functional diversity of soil microorganisms is not affected by watering with UV-LED WW when it is altered by 4 and 8 months of watering with wastewater (WW). After 12 months, functional diversity is similar regardless of the water used for watering. The persistence of faecal indicator bacteria (coliform and enterococci) was also determined and watering with UV-LED WW does not increase their number nor their diversity unlike soils irrigated with activated sludge wastewater. The study of watering-soil microcosms with UV-LED WW indicates that this system seems to be a promising alternative to the UV-lamp-treated wastewaters.

Keywords:

Wastewater reuse
Soil microbial activity
Faecal indicator
Catabolic activity
Enzymatic activities

1. Introduction

The reuse of wastewaters for irrigation is a great technological challenge for Mediterranean countries (Angelakis et al., 1999; Palese et al., 2009). This practice leads to a more efficient use of water resources in those areas where water is scarce and also to an increase in soil fertility through inputs of organic matter and nutrients present in urban wastewaters. However, the use

of wastewaters in agriculture is associated with environmental risks due to high concentrations in chemicals and in pathogenic microorganisms, which can be potentially found in such waters.

In this context, new techniques for wastewater disinfection are developed to meet the legislative requirements of each country regarding the quality of water which could be reused. French legislation (Decree of August 2nd, 2010)

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focuses on microbiological criteria, and particularly faecal enterococci and coliforms, which are the most relevant indicators of faecal pollution. For these parameters, criteria are a 2-log reduction in faecal enterococci and a faecal coliform concentration below 1,00,000 CFU/100 mL. Advanced oxidation processes based on UV radiations have been shown to be a promising wastewater disinfection technology, which could lead to an unrestricted irrigation (Lazarova and Savoye, 2004; Nasser et al., 2006). UV disinfection systems use low or medium-pressure mercury lamps, the first predominantly emitting monochromatic UV radiations at a wavelength of 254 nm and the second, polychromatic UV radiations ranging from 200 to 400 nm. However, mercury vapor lamps have many disadvantages: large size, low resistance to shock and high sensitivity to temperature variations (Oppenländer, 2003). Furthermore, these lamps contain mercury, a major environmental contaminant and since 2006, the European Union legislation states that mercury is a hazardous substance, whose use is no longer acceptable (Close et al., 2006). Recently, few studies have investigated the use of Light Emitting Diodes (LED) to replace mercury vapor lamps as UV radiation source used as a tertiary wastewater treatment (Hamamoto et al., 2007; Vilhunen et al., 2009; Chevremont et al., 2012a, 2012b). UV-LEDs have been tested for medical applications and now are produced in commercial quantities, which results in significantly reduced costs. The UV-LED systems are made of innovative materials such as aluminium nitride (AlN) or gallium and aluminium nitride (AlGaIn), which are not toxic (Vilhunen et al., 2009) and could be an advantageous alternative to mercury vapor lamps. Furthermore, the use of the UV-LED system results in good water quality meeting the legislative requirements relating to the wastewater reuse for irrigation. Irrigation with untreated wastewaters is indeed not recommended because this represents an unacceptably high risk for human health – e.g. accumulation of heavy metals at phyto-toxic concentrations in soil and contamination of soil-plant system with pathogens (Jamil et al., 2010) – and modifies drastically soil microbial activities (Prado et al., 2011) and soil physical properties (Vogeler, 2009). On the other hand, the use of secondary-treated wastewaters affects physico-chemical and biological parameters of soil only after a long-term irrigation (Rusan et al., 2007; Adrover et al., 2012). Here, our hypotheses are based on the fact that the microbial and physico-chemical composition of secondary-treated effluent may affect directly or indirectly the properties of soil. Both microorganisms and organic matter from the effluent may indeed lead to a shift in soil microbial communities. Tertiary treatment based on UV-LED system may limit these modifications by decreasing the amount of microorganisms added to the soil. Our previous study (Chevremont et al., 2012b) indeed showed that the effluents obtained after UV-LED treatment can be legally used for irrigation. Here, the aim of this study was to determine whether the supply of UV-LED-treated effluent i) altered the functional diversity of soil microbial communities and ii) affected their role in organic matter recycling. To assess changes in microbiological properties of soil, microbial activities (lignocellulolytic enzymes, proteases and ureases, both markers of the transformation of organic matter from the effluent), functional diversity via CLPP (Catabolic level

physiological profile) and persistence of faecal indicators were monitored over a 1-year period in soil microcosms watered with water (C), UV-LED tertiary-treated wastewaters (UV) and activated sludge secondary-treated wastewaters (WW). During this 1-year period of watering we also monitored the physicochemical parameters of soil by measuring pH, electrical conductivity (EC) and the evolution of organic matter by solid-state nuclear magnetic resonance (NMR) of ^{13}C .

2. Material and methods

2.1. UV-LED treatment of wastewater

The effluent used was collected from the outlet of the wastewater treatment plant of La Fare les Oliviers, Southern France, (6650 equivalent inhabitants, average flow rate of 800 m³ a day) after biological treatment by activated sludges. The experimental setup used for the UV-LED irradiation is that described by Chevremont et al. (2012b). Briefly, two LEDs (manufactured by Seoul Optodevice Co., Ltd., Korea) emitting at 280 and 365 nm respectively were used for wastewater irradiation. 250 mL of effluent were placed into a crystallizing dish (14-cm diameter) and was exposed for 30 min to the radiations of the two LED used coupled. The choice of these wavelengths was guided by the optimized protocol established in our previous study: coupling 280/365 nm led to the highest bacterial reduction compared to UV-A or UV-C used alone and other UV-A/UV-C coupling (Chevremont et al., 2012b).

2.2. Wastewater analysis

The effluent was collected each week and pH (pH meter Metrohm, 744) and electrical conductivity (EC, with a Hanna conductimeter, DIST3) were recorded *in situ*. Dissolved Organic Carbon (DOC) and Total Nitrogen (TN) measurements were made using high temperature catalytic oxidation technique (Multi N/C 2100, Analytik Jena, Germany). The pre-treated sample was injected (50 μL) into the furnace filled with a Pt preconditioned catalyst. The combustion was realized at 800 °C and the combustion products were carried by high purity oxygen (Linde Gas) allowing detection of CO₂ by non-dispersive infrared (NDIR) and detection of NO by chemiluminescence (CLD). Each sample was injected three times and the average was reported as dissolved organic carbon value (DOC, in mgC/L) or total nitrogen (NT, in mgN/L). Up to five injections were realized if the coefficient of variation was greater than 3%. The response of the NDIR detector was linear and was calibrated with potassium hydrogen phthalate solutions (Sigma Aldrich) of well-known DOC concentration, before analysis of the samples. The detection limit was 0.5 mgC/L for DOC. In the same way, the response of the CLD detector was linear ($r^2 = 0.996$) and was calibrated with a 1000 mgN/L stock solution (Analytik Jena, Germany), prepared from (NH₄)₂SO₄ (2.35965 g in 1 L) and KNO₃ (3.611 g in 1 L) and diluted as required. The limit of detection was 0.07 mgN/L for TN. The chemical oxygen demand (COD) of water was determined by the potassium dichromate method according to ISO 15705:2002. Briefly, 2 mL of sample were introduced in

COD tubes (range 0–1500 mg O₂/L) purchased from Aqualytic (Dortmund, Germany), before heating at 150 °C for 2 h (Thermoreaktor 300 from Merck, Darmstadt, Germany). COD values were then determined by an AL800 photometer from Aqualytic, previously calibrated with COD standard solutions.

Mean values (\pm standard deviation) for these parameters were: 7.60 \pm 0.34 for pH, 1032.68 \pm 57.61 μ S cm⁻¹ for EC, 11.93 \pm 3.97 mgC/L for DOC, 6.26 \pm 0.99 mgN/L for TN and 128.30 \pm 35.94 mg O₂/L for COD.

2.3. Physico-chemical characteristics of soil

Analyses were carried out in triplicate on soil dry samples. The pH (pH meter Metrohm, 744) and the Electrical Conductivity (EC, with a Hanna conductimeter, DIST3) of soil were measured in a 1:5 soil to water ratio. Total N content was determined by the Kjeldal method (AOAC, 1990). Briefly, soil samples were weighed, sieved at 2 mm and transferred into the Kjeldahl digestion flask containing 15 g of catalyst (96% K₂SO₄/4% CuSO₄·5H₂O) and 25 mL of concentrated H₂SO₄. After 2 h of digestion in a digestion unit (Büchi K-435, Büchi Labortechnik, Flawil, Switzerland) with electrical heat and fume removal and cooling to room temperature, 400 mL of distilled water, 100 mL of 50% NaOH and 0.25 g Zn were added to each flask. By distillation (Büchi K-355 distillation unit), ammonium hydroxide was trapped as ammonium borate in a 4% boric acid solution (4 g of boric acid in 100 mL deionized water (w/v)). Total nitrogen was determined by titration with standardized HCl to a mixed indicator endpoint (0.1 g/100 mL bromocresol green and 0.1 g/100 mL methyl red in 95% ethanol). Cation Exchange Capacity (CEC) in soil samples was determined by the extraction method with ammonium acetate (pH 7.0) and following the method proposed by Sumner and Miller (1996).

Phosphorous was determined by following the method developed by Olsen et al. (1954). 2.5 g of soil (previously sieved at 2 mm) mixed up with 50 mL of 0.5 M sodium bicarbonate (pH 8.5) solution, shaken together for 30 min. The mixture was then filtered through Whatman filter paper and the orthophosphate in the filtered extract was determined colorimetrically (at 630 nm by use of a Prim Advanced spectrophotometer, SECOMAM, Alès, France) by reacting it with ammonium molybdate (4.3 g in 200 mL deionized water) using ascorbic acid (1% (w/v)) as the reducing agent.

Total organic carbon (C) in the solid samples was measured using a total organic carbon analyzer (TOC-5050 A) coupled to a solid sample combustion unit (module SSM-5000 A, from Shimadzu, Marne-la-Vallée, France). The results are expressed in mg g⁻¹ of dry matter (AFNOR, 1995).

Humic substances were determined by TOC measurement on pre-treated soil samples, according to the method developed by Swift (1996). The pre-treatment consisted in the dissolution of soil samples (10 g) in a solution of NaOH 0.1 M under a nitrogen atmosphere. The solution obtained was stirred for 4 h, then left at rest for 16 h. After centrifugation at 2800 rpm the supernatant was collected and filtered at 0.45 μ m. A solution of total humic substances (HS) was thus obtained.

For the determination of total trace metal concentrations, soil samples were digested in *aqua regia* using a microwave

digestion system (MDS 2000 CEM Corporation). A chemical fractionation procedure modified from Tessier et al. (1979) was used to estimate exchangeable, acid-soluble (pH 4.7), organic, reducible, and residual forms of trace metals in soil. The chemical fractionation was carried out using 0.5 g samples in 50-mL centrifuge tubes and involved the following 5 fractions:

- F1 Exchangeable fraction: BaCl₂ 1 M, pH 7, 25 mL, 20 °C, stirring for 2 h
- F2 pH 4.7 acid-soluble fraction: acetate buffer CH₃COOH/CH₃COONa 1 M, pH 4.7, 50 mL, 20 °C, stirring for 5 h
- F3 Humic fraction: K₄P₂O₇ 0.1 M, pH 9.5, 20 mL, 20 °C, stirring for 24 h
- F4 Reducible fraction: NH₂OH–HCl 0.04 M in CH₃COOH 25%, 10 mL, 6 h at 60 °C
- F5 Residual fraction: HNO₃/HCL (1/3: *aqua regia*), 21 mL, 20 min at 150 °C

After each extraction step, samples were centrifuged at 3000 g for 25 min, the supernatant was sampled for analysis and the solid state residue was reused in the following step.

For each extraction step and for each metal, standard solutions were freshly prepared in a background solution of the extracting reagents. The Cu, Pb and Zn concentrations were determined by flameless AAS (Perkin–Elmer, model 1100B) with deuterium lamp background correction and using a matrix modifier (NH₄H₂PO₄, 40 g/L, Mg(NO₃)₂, 6H₂O, 5 g/L). For each sample, analyses were made in triplicate and differences between replicates were always less than 5%. Laboratory reference samples with known trace metal concentrations were regularly analyzed in order to test the reproducibility of the analytical method.

2.4. Microcosms

The soil microcosms were made in pots of 10-cm on a side and 10-cm in depth with 300 g of horticultural soil commonly used for vegetable crops and green spaces. Bacterial and chemical characteristics of the soil used in this study are listed in Table 1. Three types of soil microcosms were performed: i) microcosms watered with potable water i.e. tap water purified via ozonation by the Société des Eaux de Marseille, (C), ii) microcosms watered with secondary-treated wastewaters (WW) and iii) microcosms watered with UV-LED tertiary-treated wastewaters (UV). For each type of microcosms, four replicates were prepared ($n = 4$) and incubated for one year in a greenhouse where temperature ranges from 10 °C to 30 °C depending on the season. A total of 12 microcosms were thus set up for each incubation time (4, 8 and 12 months). Microcosms were watered with 60 mL of water, UV-LED tertiary-treated wastewater or activated sludge secondary-treated wastewater once a week. After 4, 8 and 12 months (which correspond to July, November and March respectively), soils (0–5 cm-depth) were screened with a mesh size of 2 mm before use.

2.5. Functional diversity of soil microbial communities

Functional diversity of bacterial communities was studied with BIOLOG[®] ECO microplates containing 96 wells corresponding to three replicates of 31 carbon sources, 3 wells

Table 1 – Microbial and physic-chemical characteristics of the soil used.

Parameters	Soil values
FU	6.5 ± 2.6*10 ²
Total coliforms (CFU/mL)	1.7 ± 0.2*10 ⁴
Faecal coliforms (CFU/mL)	1.3 ± 0.9*10 ²
pH	7.74 ± 0.08
Electrical conductivity (EC) (µS/cm)	135.5 ± 3.3
Total organic C (TOC) (g/kg)	30.06 ± 1.25
Total N (g/kg)	2.21 ± 0.01
C/N	13.60
Total P ₂ O ₅ (g/kg)	0.26 ± 0.02
Exchangeable Na (meq/100 g of soil)	0.29 ± 0.00
Exchangeable K (meq/100 g of soil)	1.97 ± 0.02
Exchangeable Mg (meq/100 g of soil)	2.34 ± 0.07
Exchangeable Ca (meq/100 g of soil)	42.31 ± 1.42
Total Cu (µg/g)	24.14 ± 0.50
Total Pb (µg/g)	12.74 ± 0.47
Total Zn (µg/g)	50.64 ± 1.06
Total Cd (µg/g)	<1
Total Ni (µg/g)	17.27 ± 0.26
Humic substances (HS) (mg C/g)	7.02 ± 0.24
Humic acids (HA) (mg C/g)	5.45 ± 0.19

containing water as controls. The use of a carbon source leads, at the end of a respiratory process, to the reduction of nitro-tetrazolium blue (colorless) into formazan absorbing at 490 nm.

To obtain cell suspensions from soil, 5 g of dry equivalent soil and 100 mL of sodium pyrophosphate (0.1%) were stirred for 1 h. The absorbance of the supernatant was spectrophotometrically measured at 590 nm. Depending on the absorbance, the supernatant was diluted with a sterile solution of NaCl (0.85%) so as to inoculate wells with a solution of 0.02 absorbance unit. Wells were inoculated with 125 µL of the suspension and microplates were incubated at 25 °C for 50 h. During incubation, absorbances of each well were measured several times (after 6 h, 10 h, 22 h, 26 h, 30 h, 34 h, 46 h and 50 h of incubation) at 490 nm (TECAN spectrophotometer, infinite M200) until the end of the growth of microorganisms. The Average Well Color Development (AWCD) was calculated according to Garland and Mills (1991) as follow: $AWCD = \sum OD_i / 31$ where OD_i is the optical density for each well. The functional diversity of soil bacterial communities was estimated using the Shannon index (Shannon and Weaver, 1949) as described by Zak et al. (1994) from:

$$H' = - \sum p_i \ln p_i$$

where p_i is the ratio of colour development of the well i to the sum of colour development of all positive wells.

2.6. Enzymatic activities

After 0, 4, 8 and 12 months of irrigation, each sample was screened with a 2 mm mesh and the degradation of organic matter by soil bacterial communities was estimated by measuring five enzymatic activities.

For laccase and cellulase activities, an enzyme extraction was first carried out using 4 g of equivalent dry soil in 100 mL of extraction buffer (CaCl₂, 2H₂O 0.2M + Tween 0.1%) shaken for 1 h. The samples were then centrifuged for 10 min at

6000 rpm. The supernatant was filtered on GF-D and GF-C filters, distributed into dialysis tubes and concentrated with polyethylene glycol. The enzyme extracts were recovered in 15 mL of Bis-Tris buffer (20 mM pH 6). Laccase activity was measured in acetate buffer 0.1 M, pH 4.5 by following the oxidation of syringaldazine to quinone ($\epsilon^M = 65\,000\text{ M}^{-1}\text{ cm}^{-1}$) at 525 nm after 1 h-incubation at 30 °C (Harkin et al., 1974). Cellulase activities were measured using the Somogy–Nelson method modified by Farnet et al. (2010a) using carboxymethyl cellulose as substrate.

Activities for lipases, ureases and proteases were measured directly in the soil. The hydrolysis activity of lipases was measured according to Farnet et al. (2010b) using *p*-nitrophenyl laurate as substrate. The urease activity was measured using the method of Kandeler and Gerber (1988) based on the determination of NH₄⁺ after enzyme action on urea. Protease activity was determined using 1 g of dry soil equivalent in a 2% casein solution in tris(hydroxymethyl)aminomethane buffer 50 mM, pH 8.1, incubated for 3 h at 50 °C. After incubation, the reaction was stopped using trichloroacetic acid (TCA) 15% and the mixture was centrifuged for 10 min at 10 000 rpm. Aromatic amino acids were detected using Folin reagent (33%) at 700 nm (Ladd and Butler, 1972). Tyrosine was used as standard.

Enzyme activities were expressed in µmoles of reaction products released per minute (U) per gram of dry soil (U g⁻¹ DS).

2.7. Chemical characterization of soils via solid-state ¹³C NMR

The solid-state ¹³C NMR spectra were obtained on a Bruker Avance 400 MHz NMR spectrometer operating at a ¹³C resonance frequency of 106 MHz and using a Bruker double-bearing probe. 80 mg of dry grinded soil were placed in a zirconium dioxide rotor of 4 mm outer diameter and spun at a magic angle spinning (MAS) rate of 10 kHz. The cross-polarization (CP) technique (Schaefer and Stejskal, 1976) was applied with a ramped ¹H-pulse starting at 100% power and decreasing until 50% during the contact time (i.e. 2 ms) in order to circumvent Hartmann–Hahn mismatches (Peersen et al., 1993; Cook et al., 1996). To improve the resolution, a dipolar decoupling GT8 pulse sequence (Gerbaud et al., 2003) was applied during the acquisition time. To obtain a good signal-to-noise ratio in ¹³C CPMAS experiment, 12 000 scans were accumulated using a delay of 2.5 s. The ¹³C chemical shifts were referenced to tetramethylsilane (at 0 ppm) and calibrated with glycine carbonyl signal set at 176.5 ppm.

According to Mathers et al. (2003), the solid-state ¹³C CP/MAS NMR spectra were divided into the four common chemical shift regions: alkyl-C (0–45 ppm), O-alkyl-C (45–112 ppm), aromatic-C (112–160 ppm) and carbonyl-C (160–210 ppm). The relative intensities of each region were determined by integration using the Dmfit program (Massiot et al., 2002). From the data of these regions, ratios of humification (HR) and aromaticity (HA) were calculated according to Baldock and Preston (1995).

2.8. Enumeration and identification of soil bacteria

For the enumeration of coliforms and enterococci, 1 g of equivalent dry soil taken from each microcosm was added to

10 mL of sodium pyrophosphate buffer (0.1%), and the mixture was stirred for 1 h. The supernatant was then diluted appropriately and plated on Slanetz and Bartley medium or medium TTC and Tergitol 7 to enumerate intestinal enterococci and coliforms respectively. Plates were incubated at 37 °C or 44 °C (faecal coliform) for 24 h. A colony of each type of coliforms per plate (size, color, thickness) was then isolated and enterobacteria identified by Api 20E gallery® (Biomérieux, France).

2.9. Statistical analysis

In order to overcome the assumption of normality of the samples, enzyme activities, Shannon indexes based on Biolog® values after 42 h incubation, pH and EC values after each incubation times were compared with non-parametric test of Kruskal and Wallis. In the case of a significant effect, a test of Dunn and Bonferroni correction were performed to further identify difference. Biolog® values (values of each well for obtaining an AWCD 0.5) and the integration percentage of area of the NMR spectra for each interval were also analyzed by principal component analysis (PCA) to explain the variance between the different microcosms. For each treatment the barycenter of the 4 replicates was calculated. All statistical tests were performed with Excel stat® software.

3. Results and discussion

The use of UV-LED system combining UV-A and UV-C after a wastewater secondary treatment provides water which fits the French legislative requirements for wastewater reuse for irrigation (Chevremont et al., 2012b). To assess possible temporal variations in the characteristics of the effluent, pH, Electrical Conductivity (EC), Dissolved Organic Carbon (DOC), Total Nitrogen (TN), number of mesophilic bacteria, enterococci, total coliforms and faecal coliforms were measured before and after UV-LED treatment (data not shown). These parameters did not change significantly over time (non-parametric test of Kruskal–Wallis, $p < 0.05$). Thus the main chemical and microbiological characteristics of the effluent remained constant throughout the experiment.

3.1. Physico-chemical characteristics of soil

In this study we tested the effect of watering with tap water, WW and UV-LED WW on physico-chemical and microbiological soil parameters. Fig. 1 shows the values of pH and electrical conductivity (EC) measured in the different microcosms. For the three types of microcosms, the main variation in both pH and EC observed was an increase between 4- and 8-month incubations. Thus, treatment (WW or UV-LED WW) did not affect pH and EC variations compared to control: this implies that soil has a very high buffering capacity which maintains pH constant (Rezapour and Samadi, 2011). Variations between 4- and 8-month incubation may be explained by a seasonal effect, since 8-month incubation corresponding to the sampling after summer time. Higher temperatures may have led to successive wetting- dehydration affecting microorganisms and thus cell lyses may have influenced EC values.

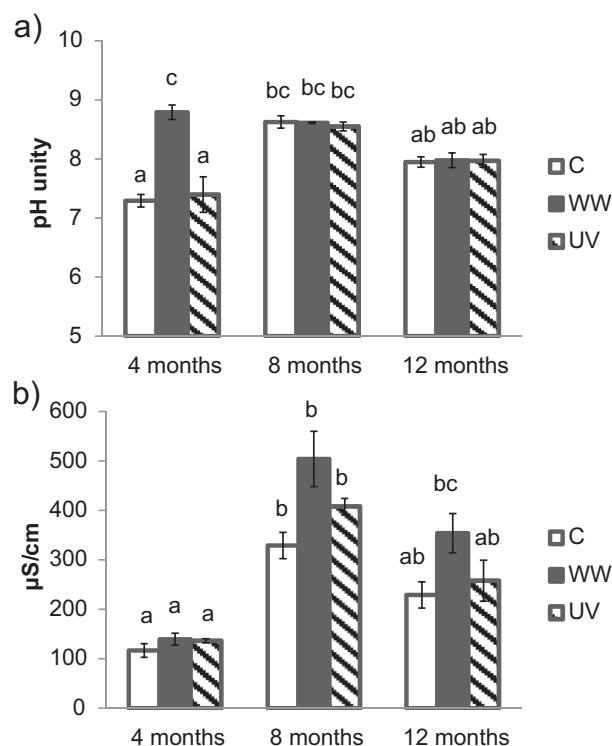


Fig. 1 – pH (a) and conductivity (b) values measured in the control microcosms (C), the microcosms watered with U-LED WW (UV) and those watered with WW (WW). The letters indicate significant differences (Kruskal–Wallis, $p < 0,05$, and multiple comparisons of means by pairs according to the Dunn procedure).

3.2. Evolution of soil organic matter quality

The evolution of organic matter composition can be studied via solid-state ^{13}C NMR. The relative percentages of the chemical functions in organic matter (alkyl-C, O-alkyl-C, aromatic-C and carbonyl-C) can be determined from the NMR spectra. Ratios of humification (HR) and aromaticity (AR) were used to study the transformation of soil organic matter plus organic matter from the effluent for UV and WW microcosms. These two ratios did not vary significantly (nonparametric test of Kruskal–Wallis, $p < 0.05$) either between the different microcosms or between the sampling times (data not shown). These results imply that the composition of organic matter in microcosms C, UV and WW was similar and that supply of the effluent treated or not, did not modify the characteristics of soil organic matter. Thus, additional organic matter from the effluent was probably efficiently transformed by soil bacterial communities. Principal component analysis (F1 and F2 explaining more than 69% of the variance) obtained from the relative percentages of alkyl-C, O-alkyl-C, aromatic-C and carboxyl-C for the different microcosms (Fig. 2) shows differences in soil organic matter composition that the ratios had not highlighted. Axis F1, which explains 56.54% of the variance, shows a difference in the organic matter composition between microcosms C and UV on one hand and microcosm WW on the other hand. This marked difference was found for

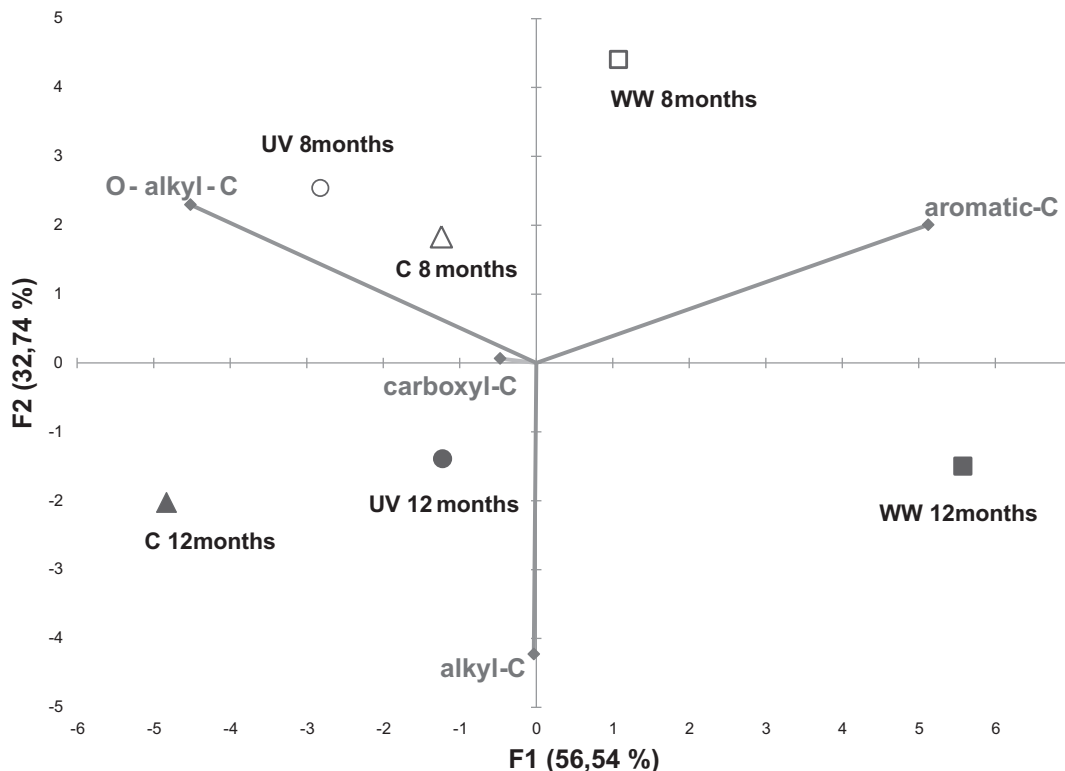


Fig. 2 – Principal component analysis of the relative percentages of alkyl C, O-alkyl C, aromatic C and carboxyl C for microcosms C (triangle), WW (square) and UV (circle) after 8 (white) and 12 months (grey) watering. For each microcosm the barycenter of points obtained for the four replicates was calculated.

both sampling times. Axis F2, which explains 32.74% of the variance, shows that the sampling time influences the composition of soil organic matter in all microcosms. After an 8-month watering period (November), microcosms C and UV were characterized by the presence of O-alkyl-C, i.e. of polysaccharides (mainly cellulose and hemicellulose) while after a 12-month watering period (March), these microcosms were characterized by alkyl-C (mainly fatty acids from lipids), which indicates a degradation of O-alkyl-C between 8 and 12 months in these microcosms. Organic matter in microcosms WW is mainly explained by aromatic-C signal after 8-month and 12-months incubation, showing an accumulation of phenolic compounds under these conditions. Thus, while aromatic molecules recalcitrant to degradation accumulate in microcosms WW, lignocellulosic material is more easily degraded by soil microorganisms in microcosms watered with UV-LED WW after a 12-month incubation.

3.3. Soil enzyme activities

Extracellular enzymes produced by soil microorganisms are involved in mineralization or storage (via humification process) of organic matter. In this study we investigated whether the type of watering influences five enzymatic activities: laccases and cellulases are enzymes involved in the degradation of lignocellulose, which is a major component of soil organic matter, while lipases, proteases and ureases are more representative of the degradation of organic matter in the effluent. The activities of these five enzymes in microcosms C, WW and

UV were measured after 4, 8 and 12 months of watering and results are shown in Fig. 3. Considering lignocellulolytic activities, laccase activity (Fig. 3a) increased after 8 and 12 months of watering in microcosms UV but not in microcosms WW. Molecules from the effluent, and in particular aromatic polymers recalcitrant to biodegradation, could be photo-oxidized during the UV-LED treatment, and aromatic by-products of oxidation can be inducers of laccase activity. This hypothesis is consistent with the results from Liu et al. (2010) who showed that high molecular weight molecules are rapidly photo-oxidized into molecules of lower molecular weight. Phenols, which can be formed from the degradation of polyphenols, are known to be inducers of laccase activities (Farnet et al., 2009; Terróna et al., 2004; Verdin et al., 2006). With cellulase activities, an increase was observed after 8 months for microcosms UV and WW (Fig. 3b), and reached the values of control after 12 months of watering. The increase in cellulase activities is often observed as a response to the input of organic matter in soil and more particularly of nitrogen, which is often a limiting nutrient in soil (Liu et al., 2011).

Thus, it is noteworthy that adding wastewaters did not affect the enzyme activities involved in the transformation of autochthonous organic matter i.e. lignocellulosic material. For instance, exogenous microorganisms from the effluent may have affected the microbial community structure in soil leading to a decrease in the enzymatic potential responsible for lignocellulose degradation. Furthermore, our results indicate that laccase activities were enhanced in microcosms UV, which can be related to the previous findings about organic

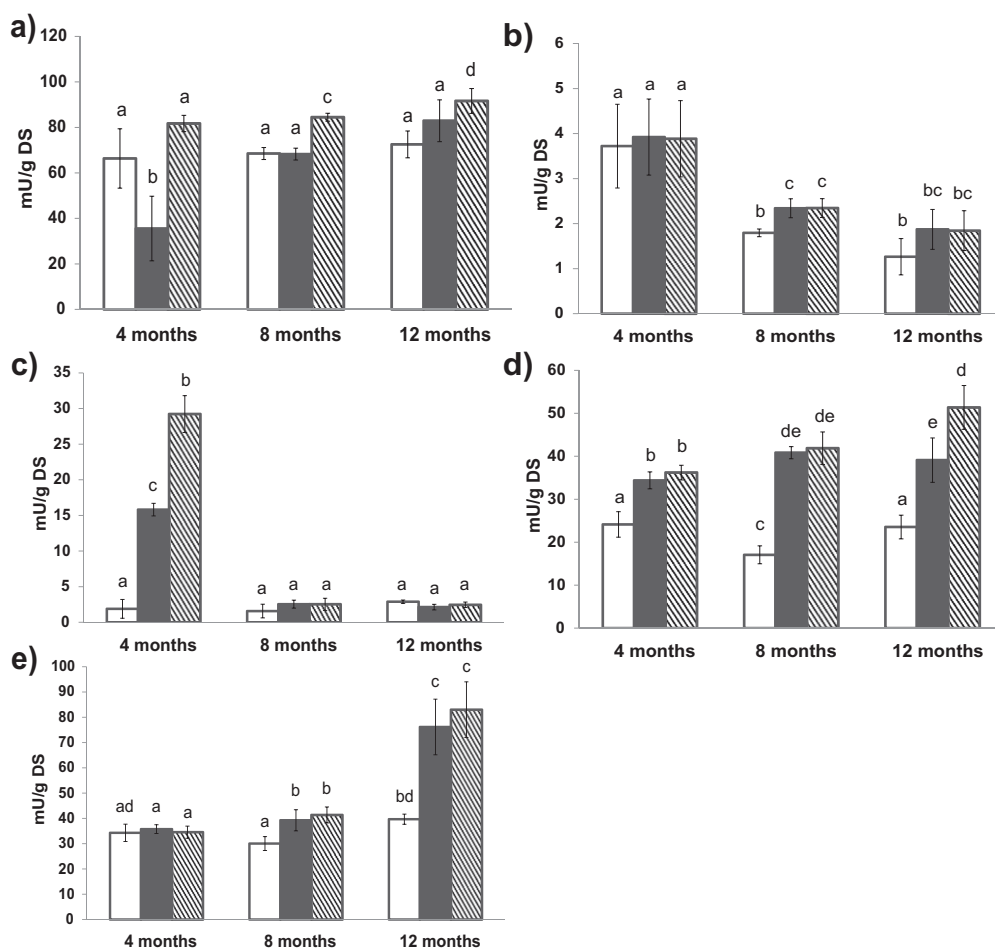


Fig. 3 – Enzymatic activities laccase (a), cellulase (b), lipase (c), urease (d) and protease (e) in soil microcosms watered with water (C), with WW (WW) and with UV-LED WW (UV). The letters represent significant differences between samples (non-parametric test of Mann–Whitney for paired comparison, $p < 0.05$).

matter degradation. The recalcitrant fraction aromatic was indeed more extensively degraded in microcosms UV. Thus, UV-LED treatment, probably via aromatic by-products of oxidation, induced laccase activities and favored aromatic compound degradation.

The hydrolytic activities more specifically involved in the transformation of organic matter from the effluent were also monitored over time. After four months of watering, lipase activity (Fig. 3c) significantly increased in the different microcosms as follows: control > microcosms UV > microcosms WW. After 8 (November) and 12 months (March) watering, no differences in lipase activity were observed in the three types of microcosms considered. The increase in lipase activity in microcosms UV and WW corresponds to June (4 month incubation), and the concentration in lipids may be greater since the effluent was collected in a tourist area in Southeastern France. However, variations in the chemical characteristics of the effluent were monitored with TOC, which is a global quantification of carbon in the effluent and does not give information about qualitative variations. Urease and protease activities increased from 4- and 8-month watering respectively, in microcosms UV and WW. The urease activity increased faster than the protease activity since transformation of polymers such as

proteins is a more complex metabolism. Zaman et al. (2002) have also observed an increase in urease and protease activities after addition of effluents onto soil. Our results indicate that organic matter from the effluent is actively transformed by microbial enzymes and thus do not accumulate in soil.

UV-LED treatment, and UV treatments generally, are supposed to form by-products of photooxidation (Liu et al., 2012; Prados-Joya et al., 2011), which may have toxic effects on microbial communities and thus on their activities. Results obtained here show that watering soil with UV-LED WW did not alter microbial activities and certain activities are higher compared to microcosms C (for the five activities monitored) and to microcosms WW (for laccase, lipase and urease activities). For instance, laccase enhancement is of major importance since these phenoloxidases are potentially involved in aromatic pollutant transformation.

3.4. Functional diversity of soil bacterial communities

To assess the modification of functional catabolic diversity of soil microbial communities in microcosms, the catabolic diversity was evaluated by testing their capacity to use different carbon sources contained in a microplate (Biolog®). The

estimation of functional diversity of soil bacterial communities was shown to be an efficient method to detect modifications linked to soil management practices (Crecchio et al., 2004; Gomez et al., 2006). To study the global activity of bacterial communities of soil, depending on the water used for watering, we calculated the AWCDs after 42 h of incubation (i.e. during the exponential growth phase) for each microcosm. The AWCD indeed represents the average oxidative capacity of soil microorganisms and thus can be used as an indicator of global microbial activity. After 4, 8 or 12 months, there was no significant difference in AWCDs between microcosms UV or WW and microcosms C (non-parametric test of Kruskal–Wallis, $p < 0.001$, data not shown). Though watering with WW and UV-LED WW modified enzymatic activities (urease and protease), as previously described, it did not affect the total microbial activity of soil. To assess the functional diversity of bacterial communities, the Shannon index was calculated based on Biolog[®] ECO values at 42 h (data not shown). The Shannon indexes in microcosms UV and WW were not significantly different from those obtained from microcosm C for each sampling time (non-parametric test of Kruskal–Wallis, $p < 0.001$, data not shown). Thus, watering with WW or UV-LED WW did not affect functional biodiversity, which can be considered as a reflect of genetic diversity, in soil microcosms. Biolog[®] Eco data were also analyzed via principal component analysis using PC1 and 2 (62% of variance). For each treatment, the barycenter of the four replicates was calculated and shown in Fig. 4. The microcosms were separated depending on the sampling time along axis F1 which explains 48.33% of the variance. This projection shows a seasonal difference in the catabolic diversity of microbial communities that was not revealed by the Shannon index and AWCDs. Moreover, after four months of

watering, microcosms C and UV are separated from microcosms WW along axis F2 which explains 13.38% of the variance. Catabolic diversity of soil bacterial communities was probably modified by the addition of microorganisms via the WW. After 8 months of watering, microcosms C are separated from microcosms UV and WW along axis F2. Microcosms WW and UV projections after 8 months are mainly explained by degradation of D-cellobiose (Fig. 4), which is confirmed by the increase in cellulase activity in these microcosms after 8 months (Fig. 3b). Moreover, after 8 months, bacterial communities in microcosms C preferentially use simple sugars (such as Mannitol, Glucose-1-Phosphate...), while those in microcosms WW and UV degrade more complex molecules (such as glycogen or cellulose) showing that these communities are more adapted to polymer transformation. After 12 months of watering, Fig. 4 shows that the projections of microcosms C, UV and WW are grouped: the functional structures of microbial communities are similar and related to the degradation of amino acids and small amino molecules (putrescine). Watering with WW changed the functional potential of soil bacterial communities after 4 and 8 months while with UV-LED WW, this effect was observed after 8 months only. However, after 12 months of watering, the functional structure in microcosms C, WW and UV were similar, showing that effluents WW and UV-LED WW had no deleterious effects on microbial functional diversity. The addition of microorganisms (mainly in effluent WW) and organic matter (once a week) may have modified the catabolic profile of soil communities. However, our results confirm those of Prado et al. (2011) who have determined that irrigation with secondary or tertiary-treated wastewater did not significantly modify soil microbial activity, while effluent with only primary treatment did.

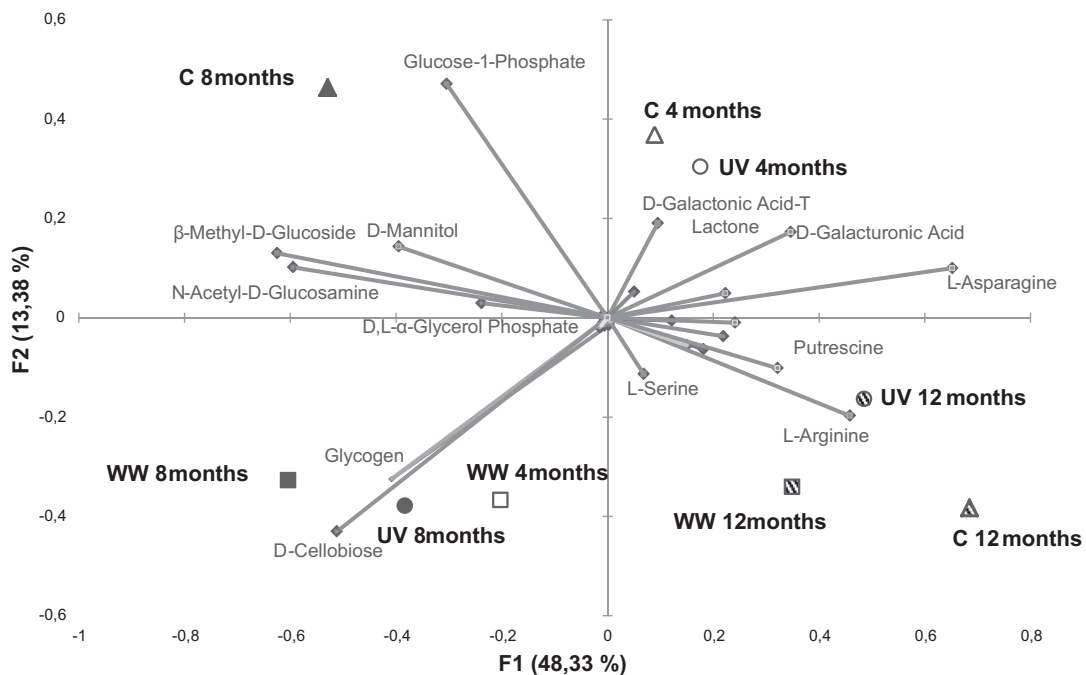


Fig. 4 – Barycenters of points representing the replicates obtained in Principal Component Analysis (PCA) based on Biolog[®] values for an AWCD of 0.5 for microcosms C (triangle), WW (square) and UV (circle) after 4 (white), 8 (grey) and 12 months (gray striped) watering.

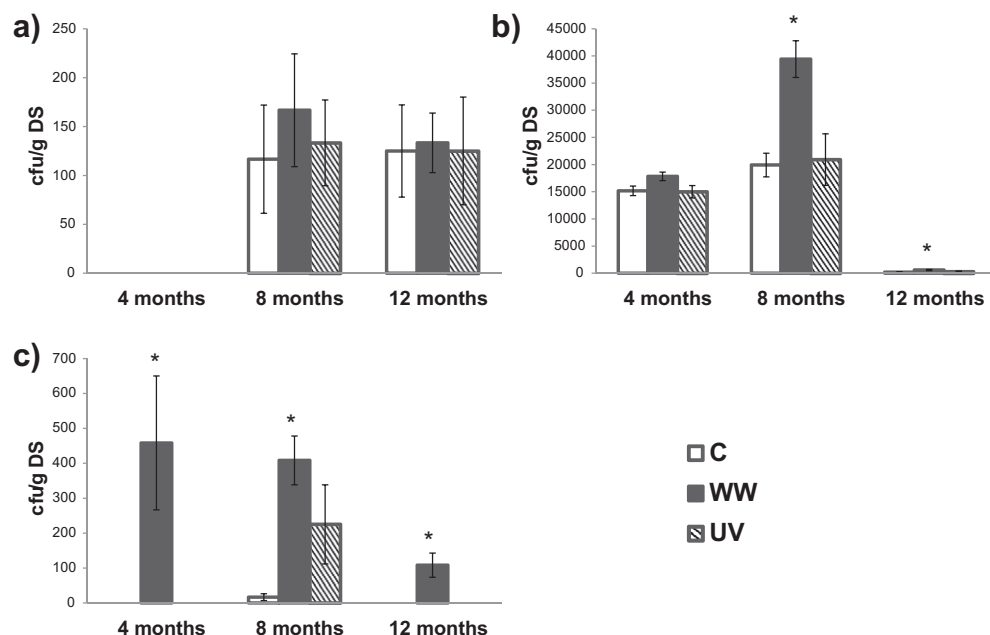


Fig. 5 – Number of faecal enterococci (a), total coliforms (b) and faecal coliforms (c) in 1 g of dry soil in microcosms C, WW and UV after 4, 8 and 12 months of watering. The asterisks represent a significant difference with the microcosms C for each sampling time (non-parametric test of Kruskal–Wallis, $p < 0.001$, followed by a multiple paired comparison according the Dunn procedure).

3.5. Persistence in soil of bacteria for detection of faecal pollution

Persistence of faecal microorganisms in natural environments has been extensively studied (Salgot et al., 2006) due to potential harmful effect on human health. They are subjected to

microbial antagonisms and competition for substrates, which can contribute to their elimination from natural ecosystems. To ensure the harmless effect of UV-LED WW watering, we checked whether faecal indicators (faecal enterococci, total coliforms and faecal coliforms) remained in soil and compared these results with those after WW watering. Fig. 5

Table 2 – Identification of coliforms (37 °C and 44 °C) counted in the microcosms C, WW and UV after 4, 8 and 12 months of watering and their percentage relative to the total number of colonies (\pm standard deviations). Bacteria different from those of microcosms C are in bold. Asterisks indicate significant differences with the control for bacteria found in all microcosms (non-parametric test of Kruskal–Wallis, $p < 0.05$).

	Sampling time	Coliforms	Relative percentage
C	4 months	- Enterobacter (Pantoea) agglomerans	100%
	8 months	- Acinetobacter lwoffii	66.4 \pm 3.7%
		- Aeromonas hydrophila	33.6 \pm 2.8%
UV	4 months	- Enterobacter (Pantoea) agglomerans	100%
	8 months	- Acinetobacter lwoffii	65.2 \pm 5.2%
		- Aeromonas hydrophila	34.8 \pm 3.7%
WW	4 months	- Enterobacter (Pantoea) agglomerans	53.8 \pm 2.4%*
	8 months	- Escherichia vulneris	22.1 \pm 1.3%
		- Enterobacter cloacae	24.1 \pm 2.0%
WW	8 months	- Acinetobacter lwoffii	31.7 \pm 2.3%*
		- Aeromonas hydrophila	21.2 \pm 1.9%*
		- Escherichia coli	14.8 \pm 1.9%
	12 months	- Enterobacter cloacae	25.3 \pm 3.2%
		- Citrobacter freundii	7.0 \pm 2.4%
		- Acinetobacter lwoffii	54.3 \pm 4.7%*
	12 months	- Aeromonas hydrophila	21.2 \pm 3.8%*
		- Enterobacter cloacae	24.5 \pm 3.9%

shows the number of faecal enterococci, total coliforms and faecal coliforms in microcosms C, WW and UV after 4, 8 and 12 months of watering. For the three bacterial types and for each sampling time, the number of faecal indicators in microcosms UV did not differ significantly from microcosms C. Though thermotolerant bacteria still remained in the effluent after UV-LED treatment (4.4 CFU/mL), they were not persistent in soil and were quickly eliminated. The effluent WW contained a higher thermotolerant bacteria concentration (1.9×10^3 CFU/mL). Microcosms WW showed a significantly higher amount of total coliforms, after 8 and 12 months of watering, and of faecal coliforms, from 4 months of watering. This demonstrates that faecal pollution, with potentially pathogenic bacteria, actually increases in soil irrigated with effluent WW. UV-LED treatment eliminates the majority of thermotolerant bacteria in the effluent (Chevremont et al., 2012b). By combining wavelengths 280 and 365 nm and using only 2 LED, UV-LED treatment provides a 4-log reduction in total coliforms, with a UV dose of 6 mW/cm² (Chevremont et al., 2012b), while the same results were obtained with standard UV lamps (254 nm) with UV dose greater than 75 mW/cm² (Oppenheimer et al., 1997). This process thus ensures a harmless mean for watering considering these microbiological parameters.

We have identified certain faecal coliforms (each type of colony per plates) obtained from microcosms C, UV and WW (Table 2). The coliforms identified in microcosms UV are identical to those found in the microcosms C and their relative percentages are similar to those of the control (non-parametric test of Kruskal–Wallis, $p < 0.05$). Thus, watering with the UV-LED WW does not change, either quantitatively or qualitatively, coliforms in soil. On the other hand, watering with WW increases the diversity of coliforms. For instance, *Escherichia vulneris*, *Enterobacter cloacae*, *Citrobacter freundii* and *Escherichia coli* are found only in microcosms WW. These bacteria can be pathogenic and antibiotic-resistant (Franck et al., 2008; Kilani et al., 2008; Domingos et al., 2011; Qureshi et al., 2011). Watering with wastewater after secondary treatment by activated sludges is thus not recommended, while after UV-LED treatment, health risks due to the persistence of faecal bacteria in soils appear to be strongly decreased.

4. Conclusion

UV-LED treatment is a new water disinfection system, which can be developed to reuse wastewater for irrigation in response to the scarcity of high quality water in Mediterranean countries. Our study indicates that this technology seems to be promising since our results show that watering with UV-LED treated wastewaters does not alter the microbial functioning of soil and even enhance recalcitrant aromatic compound degradation. Moreover, faecal contamination is limited compared to watering with wastewater. To confirm these initial findings, our further research will focus on the optimization of the UV-LED system, which will be tested on a larger scale. A prototype capable of processing 150 L of effluent will give us the opportunity to compare the efficiency of UV-LED to that of mercury vapor lamps with equal power and to focus on other criteria such as reduction in spores of anaerobic sulfate-reducing bacteria, in viruses and in protozoa.

Acknowledgments

This research was financially supported by a fellowship of Conseil Régional Provence Alpes Côte d'Azur (CR-PACA) and the European Regional Development Fund (ERDF). The authors would like to thank the assistance of Mr. Laurent Vassalo for the organic analysis.

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