Extraction of Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls from Urban and Olive Mill Wastewaters Intended for Reuse in Agricultural Irrigation

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Extraction of polycyclic aromatic hydrocarbons and polychlorinated biphenyls from urban and olive mill wastewaters intended for reuse in agricultural irrigation

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Abstract

In this paper we developed analytical procedures for the extraction of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) along a wastewater reuse chain for irrigation purposes. Besides urban wastewaters, olive mill wastewater was considered as a potential water source. Wastewaters were purified by different treatments (urban wastewater plants, pilot activated sludge and constructed wetland) and used for the irrigation of olive trees. Suitable extraction procedures were to analyze treated and untreated wastewaters, soils and for post-irrigation leachates. For wastewater and leachate samples, the optimized reverse-phase solid-phase extraction (SPE) provided recoveries up to 79%. For olive mill wastewater, the SPE procedure was preceded by a normal-phase purification stage with silica gel for the removal of polyphenols, which were as high as 8.7 g/L. After optimization, extraction recoveries in blank solutions were in the range 20-67% and moderately reduced (10-38%) in olive mill wastewater due to the matrix effect (-10 / -60%) ascribed to the very high value of chemical oxygen demand (COD 264 g/L). Detection limits of the method were below 1.1 µg/L (PAHs) and 3.2 µg/L (PCBs), using GC-MS analysis. For soil samples of different compositions, microwave assisted extraction (MAE) provided better extraction recoveries and reproducibility than the more common QuEChERS approach, which was affected by a high matrix effect. Detection limits of MAE/GC-MS method were below 4.9 µg/Kg (PAHs) and 12.3 µg/Kg (PCBs). The analytical procedures developed are a valuable tool to quantify the possible propagation of residual contamination from PAHs/PCBs with irrigation along the wastewater reuse chain.

Key words: PAHs/PCBs, urban/olive mill wastewaters, soils, leachates, SPE, MAE, wastewater treatment, reuse.

Introduction

Population growth and industrial expansion have been recognized as important pressures in determining water availability in recent decades. Likewise, it is also foreseeable that the reduction of water will be further exacerbated by the processes of desertification and drought resulting from the climate changes we are experiencing (1).

The reduced availability of water imposes sustainable strategies that can no longer be deferred, especially in specific sectors with high water intensity, such as agriculture (2) and in Countries affected by poor local precipitations and climate aridity, such as the Mediterranean regions. Even if freshwaters are considered relatively abundant in the European Union, the European Commission has recently proposed rules to encourage a wider use of reclaimed water for agricultural irrigation, thus contributing to alleviating water scarcity (3).

Treated domestic wastewaters represent the main alternative source of irrigation water both in Europe (4) and in the Mediterranean areas (5). However, depending on the peculiarities of the industrial activities of each country, also reclaimed industrial wastewater can be considered as an important irrigation source for agriculture.

As an example, olive mill industry is a strategic economic sector in the Mediterranean region where over 98% of world's olive oil is produced (6, 7). However, extraction of olive oil produces huge quantities of solid and liquid wastes called olive pomace and olive mill wastewater OMWW, respectively (8). OMWW can be generated during the extraction of olive oil using press (artisanal) or centrifuge (semi-modern) extraction methods. The quantities of OMMW produced are different due to the high consumption of water during the process, with centrifuge method consuming 1.25 to 1.75 time more water than press method (9). For the production of 1 L olive oil, around 3.5 L of water consumption is estimated, producing 4.3 Kg of OMWW (10). The estimated world production of OMWW varies between 10 to 30 million m^3 (7). Although the composition of OMWW can vary according to the climate conditions, the soil, the maturity and the variety of the fruit, the conservation conditions and the type of extraction (11), the OMWW are generally composed of water (83-94%), organic matter (4-16%) and minerals (0.4-2.5%) (12). About 2-15% of the organic matter is constituted by low and high molecular weight phenolic compounds (13). OMWW have a dark colour, high acidity and electrical conductivity. In their inorganic fraction, OMWW contain metals and major elements such as potassium, calcium and sodium (11). For the olive industry, OMWW represents a serious problem in terms of disposal and treatment (7). On the other hand, OMWW may be a suitable method both to recover soil fertility and to preserve water (14), especially in the Mediterranean basin, particularly in the southern shore (Morocco, Tunisia, etc.), where most of the soils have poor quality due to the lack of organic matter and water scarcity.

The controlled fertirrigation with OMWW can increase soil fertility (15) and carbon and nutrient soil content (16). Treated OMWW have been recently recognized useful for irrigation in arid regions (17).

Whatever the source of supply, the reuse of wastewater in agriculture must be practiced by farmers in safe conditions and must not be a threat to the health of consumers of crops irrigated with reclaimed wastewater. In this regard, it is therefore necessary to ensure that any residual contamination of wastewater does not spread either along the agricultural supply chain or in the environment.

Among chemical contaminants, polycyclic aromatic hydrocarbons (PAHs) and dioxin-like/non dioxin-like polychlorinated biphenyls (PCBs) are present in all the environmental compartments as a result of natural and anthropogenic pollution and are representative of both point source and diffuse emissions. PAHs and PCBs could still be detected in wastewater effluents, due to the not complete removal efficiency of the plant (18) (19).

To ensure the monitoring of even complex matrices within the whole agricultural supply chain fed with treated wastewaters, it is necessary to have adequate analytical procedures and methods in terms of removing the matrix and preconcentrating the pollutants to be monitored.

Many extraction approaches have been proposed for the analysis of PAHs and PCBs in environmental samples based on stir bar sorptive extraction, microwave extraction and pressurized liquid extraction, among others (20). To the best of our knowledge, in the current literature, methods for the evaluation of the residual contamination of wastewaters along refinement systems of different complexity as well as methods for the evaluation of a possible transfer of contamination through soil and leachates during the reuse of water are under-investigated (19). Furthermore, methods for the determination of PCBs and PAHs in OMWW are totally absent in the current literature.

The aim of this work is to develop extraction and analytical procedures for the analysis of PAHs and dioxin-like and non-dioxin like PCBs in different liquid and solid matrices along a pilot-scale wastewater reuse chain in agriculture.

In detail, crude olive mill wastewaters and urban wastewaters were reclaimed using a conventional wastewater treatment plant, a stabilization pond for urban wastewater and two pilot-scale plants based on (i): constructed wetland; (ii): activated sludge located in Morocco. After reclamation, these waters were used for irrigation of olive plants.

To the best of our knowledge, this work represents the first study related to the evaluation of organic micropollutants in olive mill wastewater and to the monitoring of the propagation of micropollutants along a comprehensive wastewater re-use chain of such complexity.

Experimental

Reagents and solutions

Reagent grade dichloromethane, 2-propanol, cyclohexane, acetone, were from Sigma Aldrich-Merck (Darmstadt, Germany).

Sodium chloride and magnesium sulphate, ACS reagent salts, were supplied by Riedel-de Haën (Seelze, Germany). Primary secondary amine bulk sorbent (PSA), used for the clean-up step in the QuEChERS procedure, were from Agilent Technologies (Santa Clara, CA, USA).

The PAHs studied were the 16 PAH compounds listed by EPA and were purchased from Sigma Aldrich-Merck (Darmstadt, Germany). The PCBs studied were chosen according to the results of the main environmental monitoring campaigns and were purchased from LGC Standards (Milan, Italy). They were non-dioxine like PCBs: 3,3'-dichlorobiphenyl (PCB 11), 4,4'-dichlorobiphenyl (PCB 15), 2,4,4'-trichlorobiphenyl (PCB 28), 2,2',5,5'-tetrachlorobiphenyl (PCB 52), 2,2',4,5,5'-pentachlorobiphenyl (PCB 101), 2,2',3,4,4',5-hexachlorobiphenyl (PCB 138), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169), 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180), 2,3,3',4,4',5,5'-heptachlorobiphenyl (PCB 189); and dioxine like PCBs: 3,4,4’,5-tetrachlorobiphenyl (PCB 81), 2,3’,4,4’,5-pentachlorobiphenyl (PCB 118), 2’,3,4,4’,5-pentachlorobiphenyl (PCB 123), 2,3’,4,4’,5,5’-hexachlorobiphenyl (PCB 167).

Labelled isotope compounds for PCBs (2 mg/L) and for PAHs (5 mg/L), Wellington Laboratories, Ontario, Canada, were used as internal standards and surrogates in order to obtain calibration curves and extraction recoveries, respectively. The ^{13}C surrogate solutions used contained: ^{13}benzo(a)anthracene [BaA], ^{13}chrisene [Chr], ^{13}benzo(b)fluoranthene [BbFl], ^{13}benzo(k)fluoranthene [BkFl], ^{13}benzo(a)pyrene [BaP], ^{13}indene [Ind], ^{13}dibenzoanthracene [DBA], ^{13}benzoperylene [BP] and ^{13}PCB28, ^{13}PCB52, ^{13}PCB118, ^{13}PCB153, ^{13}PCB180.

High-purity water (18.2 MΩ cm resistivity at 25 °C), produced by an Elix-Milli Q Academic system (Millipore-Merck, Vimodrone, MI, Italy) was used.

Instrumentation

For PAHs and PCBs analysis, a gas chromatographic-mass spectrometric (GC-MS) method was used, moving from EPA 8275A procedure (21).

Analysis were performed on an Agilent 6980 series gas chromatograph coupled with an Agilent 5973 Network mass spectrometer detector.

The GC column was a (5%-Phenyl)-methylpolysiloxane column (HP 5ms, 30 m x 0.25 mm x 25 µm, Agilent), with He used as gas carrier (1 mL/min). MS detection was performed in Single Ion Monitoring (SIM) mode. For each analyte, proper m/z ratio (m/z ratio available upon request) was selected. Injections (2 µL) were performed by the Pulsed Splitless mode (pressure at 40 psi for 2.5 minutes). The oven ramp was set as follows: starting temperature: 40°C, hold for 2 min; ramp to 176 °C, 12 °C/min rate; ramp to 196°C, 5 °C/min rate, hold for 3 mins; ramp to 224°C, 12 °C/min rate; ramp to 244 °C, 12°C/min rate, hold for 3 min; ramp to 270 °C, 7°C/min rate, hold for 3 min; final ramp to 300 °C, 5°C/min, hold for 10 min to completely clean and restore the GC column. The complete separation of PAHs and PCBs was obtained within 52 min.

Wastewater reclamation systems and olive tree installation

The following reclamation systems were used: urban wastewater treatment plants, pilot constructed wetland, pilot activated sludge system. Their details are discussed in the Supplementary Material Section (see also Figures 1S-2S).

The details of the olive tree installation are described in the Supplementary Material Section (Figure 3S).

Samples

The soil and water samples (including leachates after irrigation) analysed are detailed in Table 1. Main physicochemical and microbiological characteristics of olive mill and urban wastewater samples were measured and reported in Table 1S (Supplementary Material section).

Table 1

Extraction of PAHs and PCBs

For each matrix considered within this work (see Figure 1), extraction procedures were appositively optimized, and hereafter summarized.

Figure 1

Wastewater samples and leachates.

Water samples were initially filtered through nylon filters (0.45 µm). PAHs and PCBs were extracted by solid-phase extraction (SPE) using a SPE Vacuum manifold and a polymeric reversed-phase cartridge (STRATA XL-100 µm, Phenomenex, Torrance, USA), The cartridge, conditioned (20 psi) with 5 mL CH_2Cl_2, 5 mL 2-propanol, 5 mL H_2O, was loaded (50 psi) with 200 ml of samples added with 20 mL 2-propanol. The water sample container was subsequently washed with 20 mL of a 2-propanol-water solution (10:90, v/v). After loading, the cartridge was washed (20 psi) with 5 ml H_2O, and 5 mL of a 2-propanol-water solution (85:15, v/v). The cartridge was dried for 10 min and analytes were finally eluted with two aliquots of 1.0 mL CH_2Cl_2. The eluted extract was finally spiked with the internal standard solution of PAHs and PCBs to achieve a final concentration of 5 µg/L and injected for GC/MS analysis.

To evaluate the extraction recoveries of PAHs and PCBs, before extraction, the water samples were spiked with ^{13}C surrogate solutions of PAHs and PCBs in order to achieve a final concentration of 5 µg/L.

Olive mill wastewater sample

Olive mill wastewater was initially filtered through paper filters and subsequently through nylon filters (0.45 µm). The filtered sample (with or without solvent, see results and discussion section) was loaded (70 psi) through a Sep-Pak Silica cartridge (previously conditioned with H_2O) to retain polyphenols. The eluate exiting from the silica cartridge was hence loaded onto STRATA XL-100 µm cartridge, following the procedure adopted for wastewater samples and leachates. The eluted extract was finally spiked with the internal standard solution of PAHs and PCBs to achieve a final concentration of 5 µg/L and injected for GC/MS analysis.

To evaluate the extraction recoveries of PAHs and PCBs, before extraction, the olive mill wastewater samples were spiked with ^{13}C surrogate solutions of PAHs and PCBs to achieve a final concentration of 5 µg/L.

Soil samples

QuEChERS extraction

QuEChERS approach, historically developed for the rapid extraction of contaminants from food matrices, has been extensively applied also to different environmental matrices such as soils (22).

According to a previously optimized procedure (23), with minor modifications, a known amount of previously sieved soil (5 g) was put in a vial containing 10 mL CH_2Cl_2, 400 mg $MgSO_4$ and 1 g NaCl. The tube was vigorously shaken and centrifuged at 1500xg for 5 min. The supernatant was then transferred for the clean-up step in a new vial containing 50 mg of Primary and Secondary Amine (PSA) sorbent and 150 mg of $MgSO_4$. Again, the tube was shaken and centrifuged at 7900xg for 10 minutes. Aliquots of 5 mL of the supernatant were collected and evaporated to 0.5 mL.

Microwave assisted extraction (MAE)

Organic micropollutants were extracted by MAE following a procedure previously developed for PCBs (24).

Aliquots of 0.4 g of soil, previously sieved at 2 mm, were put in a disposable Pyrex vessel with 5 ml of a 3:2 acetone-cyclohexane solution. Sample was digested in microwave oven at the following conditions (250 psi, 300W): 0-10 min up to 130°C, 10-15 min T=130°C, 15-25 min decrease to 60°C). Afterwards, the solution was centrifuged at 1580xg for 5 min and heated at 60°C to evaporate acetone. The extract was made up at 5 mL with cyclohexane and cleaned up with 2 mL of H_2SO_4. This treatment is intended to remove co-extracted hydrocarbons and other organic compounds, as well as water, being sulfuric acid a dehydrating acid.

In both MAE and QuEChERS procedures, before injection in GC-MS, 1 mL of the extract was spiked with the internal standard solution of PAHs and PCBs to achieve a final concentration of 5 µg/L.

For both MAE and QuEChERS procedures, recoveries of PAHs and PCBs were determined by ^{13}C surrogate solutions of PAHs and PCBs spiking both soil samples to achieve a final concentration of 5 µg/L in the extract. After extraction, concentrations were calculated by using an external standard calibration curve. Two different curves were used to quantify surrogate standards in cyclohexane and CH_2Cl_2, depending on the solvent used for the extraction procedure.

The method which provided higher extraction yields and better reproducibility was chosen for the final quantitation of PAHs and PCBs in soil samples.

Matrix effect evaluation

For soil samples, matrix effect (ME) was evaluated for MAE and QueChERS procedures. For wastewater samples, ME was evaluated for the most difficult sample (olive mill wastewater) which appeared viscous and dark.

For both soils and olive mill wastewater, the post-extracted solutions were added with ^{13}C surrogate solutions to achieve a final concentration of 5 µg/L and injected for GC-MS analysis. The concentration obtained ($C_{\text{surrogates,matrix}}$) was compared with the one attained after spiking the same amount of ^{13}C surrogates in the extraction solvent, which was cyclohexane for soil extracted by MAE digestion and CH_2Cl_2 for soil extracted by QuEChERS and for olive mill wastewater ($C_{\text{surrogates,solvent}}$) and ME was calculated according to the following equation:

$$ME(\%) = 100 \cdot (C_{\text{surrogates,matrix}} - C_{\text{surrogates,solvent}}) / C_{\text{surrogates,solvent}}$$

Results and discussion

Extraction of PAHs and PCBs from wastewater samples and leachates from the olive tree cultivation

As described in Table 1 and, hereafter represented in the experimental plan of Figure 1, different types of waters were used to irrigate installed olive trees, namely: urban wastewaters reclaimed by: i) a municipal treatment plant (at different purification stages), ii) a stabilization pond; and urban wastewaters mixed with olive mill wastewaters reclaimed by: i) by a pilot activated sludge system and ii) a CW plant. Urban and olive mill wastewaters were characterized, and the main parameters are reported in Table 1S of the Supplementary Material section. As shown, they are very different in physico-chemical composition, and the same is expected for leachates obtained from olive trees after irrigation.

Reversed-phase SPE with hydrophobic polymeric (19) or functionalized silica based (25) substrates proved useful to extract PAHs and PCBs from wastewater matrices. Considering the matrices selected within this work, which also include acidic olive mill wastewater, we have chosen hydrophobic polymeric substrate (Strata-XL cartridge). According to manufacturer's information, the use of this substrate is indicated for matrices containing high quantities of organic compounds and for viscous samples (such as olive mill wastewater samples).

The effect of sample volume to be loaded (200 mL or 1000 mL) on extraction recoveries was initially studied. Experimental tests were performed on W_2 treated wastewater using the procedure shown in the Experimental section. Experimental results showed that for 200 mL sample loading, recoveries ranged from 59.4 % (^{13}PCB180) to 88.5 % (^{13}BaA), with an average value of 70.9 %. For 1000 mL sample loading, recoveries ranged from 48.7 % (^{13}PCB180) to 85.6 %(BaA), with an average value of 65.8%. Retention mechanisms typical of this phase, i.e. π-π, hydrogen bonding, dipole-dipole and hydrophobic interactions, seem to be little influenced by the amount of matrix loaded. Based on these results, considering the lower impact of a lower sample volumes, the optimal volume to be treated was chosen at 200 mL.

The optimized extraction procedure was tested for the analysis of the eleven water samples (including untreated, treated wastewaters, leachates after irrigation). Despite the variety of samples processed, the SPE extraction showed to be robust in terms of both extraction recoveries and reproducibility. Table 2 presents the extraction recoveries and relative standard deviation obtained for each labelled surrogate, expressed as average of the eleven samples analysed.

Table 2

As shown, for all the 13 PAH and PCB congeners, the average extraction yield in all the wastewaters and leachates approaches 64% (with the highest recovery yield obtained for ^{13}BaA with almost 80%. Excellent relative standard deviations (< 7%) were obtained for such large sample variety. Recoveries slightly decreased for more hydrophobic compounds (^{13}PCB180 and ^{13}BP) probably due to the weaker competition (i.e. elution efficiency) exhibited by dichloromethane for these compounds, which, therefore, are stronger retained on the reversed-phase SPE cartridge.

Detection limits (DLs) of the overall reversed-phase SPE-GC/MS method were finally calculated by means of the response error (sy) and the slope of the calibration curve (m), according to the following expression: DL=3.3*sy/m (26). Calculated values were in the range 0.43 ng/L (Phe) – 1.11 ng/L (BP) for PAHs and 0.78 ng/L (PCB138) – 3.18 ng/L (PCB11) for PCBs, fully satisfying the limits fixed by Italian regulation on maximum admitted concentration of pollutants in treated waters to be reused for irrigation (IT D. Lgs 185/2003) (27) and the stricter, more precautionary, EU regulation to be applied for waters intended for human consumption (98/83/CE) (28).

Figure 2 shows the content of PAHs and PCBs in wastewaters and olive tree leachates determined by the method developed.

Figure 2

Olive tree leachates are characterized by a lower quantity of PAHs and PCBs and by a reduced numbers of congeners in respect of wastewaters. This phenomenon must be ascribed to the fact that, as expected, during irrigation, the soil provides a further refinement of wastewaters.

The possible contribution of the virgin olive tree soil (before irrigation) to the release of PAHs and PCBs in leachates, was also assessed, which is negligible, with the exception of Flu, which was observed in each leachate.

In leachates, for both PAHs and PCBs, the concentrations found were all less than 0.05 µg/L, with the exception of Phe in the leachate deriving from irrigation with the CW outlet, which is less than 0.1 µg/L. It is interesting to observe how PAHs with the highest number of aromatic rings and highly Cl-substituted PCBs have never been detected, thus demonstrating the effectiveness of the different wastewaters treatment systems in the removal of these compounds.

Extraction of PAHs and PCBs from olive mill wastewater

Olive mill wastewater is as a dark and viscous sample. Olive mill wastewater was initially characterized, as detailed in Table 1S of the Supplementary Material section. Its unbalanced salinity (22 g/L), organic matter content (264 g/L chemical oxygen demand) and polyphenols (8.7 g/L) concentrations in respect to the expected low µg/L levels of PAHs and PCBs make impossible the analysis of PAHs and PCBs micropollutants by simple matrix dilution. As shown in previous section, the determination of PAHs and PCBs is based on a SPE with a highly hydrophobic substrate for the simultaneous enrichment of target analytes and matrix removal. However, it is expected that the high content of organic matter and the considerable quantity of polyphenolic compounds can rapidly saturate the SPE cartridge and preconcentrate, thus heavily interfering with the subsequent GC-MS determination. Hence, extraction of target micropollutants from olive mill wastewater requires further purification steps in addition to SPE on RP substrate.

On the basis of the above considerations, a preliminary clean-up step has been introduced, aimed to remove polyphenols. A normal phase SPE with silica gel cartridge was used, exploiting the polarity of polyphenols introduced by the -OH moieties. After loading the sample on the direct-phase SPE cartridge, the solution obtained was loaded on the reversed-phase cartridge as described above (see Experimental section).

The optimization of the extraction procedure was initially performed on a blank consisting of ultrapure water spiked with PAH and PCBs surrogates (Table 3).

Table 3

For all the congeners, recoveries obtained after normal-phase and reversed-phase steps were in the range 11-38%. The lowest recoveries were observed for PCBs, since three congeners (^{13}PCB53, ^{13}PCB118 and ^{13}PCB180) were not extracted. Since good recoveries (52-79%, see Table 2) were obtained for water samples of very different compositions extracted with RP-SPE step only, the lowest recoveries obtained coupling the normal-phase SPE step (see Table 3) could be explained by the interaction between silica phase and PCBs/PAHs, which are definitely retained by the cartridge. For PAHs, interactions between π electrons of their aromatics rings and acidic centres of silica can be hypothesized (29). For PCBs, additional interactions can be ascribed to hydrogen bonds between Cl-atoms of PCBs and the -OH groups of the silica cartridge (30). The co-existence of the above-mentioned interactions and the relatively higher strength of H-bonding over π-π interactions, justify a stronger retention for PCBs (lower recoveries) than PAHs in the cartridge.

In order to improve extraction recoveries, acetonitrile was added to the water sample (10% volume) before loading on the silica cartridge. The organic modifier is expected to compete with silica for the interaction with PAHs and PCBs. As expected, retention of organic micropollutants onto silica decreased, thus strongly increasing extraction recoveries (Table 3), boosting them in the ranges 41-72% for PAHs, and from 16-49% for PCBs, thus solving the problem of the PCB congeners not detected under previous experimental conditions. This experimental procedure was followed for the analysis of the olive mill wastewater sample (Table 3). The extraction recoveries obtained in olive mill wastewater and acetonitrile overall decreased in respect to ultrapure water and acetonitrile for all analytes, except ^{13}BaA.

Since the contribution of silica cartridge was already assessed, the decrease of recovery yields should be addressed to the complexity of the matrix (see Table 1S, Supplementary Material section). Hence, matrix effect was calculated (Figure 3), revealing an underestimation in the range 10-60%, thus confirming the reduction in extraction efficiencies. Only for ^{13}BaA, the matrix effect leads to a partial overestimation of signal thus justifying the increase of its apparent recoveries shown in Table 3.

Figure 3

DLs of the overall normal/reversed-phase SPE/GC-MS method were calculated and were in the same range of DLs calculated for wastewaters and leachates, thus still satisfying previously mentioned legislative requirements (see paragraph "Extraction of PAHs and PCBs from wastewater samples and leachates from the olive tree cultivation").

Finally, the optimized extraction approach was successfully applied for the determination of PAHs and PCBs in the olive mill wastewater sample. Only few congeners were detected at concentration ranges between 59 and 108 ng/L (AcPY, 100 ng/L; AcPh, 108 ng/L; PCB11, 102 ng/L; PCB15 95.3 ng/L; PCB28, 59.4 ng/L). Their presence in such type of samples does not seem surprising given the possible direct contamination during the mill processing or the indirect olive skin contamination by environmental sources (31).

Extraction of PAHs and PCBs from soils of constructed wetlands

In order to be able to apply an extraction procedure for PAHs and PCBs usable on soils typical of constructed wetlands, the method was initially optimized on two soils of completely different composition: *Soil1* (clay soil) and *Soil2* (humic soil).

Although an EPA method, based on Soxhlet extraction, is available (32), this method has some disadvantages such as the low eco-friendly profile, since a large quantity of solvent is used, the long extraction times and the modest automation. To go beyond these methodological limits, the following alternative approaches were tested in this work: microwave assisted extraction, MAE, and QuEChERS extraction.

Experimental results proved that the composition of the soil played an important role in the extraction rates (Figure 4).

Figure 4

Indeed, for the clay-based *Soil1* (Figure 3A), both methods provided similar extraction yields (average extraction for PCBs is 109.4%, for PAHs is 86.6%, almost quantitative). However, for ^{13}Ind, ^{13}DBA and ^{13}BP, the PAH congeners characterized by the highest hydrophobicity, the extraction efficiency for QuEChERS is 30% lower in respect to MAE. This behaviour could be explained by two intrinsic difference of the two methods tested. Through heating and longer extraction times, MAE promotes a more efficient extraction of highly hydrophobic compounds from sediment-like matrices than QuEChERS, which operates at room temperature for a short interval time (5 minutes against to 25 of MAE). For both procedures, repeatability was higher than 80%, as demonstrated by the standard deviation bars (Figure 4A).

Differently, for the humic soil type (*Soil2*, figure 4B), QuEChERS procedure exhibits poor reproducibility and overestimation with extraction recoveries even reaching 240%, for 13BaA. On the contrary, MAE is characterized by good extraction recoveries (50-70%) and reproducibility, with matrix effect in the range ±20% for all the analytes.

It is hypothesized that the different performance between the two extraction techniques relies on the complexity of the humic matrix and on the ability to remove the interfering matrix. Even if both procedures co-extract humic acids, the clean-up step of QuEChERS with PSA does not efficiently remove humic acids, resulting in an overestimation of the signal. Conversely, co-extracted humic acids, are degraded under MAE conditions due to the high temperature reached during the extraction (up to 250°C). Previous studies demonstrated the role of temperatures higher than 110 °C in the gradual decomposition of humic acids to carbon monoxide and dioxide (in the range 110–240 °C) (33).

The matrix effect and its role in the overestimation of extraction yield in QuEChERS approach was studied (Figure 5).

Figure 5

The positive matrix effect observed for each analyte, resulted to be almost proportional to the surplus of extraction recoveries observed. As an example, the +94% matrix effect calculated for ^{13}PCB118 well compares with the overestimation of extraction yield (about 200%).

Based on the results discussed above, the MAE procedure has proved to be more robust in a wider spectrum of soil compositions and more suitable for the subsequent determination of PAHs and PCBs in CWs soils.

DLs of the overall MAE/GC-MS method were calculated and were in the range 0.94 µg/Kg (Ind) - 4.94 µg/Kg (BaA) for PAHs and 0.37 µg/Kg (PCB15) - 12.31 µg/Kg (PCB118) for PCBs, far lower than the detection limits reported by EPA 8275A method, for the determination of PAHs and PCBs in soils (from 0.01 to 0.5 mg/Kg) (34).

Soils were sampled from constructed wetland at a 0-10 cm depth, before and after irrigation with OW_1, and spiked with surrogate PAHs and PCBs for the evaluation of recoveries. As expected, mean recoveries were 93.2% for PAHs and 89.8% for PCBs, confirming the values previously obtained for model soils (*Soil1* and *Soil2*). Relative standard deviations were below 10% for all the analytes, supporting the good reproducibility and the robustness of the optimized MAE extraction method.

As regards the efficiency of the constructed wetland for PAHs and PCBs removal, Figure 6 shows that before the remediation treatment, few congeners of PAHs and PCBs are present each one below 10 µg/Kg.

Figure 6

After remediation treatment, an increase in the number and in the concentration of congeners was actually detected. Such results demonstrated the role of the CW in the abatement of PAHs and PCBs from wastewaters. The analysis of PAH and PCB concentrations in the more depth layers (data not shown) demonstrated that these micropollutants were accumulated preferably in the first 10 cm rather than in deeper layers, in accordance with other CW models (35).

Conclusions

The availability of extraction procedures suitable to be applied in the wastewater reuse agricultural chain will be important and necessary to evaluate any possible propagation of residual micropollutants from wastewaters. The SPE methods here developed allow to measure trace amounts of PAHs and PCBs not only in domestic wastewaters, or in leachates, but also in olive mill wastewaters which, despite represent an important source for water reuse, have a very complex composition. Through the development of microwave assisted extraction, the overall method derived allows accurate and reproducible determination of PAHs and PCBs in soils which were used to remediate complex wastewaters.

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Table 1. Description of the soils, wastewaters, treated wastewaters and leachates studied.

Sample	Description
W_0	inlet of wastewater treatment plant (Morocco)
W_2	outlet of secondary (activated sludge) stage of wastewater treatment plant (Morocco)
L_$_{W2}$	leachate from olive tree pots irrigated with W_2
W_3	outlet of tertiary (microfiltration by sand filter + UV + chlorination disinfection) stage of wastewater treatment plant (Morocco)
L_$_{W3}$	leachate from olive tree pots irrigated with W_3
OW_1	mixture of untreated OMWW (1%) and W_0 (99%)
CW_{OW1}	OW_1 after remediation with constructed wetland
L_$_{CW\ OW1}$	leachate from olive tree pots irrigated with CW_{OW1}
$W_{out}P$	outlet of the stabilization pond (Morocco)
L_$_{Wout}$P	leachate from olive tree pots irrigated with $W_{out}P$
$W_{out}AS$	OW_1 treated by pilot activated sludge system
L_W_{out}AS	leachate from olive tree pots irrigated with $W_{out}AS$
$CW_{soil\_0\text{-}10cm}$	Soil of CW after reclamation, sampled at 0-10 cm depth
CW_{soil_V}	Soil of CW before reclamation (virgin soil)

Table 2. Recoveries and relative standard deviation (RSD%) of labelled PAH and PCB surrogates obtained for wastewaters and leachates samples using the reversed-phase SPE procedure. Recoveries are expressed as averages of 11 samples.

Surrogated compound	Extraction recovery [%]	RSD [%]
^{13}PCB28	62.4± 2.4	3.8
^{13} PCB52	69.2± 2.5	3.6
^{13}PCB118	60.9± 2.4	3.9
^{13}PCB153	76.8± 2.4	3.0
^{13}PCB180	54.0± 1.7	3.1
^{13}BaA	79.3± 2.3	2.9
^{13}Chr	58.0± 2.3	3.9
^{13}BbFl	61.7± 2.4	3.8
^{13}BkFl	61.6± 2.5	4.0
^{13}BaP	72.0± 3.1	4.2
^{13}Ind	61.6± 4.0	6.4
^{13}DBA	60.8± 3.5	5.6
^{13}BP	52.0± 3.7	5.9

Table 3. Extraction recoveries [%] of PAH and PCB surrogates obtained with silica clean-up, followed by reversed-phase SPE for ultrapure water (Blank), ultrapure water with acetonitrile (Blank with CH_3CN) and olive mill wastewater sample added with acetonitrile (Olive mill with CH_3CN); n.d.= not detected.

Surrogated compound	**Extraction recovery [%]**		
	Blank (ultrapure water)	**Blank with CH_3CN**	**Olive mill with CH_3CN)**
^{13}PCB28	12.8	48.9	26.6
^{13} PCB52	9.9	52.0	25.9
^{13}PCB118	n.d.	31.8	24.1
^{13}PCB153	n.d.	19.8	13.9
^{13}PCB180	n.d.	15.9	9.8
^{13}BaA	33.6	72.0	96.2
^{13}Chr	38.5	63.2	37.9
^{13}BbFl	22.3	62.9	29.9
^{13}BkFl	19.2	66.8	24.0
^{13}BaP	15.6	61.5	21.4
^{13}Ind	11.9	41.5	15.7
^{13}DBA	13.0	49.3	18.7
^{13}BP	11.1	43.5	9.7

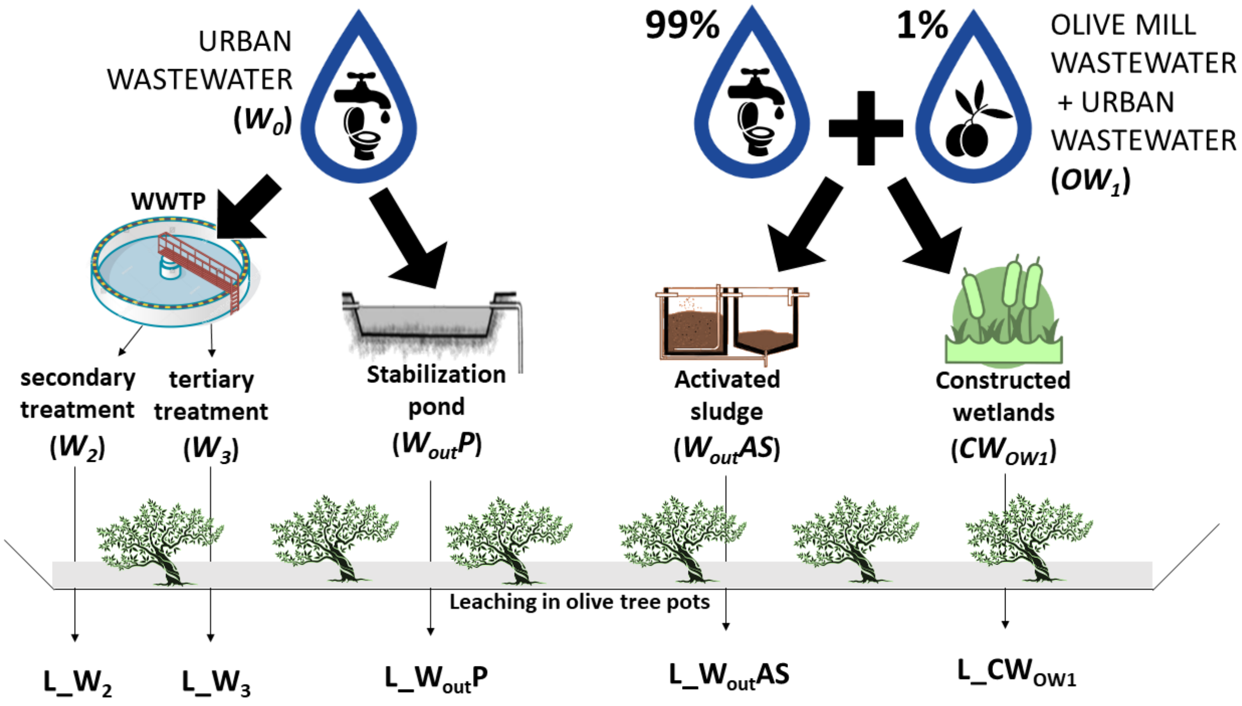

Figure 1. Schematic design of the reclamation of urban and olive mill wastewaters and of the use of treated waters for irrigation. Samples analysed, including leachates, are also indicated.

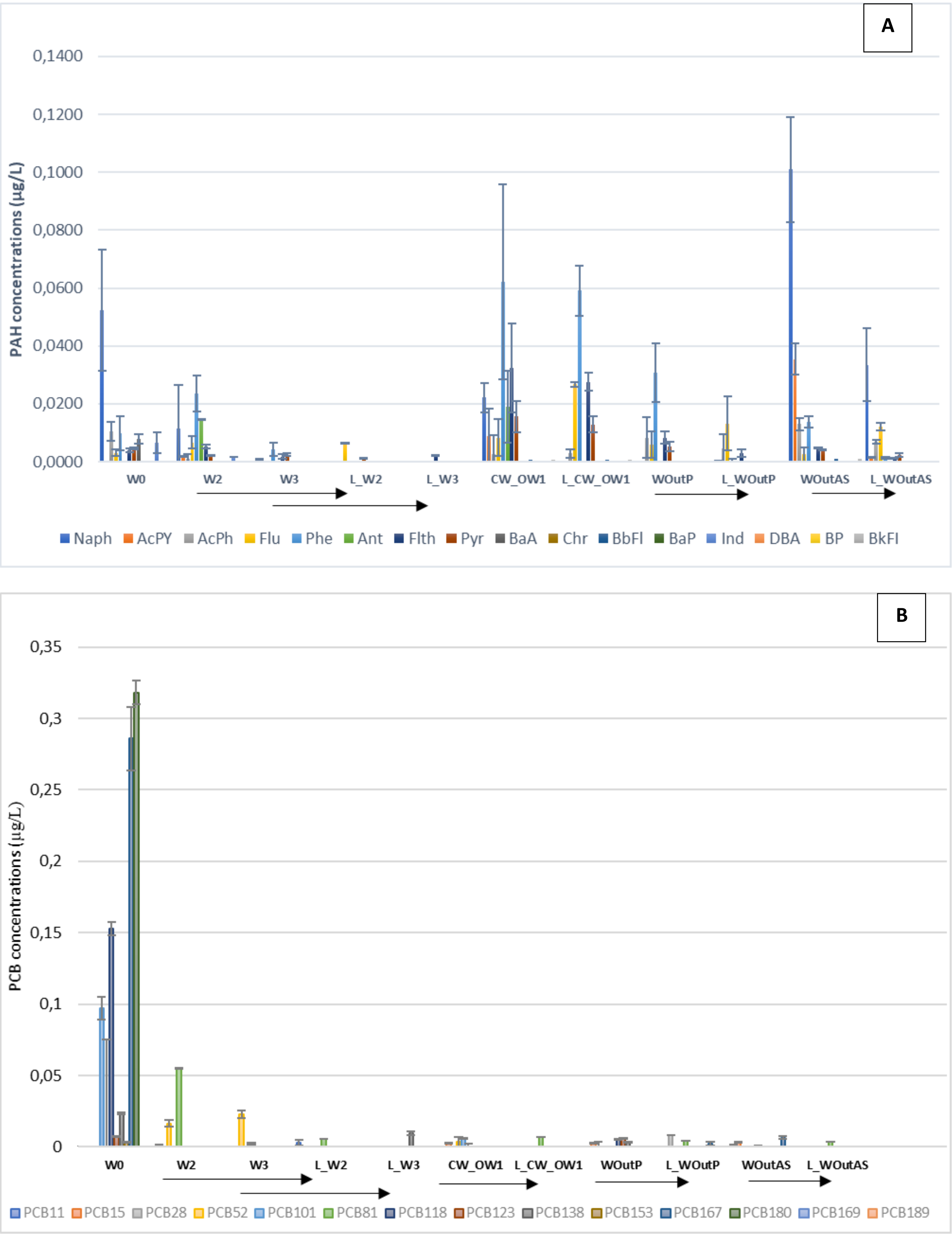
A
0,1400
0,1200
0,1000
0,0800
0,0600
0,0400
0,0200
0,0000
PAH concentrations (µg/L)
W0
W2
W3
L_W2
L_W3
CW_OW1
L_CW_OW1
WOutP
L_WOutP
WOutAS
L_WOutAS
Naph
AcPY
AcPh
Flu
Phe
Ant
Flth
Pyr
BaA
Chr
BbFl
BaP
Ind
DBA
BP
BkFl
B
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
PCB concentrations (µg/L)
W0
W2
W3
L_W2
L_W3
CW_OW1
L_CW_OW1
WOutP
L_WOutP
WOutAS
L_WOutAS
PCB11
PCB15
PCB28
PCB52
PCB101
PCB81
PCB118
PCB123
PCB138
PCB153
PCB167
PCB180
PCB169
PCB189

Figure 2. Concentrations of PAHs (A) and PCBs (B) in treated wastewaters and in their respective leachates, using the reversed-phase SPE procedure. Black arrows pointed out wastewaters-leachate couples. Sample names are summarized in Table 1.

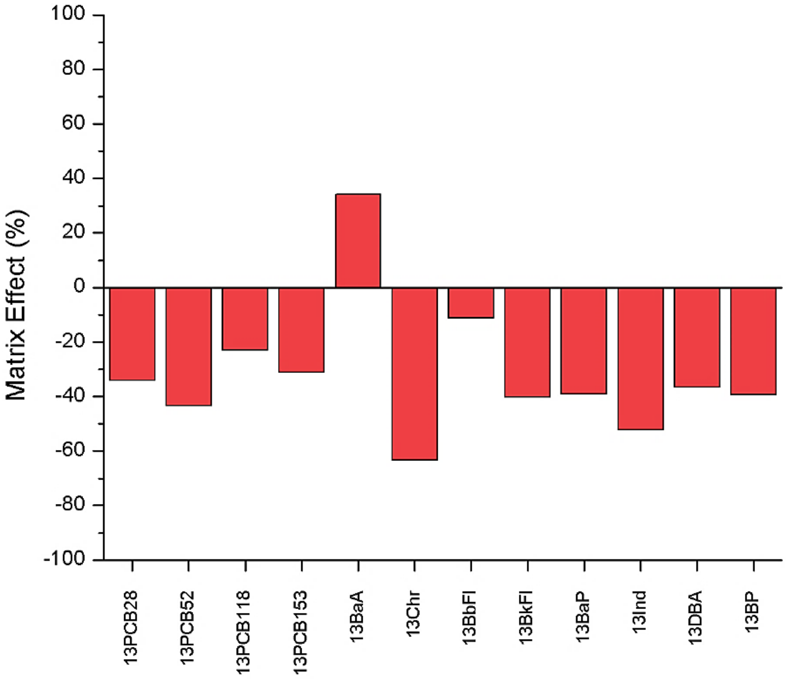

Figure 3. Matrix effect for labelled PAHs and PCBs surrogates in olive mill wastewater, using optimized silica clean-up, followed by reversed-phase SPE approach.

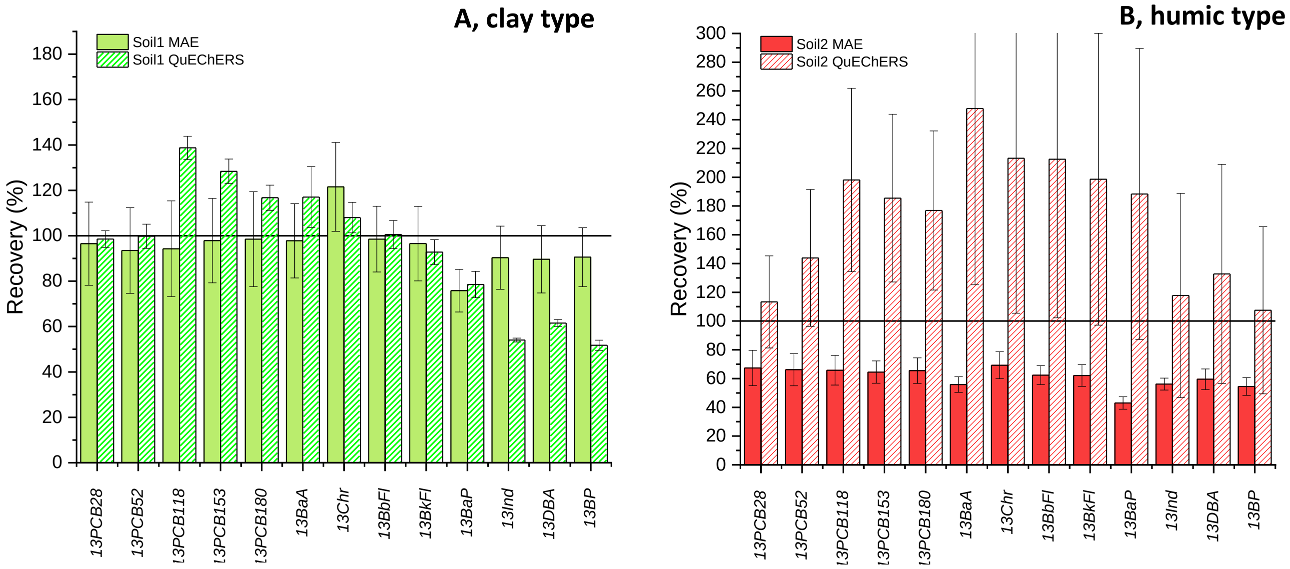

Figure 4. Extraction recoveries for labelled PAHs and PCBs surrogates from Soil1 (A, clay type) and Soil2 (B, humic type), using MAE and QuEChERS approaches.

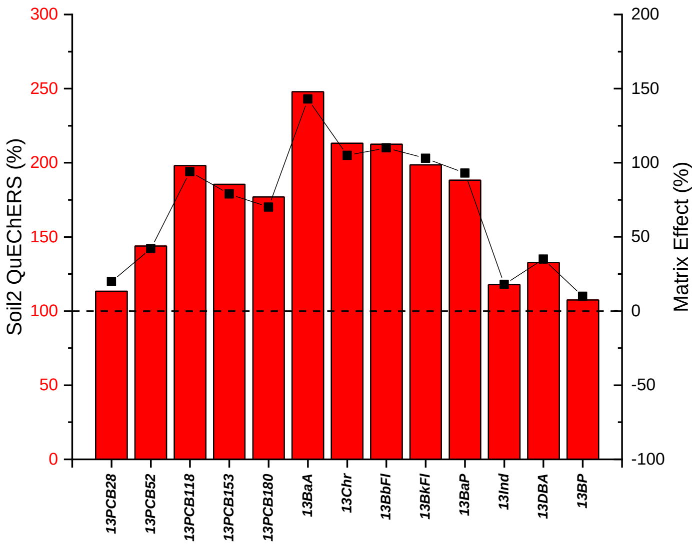

Figure 5. QuEChERS approach: overlay of the extraction recoveries (red bars with scale on the left axis) of labelled PAHs and PCBs surrogates for humic type soil (*Soil2*) with their calculated matrix effect (black squares with scale on the right axis). The correlation between the two parameters is clearly highlighted.

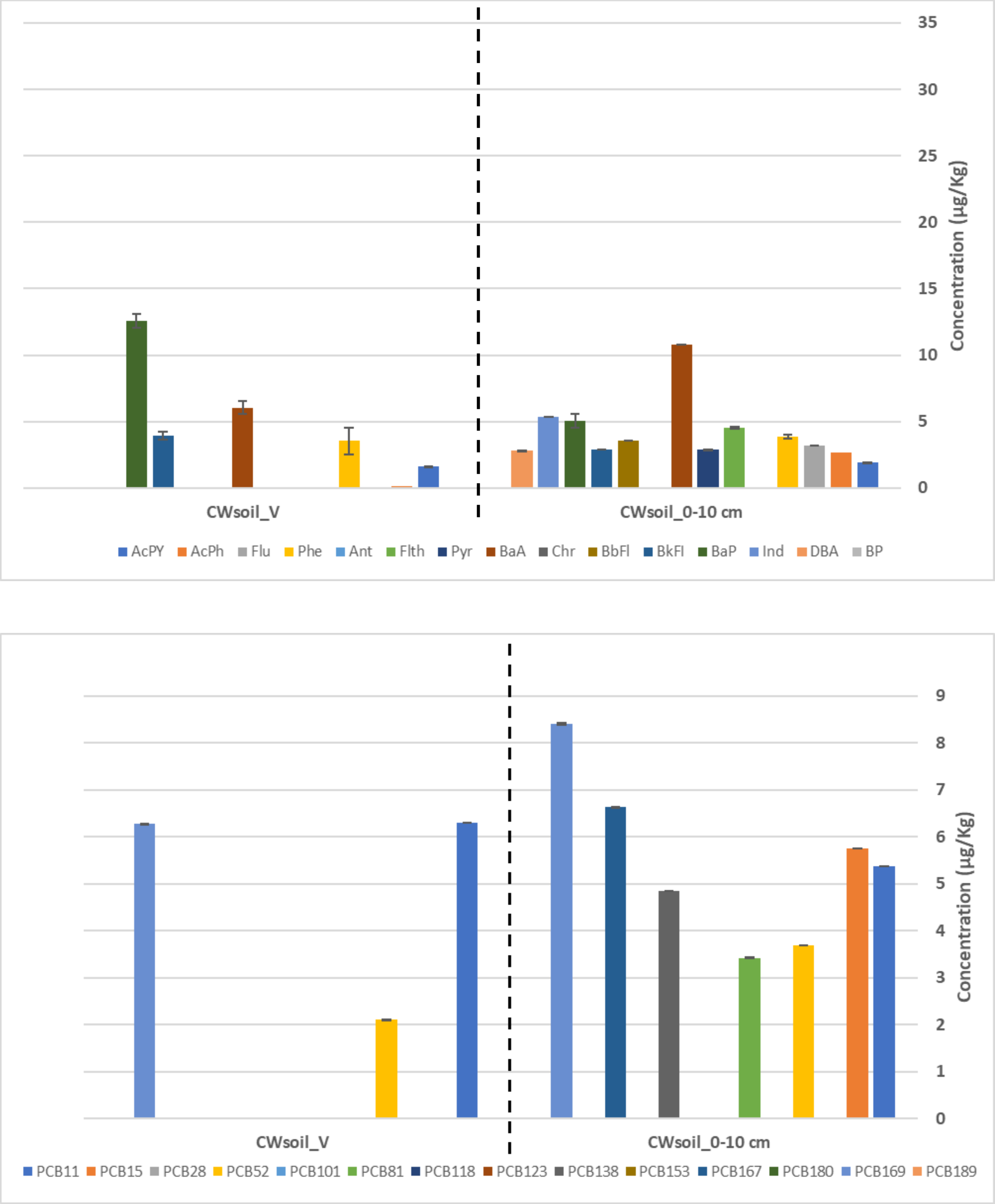

Figure 6. Concentrations of PAHs and PCBs in CW soils, before and after treatment, using the optimized MAE procedure. CWsoil_V, soil before the wastewater treatment.

Supplementary Material

Extraction of polycyclic aromatic hydrocarbons and polychlorinated biphenyls from urban and olive mill wastewaters intended for reuse in agricultural irrigation

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WASTEWATER SAMPLES

Olive mill wastewater

Olive mill wastewater was sampled from a traditional olive mill in Marrakech region (Morocco) and had the following chemical and biological characteristics:

chemical oxygen demand (COD): 264 g.L^{-1}, electric conductivity: 28.23 mS.cm^{-1}, phenolic compounds: 8.7 g.L^{-1}, pH 5.01. The main indicators of fecal contamination including total coliforms, fecal coliforms, and streptococcus were absent. Details are shown in Table 1S.

Urban wastewater (W_0)

Urban wastewater was sampled from the inlet of Marrakech wastewater treatment plant (Morocco) and had the chemical and biological characteristics reported in Table 1S.

Table 1S. Physico-chemical and microbiological characteristics of olive mill and urban wastewaters.

Parameters	Unit	Olive mill wastewater	Urban wastewater
pH		5,01	7,07±0,02
Dissolved oxygen	**mg/L**	0,70	0,91±0,13
Conductivity	**ms/cm**	28,23	4,36±0,17
Total dissolved salts	**g/L**	22,10	2,80±0,33
Suspended matter	**mg/L**	2066,00±11,269	519,33±7,11
Total polyphenol	**g/L**	8,73±0,434	0
Chemical oxygen demand	**g/L**	264,05±11,498	0,539±0,95
Orthophosphate	**mg/l**	31,14±0,651	1,44±0,08
Sulfate		1320±0,055	402,00±9,33
Ammonium		6,33±0,306	24,96±0,05
Nitrates		1,32±0,055	6,01±0,25
Nitrites		96,23±9,416	0,04±0,00
Total phosphorus		41,61±4,376	1,95±0,04
Total coliform	**UFC/100ml**	0	$2,13.10^{7}\pm3,71.10^{6}$
Fecal coliform	**UFC/100ml**	0	$8,67.10^{6}\pm1,11.10^{5}$
Streptococcus	**UFC/100ml**	0	$1.10^{3}\pm4,44.10$

Mixed samples (Olive mill wastewater + Urban wastewater)

In order to test the treatment feasibility of the harsh and toxic olive mill wastewater in low cost biological wastewater treatment processes, we have diluted OMWW by urban wastewater:

1% OMWW + 99 % urban wastewater = OW_1

The dilution rate was chosen according to the nominal organic load allowed to be received by each biological treatment.

URBAN WASTEWATER TREATMENT PLANTS:

Stabilization pond:

Sidi Mokhtar Stabilization ponds: the plant is constituted of anaerobic pond, followed by facultative ponds (Total area: 4 ha). There is no tertiary treatment facility. The treated wastewater flow is around 350-400 m3/day. Treatment allows 60% removal of organic matter. The treated wastewater is currently discharged into Lahmer river and a part of the treated wastewater is reused in irrigation.

Conventional wastewater treatment plant

Marrakech wastewater treatment plant: in this plant, about 120,000 m^3/d of wastewater are treated in four stages: 1) a pre-treatment 2) a primary treatment in sedimentation tank 3) a secondary treatment (activated sludge) 4) a tertiary treatment, which consists of microfiltration by sand filter and disinfection by UV+ Chlorination. This last process raises the effluent quality before it is reuse in irrigation of golf courses.

PILOT SCALE PLANTS FOR MIXTURE OF URBAN and OLIVE MILL WASTEWATER TREATMENT:

Constructed wetland:

The constructed wetland pilot, Figure 1S, was a PVC tank (height: 0.60m, diameter: 0.56m, volume: $0.14m^3$ and area: $0.25m^2$). The CW was filled from the bottom with a first layer (draining layer) made of 10 cm of 20/40mm gravel; the second layer (transition layer) was 10cm of 5/20mm pozzolan; the third layer (infiltration layer) was composed of 30cm of 0.25/0.40mm sand. The system was equipped with a drain to collect the water after treatment and ventilation pipe to insure the oxygenation of the different layers. The plant used was *Phragmites australis*.

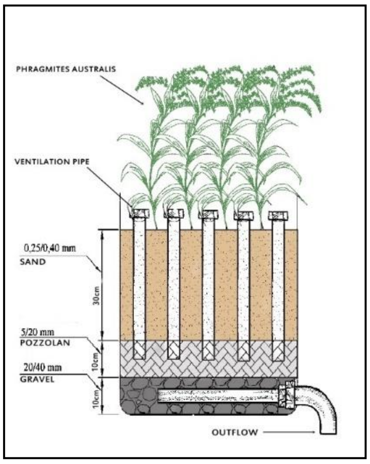

Figure 1S. Schematic picture of the constructed wetland (CW) for water reclamation

Activated sludge pilot:

The pilot-scale automated activated sludge (AS) plant, Figure 2S, consisted of a feed tank (100L), an anoxic tank (18 L), an aerobic tank (60L), a settling tank (30L), alimentation pumps, various recycling flows, agitators and an aeration system.

The pilot plant was inoculated with activated sludge (MLLS 2.20 $g.L^{-1}$) sampled from the aeration basin of Marrakech wastewater treatment plant (Morocco). The plant was fed by a mixture of urban wastewater collected after the primary settling tank of the same WWTP.

Food to Microorganisms (F/M) ratio was maintained between 0.1 and 0.3 $kg_{COD}.kg_{MLVSS}^{-1}.d^{-1}$. The hydraulic retention time (HRT) in the bioreactor was around 0.7d.

The sludge retention time (SRT) was set at 35 days by controlling sludge wastage. Mixed liquor suspended solids (MLSS) concentration in the aeration reactor was about 5.5g.L^{-1}.

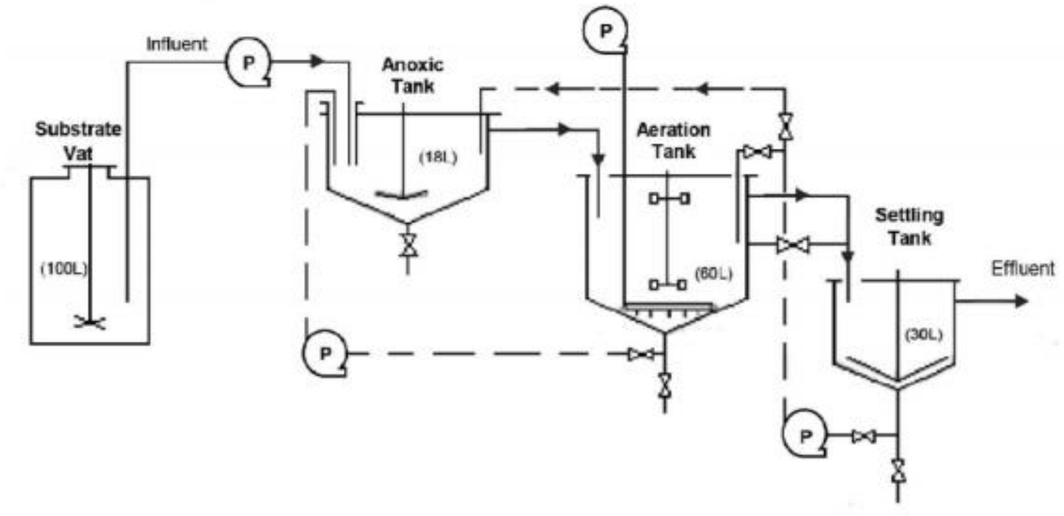

Figure 2S. Schematic picture of the pilot-scale activated sludge plant for water reclamation

MODEL PLANT IRRIGATED WITH CRUDE AND RECLAIMED WASTEWATERS

Olive tree pots

Treated wastewaters and mixtures of crude olive mill and urban wastewaters were reused to irrigate *Koroneiki* olive trees.

Trees were planted in 30 L capacity PCV pots with the following dimensions: 37 cm diameter and 38 cm height. Each pot was filled with 40 kg soil. The experimental design comprised 40 pots. A drain was installed in each pot to collect leachates. The installation is shown in Figure 3S.

Figure 3S. Installation of olive plants irrigated with different types of crude and reclaimed wastewaters