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IMPACT OF EFFLUENTS FROM WASTEWATER TREATMENTS REUSED FOR IRRIGATION: STRAWBERRY AS CASE STUDY

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Abstract

This research is intended to study the possible transfer of the residual chemical contamination from treated wastewaters reused for irrigation purposes of Fragaria x ananassa strawberry (cv. *Camarosa*). Different sewages from urban, and mixed urban-textile origins treated according to different treatment trainswere used for the irrigation of strawberry in pots. Organic and inorganic chemical contamination indicators, i.e.: PCBs, including dioxin-like congeners, PAHs, and Cr(VI), were monitored along the whole agricultural production chain (wastewater treatment effluents, soil and crop). Robust analytical procedures were specifically developed for the determination of contaminants in the above-mentioned matrices with quantitation limits (MQLs) ranging from 1.3 (Phe) to 11.0 ng/L (PCB169) for wastewaters; from 3 (PCB180) to 10 µg/kg (BbFl) for soils; from 1.0 (Phe) to 10.9 µg/Kg (PCB169) for strawberries. For Cr(VI), limits were 0.15 µg/L (waters) and 0.018mg/kg (soils). These performances fully satisfy limits fixed by Italian or European regulations on maximum admitted concentration of pollutants in treated water intended for irrigation, in soils and crops. Even if selected PAHs and PCBs were detected in wastewaters (highest concentrations observed for phenanthrene, 429 µg/L, and PCB52, 110 µg/L) their presence was not observed in soils and in strawberries above the MQLs. On the contrary, chromium content in strawberries and soils irrigated with TWs suggested a possible transfer of the metal during irrigation, which however does not represent a hazardous situation for consumers since calculated daily intake does not exceed the Tolerable Daily Intake of 300 µg/kg b.w.

Key words: food chain; reuse impact; strawberry; treated wastewater

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1. Introduction

Agriculture is characterized by a high-water demand; one third of the water use in Europe is addressed to the agricultural sector, most of it for crop irrigation EEA, 2012). Italy is one of the Countries with the highest water footprint in Europe, 25% above the European Union average, and, at a global level, 66% above the world average (Sartori et al., 2014). It is worth to be mentioned that agriculture in Italy accounts for about 85-89% of the water footprint of consumption.

The availability of fresh water (FW) is a problem of increasing concern in the world and

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climate change will likely introduce significant variations in water availability in the Mediterranean region, where aridity or droughts periods could originate conflicts among water uses. Water reuse is a possible solution to face water scarcity in view of preserving high-quality water that can be used for human consumption. EU is defining minimum requirements for water reuse, with the general objective to alleviate water scarcity across the EU, in the context of adaptation to climate change, notably by increasing the uptake of water reuse (European Commission, 2018), in particular for agricultural irrigation. In this context, treated wastewater (TW) might provide a reliable supply. Area under wastewater irrigation has increased in arid developing countries, with Tunisia having the largest experience in wastewater reuse in agriculture dated back to the early ’60 (Haddaoui et al., 2016).

The reuse of municipal or mixed municipal/industrial TWs for irrigation could be an efficient tool of reducing water shortage, but can negatively affect plant growth and productivity, so that a number of concerns regarding environmental and health aspects should be taken into account (Norton-Brandão et al., 2013).

Among chemicals of high environmental concern, still potentially present in TWs, Cr(VI) and organic micropollutants arouse a great attention. Cr(VI) is a hazardous compound for human health, frequently used for chrome plating, dyes and pigments, leather tanning, and wood preserving. Cr(VI) is mobile in the environment and can easily penetrate the cell wall, exerting its noxious influence in the cell itself, being also a source of various cancer diseases [IARC Group 1] (Straif et al., 2009). Also polycyclic aromatic hydrocarbons (PAHs) are a frequently detected class of water pollutants (Bruzzoniti et al., 2010); they are mainly formed by the incomplete/inefficient combustion of organic material (anthropogenic source), by diagenesis and biosynthesis. (Boehm, 1964). It is well known that PAHs are recalcitrant and that they are mutagenic/carcinogenic pollutants. Hence there is serious concern about their presence in the environment, especially for their tendency for bioaccumulation in food chains (Boga et al., 2018; Yan et al., 2004), particularly due to their lipophilicity (Balducci, 2008). Similar behaviour can be pointed out for polychlorinated biphenyls (PCBs). PCBs were manufactured and used in industry as heat transfer fluids, hydraulic lubricants, dielectric fluids for transformers and capacitors, plasticizers, pesticide extenders and were detected in the environment since 1966 (Jensen, 1972). This class of pollutants is divided into dioxin-like (polychlorinated non-ortho and mono-ortho biphenyls) and non-dioxin-like PCBs. The higher toxicity of dioxin-like PCB compounds is ascribed to their role in the activation of the Ah receptor (AhR), responsible for gene expression (Giesy and Kannan, 1998).

Several recent studies showed that PAHs/PCBs are still found in TWs even after tertiary refinement processes such as filtration by activated carbons (Dimpe and Nomngongo, 2016, Petrie et al., 2015). Cr(VI) can still be detected in wastewater effluents of textile districts. (Fibbi et al., 2012). If PAHs and PCBs are present in waters as a result of natural and anthropogenic processes which produce both point source and diffuse emissions, Cr(VI) is the result of point source emission mainly through textile waters. Based on the aforementioned considerations, the aim of this research was to investigate possible transfer effects along an agricultural production chain under irrigation with municipal and mixed textile TWs. *Fragaria x ananassa, Camarosa* cultivar was chosen as model plant, since it accounts for about 60% of the world's production and it adapts greatly to wide climate and growth conditions. A comprehensive monitoring approach was followed, considering the residual contamination of PAHs, PCBs, and Cr(VI) in: (i) TWs used for irrigation; (ii) soils where strawberry cultivars grown up and (iii) the strawberry crop.

For this purpose, suitable extraction protocols and analytical methods were appositively developed. To the best of our knowledge, the use of industrial or mixed municipal/industrial TWs for crop irrigation or plant nursery is under-investigated (Gori and Caretti, 2008, Hashem et al., 2013, Lin et al., 2000, Sou et al., 2013, Vergine et al., 2017). In fact, in no case, studies about the fate and the accumulation of pollutants in the agricultural and food chain are as comprehensive as the one presented in this manuscript, since only a partial uptake evaluation (i.e. water-soil or water-crop transfer) is presented. Moreover, organic and inorganic pollutants fate was also not considered simultaneously (Amin et al., 2013; Arora et al., 2008; Khan et al., 2008; Kipopoulou et al., 1999; Song et al., 2006).

2. Material and methods

2.1. Treated waters and sampling campaign

Five types of TWs were used to irrigate strawberry plants. TW1: mixed urban/industrial wastewater treated by primary settling, biological oxidation, secondary settling, clariflocculation, ozonization. TW2: TW1+ clariflocculation, sand filtration, activated carbon, disinfection with hypochlorite. TW3: TW1+ clariflocculation, sand filtration, dilution with river water, disinfection with hypochlorite. TW4: as TW1 with sewage incoming composed by mixed urban/industrial, aerated septic tank wastewaters and by landfill leachate pre-filtered with a membrane biological reactor. FW: drinking water (control).

TWs were provided by the wastewater treatment plant GIDA (Prato) every fifteen days and stored in dark tanks in the irrigation site. Samples were withdrawn at the 1st and 8th day from the filling of the tanks, stored at -10°C until analysis. Physicochemical, chemical and microbiological (data not reported) characteristics of TWs and FW were monitored within the irrigation period (2017) which is labelled as: A, May; B, June; C and D, July; E, August; F, September; G, October. Analyses were performed in triplicate.

2.2. Experimental cultivation plant

The experimental site (Fig. 1), implemented at the scientific campus of the University of Florence, consists of five rows, composed by a water tank for each TW studied and seven pots (each one containing 10 strawberry plants).

Certified plantlets of strawberry (*Fragaria x ananassa*, cultivar "*Camarosa*"), purchased from Vivai Fratelli Zanzi (Ferrara, Italy) were transferred in 80-liters pots (ten plantlets per pot) filled with commercially available top-soil for fruit and vegetable nursery. A porous, expanded commercial perlite (AGRILIT 3) with a grain size of 2 -6 mm, specifically developed to be used as a growing medium and/or soil improver, was added to each strawberry pot. Plantlets were irrigated with four TWs, and FW as control. Pots were covered with a plastic tunnel in order to protect the plants from animals and to avoid the direct contact with rain water, which interfere with the experimentation. Pots were regularly irrigated from May to October 2017. Contamination of soils was evaluated before (April 2017) and after irrigation (November 2017). The post-irrigation substrate was collected in a depth range of 0-15 cm; for each TW and pot, three different substrate cores were sampled. For each TW irrigation line, the collected substrate portions were mixed, homogenized, freeze-dried and stored at -20 °C until analysis.

After the harvest, strawberries were washed, dried and frozen at -4°C, until analysis.

2.3. Reagents and solutions

The complete list of 13 PAHs and 14 PCBs analysed in this study is reported in Table 1. PAHs standards were from Sigma Aldrich-Merck (Darmstadt, Germany) and contain the priority compounds listed by EPA. PCBs were from LGC Standards (Milan, Italy), and were chosen according to the results of the main environmental monitoring campaigns carried out in Italy. The PCB congeners chosen represent chlorine (from mono to epta) substitution classes and include dioxin-like compounds (marked with an asterisk in Table 1).

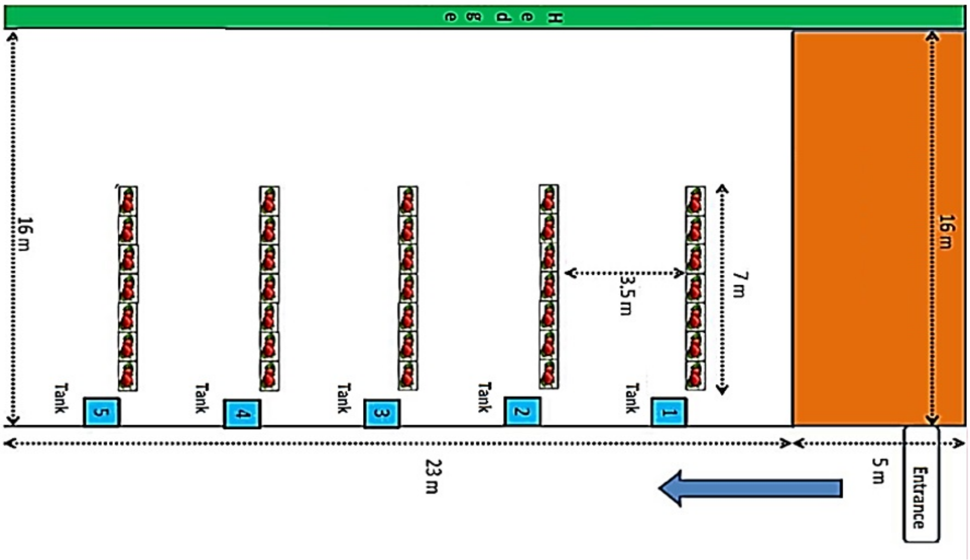


Fig. 1. The experimental site for strawberry cultivation

Table 1. Target PAHs and PCBs studied in waters, soils and strawberries, together with labelled compounds used as internal standards (IS) and surrogates

PAH ANALYTE	***PCB CONGENER***	***SURROGATES AND IS***
Acenaphthylene (AcPY)	*3,3'-dichlorobiphenyl (PCB 11)*	*13BaA*
Fluorene (Flu)	*4,4'-dichlorobiphenyl (PCB 15)*	*13Chr*
Phenantrene (Phe)	*2,4,4'-trichlorobiphenyl (PCB 28)*	*13BbFl*
Anthracene (Ant)	*2,2',5,5'-tetrachlorobiphenyl (PCB 52)*	*13BkFl*
Pyrene (Pyr)	*3,4,4',5-tetrachlorobiphenyl (PCB 81*)*	*13BaP*
Benzo[a]anthracene (BaA)	*2,2',4,5,5'-pentachlorobiphenyl (PCB 101)*	13Ind
Chrysene (Chr)	*2,3',4,4',5-pentachlorobiphenyl (PCB 118*)*	13DBA
Benzo[b]fluoranthene (BbFL)	*2',3,4,4',5-pentachlorobiphenyl (PCB 123*)*	13BP
Benzo[k]fluoranthene (BkFL)	*2,2',3,4,4',5-hexachlorobiphenyl (PCB 138)*	^{13}C PCB 28
Benzo[a]pyrene (BaP)	*2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153)*	^{13}C PCB 52
Indeno[1,2,3-cd]pyrene (Ind)	*2,3',4,4',5,5'-hexachlorobiphenyl (PCB 167*)*	^{13}C PCB 118
Dibenzo[a,h]anthracene (DBA)	*3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169)*	^{13}C PCB 153
Benzo[ghi]perylene (BP)	*2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180)*	^{13}C PCB 180
	2,3,3',4,4',5,5'-heptachlorobiphenyl (PCB 189)	

(*): dioxin like PCBs

Labelled isotope compounds from both categories, (Wellington Laboratories, Ontario, Canada), were used as internal standards and surrogates in order to obtain calibration curves and extraction recoveries, respectively. From chromium analysis, Cr(VI) standard solution was prepared from K_2CrO_4 (Alfa Aesar, Haverhill, USA). Reagent grade dichloromethane, 2-propanol, cyclohexane, acetone, Na_3PO_4 were from Sigma Aldrich-Merck (Darmstadt, Germany). 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.

2.4. Instrumentation

For PAHs and PCBs analysis, a gas chromatographic-mass spectrometric (GC-MS) method was optimized, moving from both EPA 8275A procedure (EPA, 1996) and Zhang et al. results (Zhang et al., 2007). A (5%-Phenyl)-methylpolysiloxane column (HP 5ms, 30 m x 0.25 mm x 25 µm, Agilent) was used. Analysis was performed in Single Ion Monitoring (SIM) mode, selecting for each analyte its proper m/z ratio (m/z ratio available upon request). 2 µL of each sample were injected using 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, 5°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 total run time for the complete separation of PAHs and PCBs is 52 min. The determination of Cr(VI) was performed by ion chromatography (IC) with post-column derivatization and spectrophotometric detection, as developed by our research group (Bruzzoniti et al., 2017).

2.5. Extraction Procedures

Due to the comprehensive aim of the presented study, several matrices were analyzed (water, soil, strawberries). For each matrix, extraction procedures were appositively optimized, as summarized hereafter.

Water samples. PAHs and PCBs were extracted from TWs and FW by solid-phase extraction (SPE) on a polymeric reversed-phase cartridge (STRATA XL, Phenomenex, Torrance, USA), as schematized in Fig. 2, and injected for GC-MS analysis. Cr(VI) was analysed by direct injection IC of filtered samples (nylon filters, 0.45 µm).

Soil samples. PAHs and PCBs were extracted by microwave assisted extraction (MAE). The procedure, developed for PCB extraction (Bruzzoniti et al., 2012), was here tested for the extraction of PAHs as well. Briefly, 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. After a temperature ramp (0-10 min up to 130°C, 10-15 min T=130°C, 15-25 min decrease to 60°C) in microwave oven, the vessel was centrifuged at 3850 rpm for 5 min, acetone was evaporated by heating at 60°C and the solution made up at 5 mL with cyclohexane. Finally, 2 mL of H_2SO_4 were added, as a clean-up step, and 1 mL of supernatant was withdrawn and injected for GC/MS analysis.

Cr(VI) was extracted using a Na_3PO_4 solution (Bruzzoniti et al., 2017). In detail, two aliquots of the same soil were extracted at the same time. The first aliquot (0.5 g) was extracted with 50 mL Na_3PO_4 (10 minutes, 100°C), filtered with a 0.45-µm nylon syringe filter and injected in IC system. To evaluate the extraction yield, the second aliquot (0.5g) was spiked with Cr(VI) to obtain 1 µg/L in the final extract and then extracted as previously described.

Strawberry samples. PAHs and PCBs were extracted using the QuEChERS approach (Bruzzoniti et al., 2014, De Carlo et al., 2015). Briefly, 5 g of strawberries were put in a vial containing 10 mL dichloromethane, 400 mg $MgSO_4$ and 1 g NaCl. The tube was vigorously shaken and centrifuged at 1507 xg for 5 minutes. The supernatant was then transferred for clean-up in a new vial containing 50 mg Primary and Secondary Amine (PSA) sorbent and 150 mg $MgSO_4$. Again, the tube was shaken and centrifuged (7871 xg, 10 minutes). 1 mL of the supernatant was directly analyzed by GC-MS.

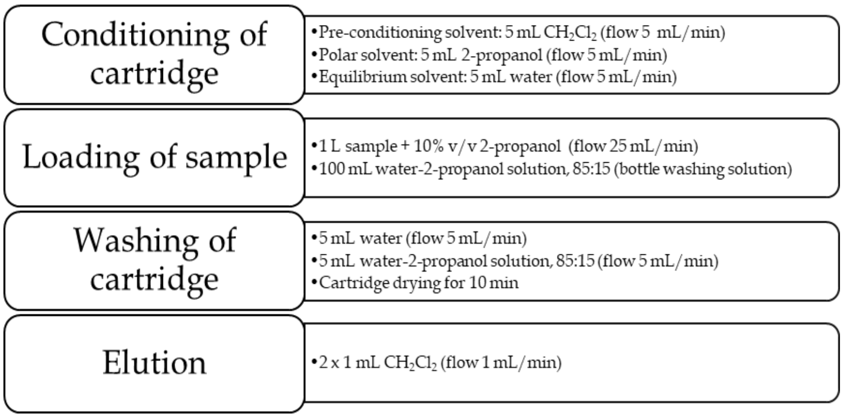


Fig. 2. Protocol for the SPE extraction of PAHs and PCBs

On the opposite, the determination of Cr(VI) on strawberry was not successfully achieved by transferring the soil extraction procedure to crops, due to a residual colour interference of the matrix. Hence, the determination was performed measuring total Cr by means of an acid MAE digestion (HNO_3/H_2O_2 mixture) followed by ICP-MS detection.

3. Results and discussion

3.1. Performances of analytical methods

To verify that the methods developed fulfil limits imposed by regulations (where present), extraction yields, methods detection (MDL) and quantitation limits (MQL) were tested.

For PAHs and PCBs extraction recoveries for each matrix were in the following ranges, waters: 60% (13BP)- 99% (13PCB52); soils: 45% (13BP) – 95% (13PCB52); strawberries: 62% (13Chr) – 102% (13PCB118). For Cr(VI), recoveries were 85% (waters) and 30% (soils).

For PAHs and PCBs, MQLs for each matrix were in the following ranges, waters: 1.3 (Phe)- 11.0 ng/L (PCB169); soils: 3 (PCB180) - 10 μg/Kg (BbFl); strawberries: 1.0 (Phe) - 10.9 μg/Kg (PCB169). For Cr(VI), MQLs were 0.15 μg/L (waters) and 0.018 mg/kg (soils). These performances fully satisfy limits fixed by Italian or European regulations on maximum admitted concentration of pollutants in i) treated waters to be reused for irrigation (D. Lgs 185/2003); ii) private and commercial soils (D.Lgs 152/2006), since limits are not present for soils intended for agricultural aims; iii) fruit crops (CE Regulation 1881, 2006). These limits will be fully discussed in the subsequent sections.

3.2. Chemical characterization of TWs

PAHs and PCBs. Data for PAHs found in TWs and FWs are summarized in Table 2.

Table 2. Concentration (ng/L) and standard deviations in brackets of PAHs in TWs and FW, for each sampling period (see Materials and Methods section). Average data for the whole campaign is also shown

	AcPy	*Flu*	*Phe*	*Ant*	*Pyr*	*Chr*	*BaA*
FWA	1.42 (0.01)	12.54 (4.6)	66.23 (25)	10.73 (2.7)	2.91 (0.96)	1.30 (0.14)	0.79 (0.04)
FWB	3.47 (0.02)	50.68 (35)	33.9 (0.08)	6.53 (1.8)	2.00 (2.0)	25.01 (28)	10.8 (8.4)
FWC	16.34 (2.5)	38.90 (16)	44.3 (20.6)	19.99 (4.8)	12.87 (4.3)	6.49 (2.4)	9.2 (2.6)
FWD	24.49 (3.8)	98.08 (30)	633.7 (180)	45.03 (7.6)	9.31 (1.2)	1.00 (0.12)	0.86 (0.32)
FWE	11.3 (0.04)	38.21 (3)	201.3 (29)	24.18 (4)	11.26 (2.4)	2.54 (0.3)	2.65 (0.3)
FWF	0.49 (0.09)	7.21 (0.30)	4.39 (0.32)	nd	0.50 (0.15)	nd	nd
FWG	6.18 (0.15)	8.0 (1.2)	31.3 (0.89)	nd	1.63 (0.02)	nd	nd
AVERAGE	***20.35 (1.45)***	***36.23 (7.02)***	***145.02 (26.45)***	***15.20 (1.48)***	***5.78 (0.79)***	***5.19 (4.71)***	***3.47 (2.62)***
TW1A	57.79 (5.2)	157.18 (4.9)	858.93 (37)	79.72 (2.6)	39.82 (1.8)	1.75 (0.44)	17.6 (1.8)
TW1B	17.70 (0.5)	131.56 (39)	477.76 (142)	37.57 (3.4)	21.21 (3.4)	3.97 (0.4)	3.45 (0.52)
TW1C	10.79 (5.0)	101.13 (38)	391.89 (180)	17.86 (6.8)	15.20 (6.9)	1.39 (0.4)	1.23 (0.8)
TW1D	21.6 (7.4)	177 (28)	1160 (168)	83.2 (12.8)	15.70 (3.0)	1.29 (0.06)	nd
TW1E	7.12 (0.6)	24.19 (1.6)	103.06 (3.2)	8.48 (0.4)	2.57 (0.4)	nd	nd
TW1F	2.52 (0.19)	13.92 (0.56)	7.19 (1.13)	nd	1.80 (0.5)	nd	nd
TW1G	1.01 (0.03)	15.41 (2.35)	5.73 (0.32)	nd	nd	nd	nd
AVERAGE	***16.93 (1.48)***	***88.53 (8.78)***	***428.85 (40.91)***	***32.17 (2.14)***	***13.48 (1.19)***	***1.2 (0.10)***	***3.18 (2.08)***
TW2A	20.60	76.54	340.64	54.95	32.24	0.74	1.78
TW2B	1.18	4.97	22.45	7.39	5.0	1.83	4.1
TW2C	37.94 (4.1)	173.26 (14.8)	739.01 (94)	46.10 (6.7)	40.58 (3.0)	1.71 (0.3)	1.36 (1.08)
TW2D	31.99 (4.6)	130.85 (24)	477.80 (165)	65.17 (14.2)	15.01 (3.2)	2.34 (0.8)	1.13 (0.26)
TW2E	9.83 (0.5)	27.27 (1)	145.64 (5.6)	17.54 (2.4)	6.09 (1.0)	1.51 (0.3)	1.80 (0.56)
TW2F	1.03 (0.28)	5.04 (0.13)	2.40 (0.92)	nd	nd	nd	nd
TW2G	nd	2.16 (0.46)	1.44 (0.15)	nd	nd	nd	nd
AVERAGE	***13.38 (0.82)***	***59.69 (5.85)***	***246.69 (27.95)***	***27.19 (2.48)***	***14.33 (0.76)***	***1.10 (0.13)***	***1.45 (0.5)***
TW3A	0.76 (0.02)	2.90 (0.24)	13.73 (1.4)	2.10 (0.02)	0.55 (0.1)	nd	nd
TW3B	3.24	20.66	51.38	2.19	0.45	nd	nd
TW3C	24.07 (2.8)	67.11 (8.8)	328.11 (43)	33.62 (1.8)	9.55 (0.3)	nd	nd
TW3D	10.2 (0.48)	34.65 (2.1)	181.27 (21)	13.46 (1.8)	3.36 (0.6)	nd	nd
TW3E	12.95 (2.4)	39.15 (9.8)	201.92 (54)	16.15 (4.6)	4.06 (1.0)	nd	nd
TW3F	1.53 (1.05)	3.89 (2.24)	13.3 (1.23)	nd	1.44 (0.09)	nd	nd
TW3G	nd	3.04 (0.32)	nd	nd	nd	nd	nd
AVERAGE	***7.54 (0.63)***	***24.74 (1.99)***	***112.61 (10.48)***	***9.65 (0.75)***	***2.77 (0.17)***	***nd***	***nd***
TW4A	65.64 (17)	169.36 (40)	926.91 (224)	93.07 (17.7)	45.24	1.29 (0.2)	24.2 (5.2)
TW4B	24.4 (0.24)	92.83 (7.8)	468.21 (24)	35.24 (0.92)	13.50	1.14 (0.1)	nd
TW4C	34.85 (0.6)	106.35 (2.0)	594.65 (7.4)	50.38 (2.1)	18.94	1.41 (0.16)	0.68 (0.14)
TW4D	6.76 (3.6)	23.93 (10.2)	112.84 (51)	69.13 (4.6)	1.40	nd	nd
TW4E	9.83 (0.34)	39.75 (10.2)	251.75 (8.4)	19.19 (0.7)	10.00	0.50 (0.06)	0.22 (0.04)
TW4F	1.05 (0.22)	9.19 (0.07)	nd	nd	nd	nd	nd
TW4G	nd	nd	nd	nd	nd	nd	nd
AVERAGE	***20.36 (2.48)***	***63.92 (6.27)***	***335.86 (35.08)***	***38.14(2.63)***	***12.73***	***0.45 (0.03)***	***3.58 (0.74)***

It is interesting to observe how PAHs with higher aromatic ring number (BbFL, BkFl, BaP, DBA, BP and Ind) were never detected in TWs; moreover, a variability of PAH concentrations among the different months is also present for all the analytes. The absence of several PAHs in TWs demonstrated the efficacy of the WWTPs in the removal of PAHs from wastewaters. In fact, previous studies demonstrated that high molecular weight compounds are better removed during the treatment, and probably transferred to sludges (Yan et al., 2016), in respect to low molecular weight ones, which still remain in the final effluent. Results are also in good agreement with previously obtained studies on TWs (Mezzanotte et al., 2016, Yan et al., 2016), even if concentrations of Flu and Chr here detected are higher than data collected in the above-mentioned works.

Some concentration trends could be highlighted. PAH concentrations in all TWs reached a maximum in the summer months (July and August), for decreasing in September and October. This trend could be explained by summer storms phenomena affecting Mediterranean countries, included Italy, where concentration level of PAHs in wastewater could greatly increase up to 10–100 fold (Blanchard et al., 2001). Another trend is highlighted for AcPY, Flu, Phe and Ant concentrations which are higher in TW1 and TW4.This behaviour can be correlated with the Total Suspended Solid (TSS) parameter that for TW1 and TW4 was 1.5 order of magnitude higher than the other TWs (21.1 mg/L and 19.8 mg/L against an average of 1.2 mg/L). These PAHs with low aromatic ring number are known to slightly interact with particulates (Li et al., 2010): therefore, for high TSS contents in the water, these compounds are expected to be present in higher concentrations (Sangster, 1989).

As previously discussed, admitted concentrations of PAHs in treated waters reused for irrigation are included in the D.M. 185/2003, that, however, regulates only the presence of Benzo[a]pyrene, to a fixed limit of 10 ng/L (Italian Republic, 2003). In this regard, it is important to highlight the absence of benzo[a]pyrene in the four TWs and in the control. To follow a precautionary approach, data in Table 2 were also compared to the more strict EU Directive on waters intended for human consumption (98/83/CE), which regulates not only benzo[a]pyrene (10 ng/L), but also benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene and Indeno[1,2,3-cd]pyrene, whose sum should not exceed 100 ng/L (European Council, 1998). Again, none of the above-mentioned compounds was detected in TWs and FW.

PCB concentrations (data not shown but available upon request) for all the samples ranged from below detection limits (1-2 ng/L) to about 200 ng/L (PCB52) with concentrations in FW lower in respect to TWs. PCBs are not included in the D.M. 185/2003 and, therefore, no comparison with maximum admitted concentrations could be discussed. As for PAHs, the removal of PCBs in WWTPs is strongly dependent on their sorptive behaviours, affected by their octanol-water partition coefficients. However, contrary to what evidenced for PAHs, no seasonal trend could be observed. It is important to underline that most PCB presence is expressed by PCB11 and PCB52; in particular, the predominance of the PCB11 species (which however is not a dioxin-like PCB) is in good agreement with data on WWTPs effluents, (Balasubramani et al., 2014, Yao et al., 2014). Its presence can be explained with the wastewater treatment processes, as a result of dechlorinating of heavier PCBs into lighter PCBs (Balasubramani et al., 2014). Furthermore, the presence of PCB11 can be ascribed also to the peculiarity of the influent (domestic/textile waters). In fact, PCB11 is known to be produced in the manufacture of diarylide yellow pigments, used in textile industries (Grossman, 2013). Pigments are a point source of other dioxin-like congeners, such PCB167, thus justifying its detection even if sporadic and at trace levels, in the October sampling.

Chromium(VI). Hexavalent chromium concentrations found in irrigating waters are summarized in Fig. 3. Samples withdrawn in September and October (F and G) are not presented in figure, due to fail of storage conditions during transportation to our laboratory. The colour of some TW samples interfered in Cr(VI) determination by IC at concentrations lower than 1 µg/L, hence for these samples, 1 µg/L was assigned as a precautionary value. The presence of Cr(VI) in the TWs effluents is not surprising, since it is well known how traditional removal treatments could be affected by incomplete removal of heavy metals (Barakat, 2011), especially in textile districts. It should be remarked, however, that for all the tested samples, Cr(VI) concentration is under the limits set by Italian decree 185/2003 on the reuse of treated waters for irrigation (5 µg/L).

TW3 sample exhibits a higher Cr(VI) contamination than TW1, although it derives from a further refinement of TW1 (see Material and Methods section). This behaviour could be ascribed to the treatment process that relies on dilution of TW1 with an adjacent river. Indeed, it is not unusual to detect hexavalent chromium in natural waters basins (Hemmatkhah et al., 2009, Vasilatos et al., 2008).

3.3. Soil chemical characterization

PAHs and PCBs. The presence of PAHs, PCBs and Cr(VI) was evidenced in TW samples. Therefore, the analysis of soils irrigated with TWs is fully justified to evaluate whether a contamination took place or not from waters.

Soils used for the cultivation of strawberry plants were analysed before the irrigation period started (to obtain a “zero level” characterization) and at its end (Fig. 4).

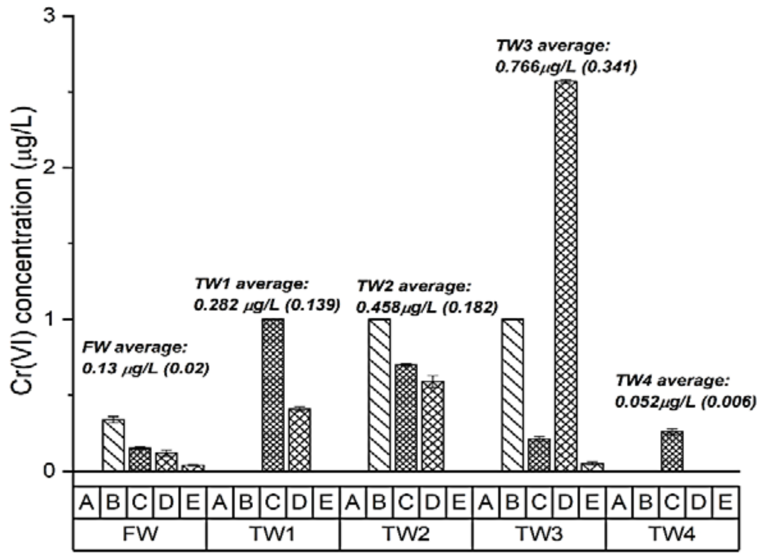
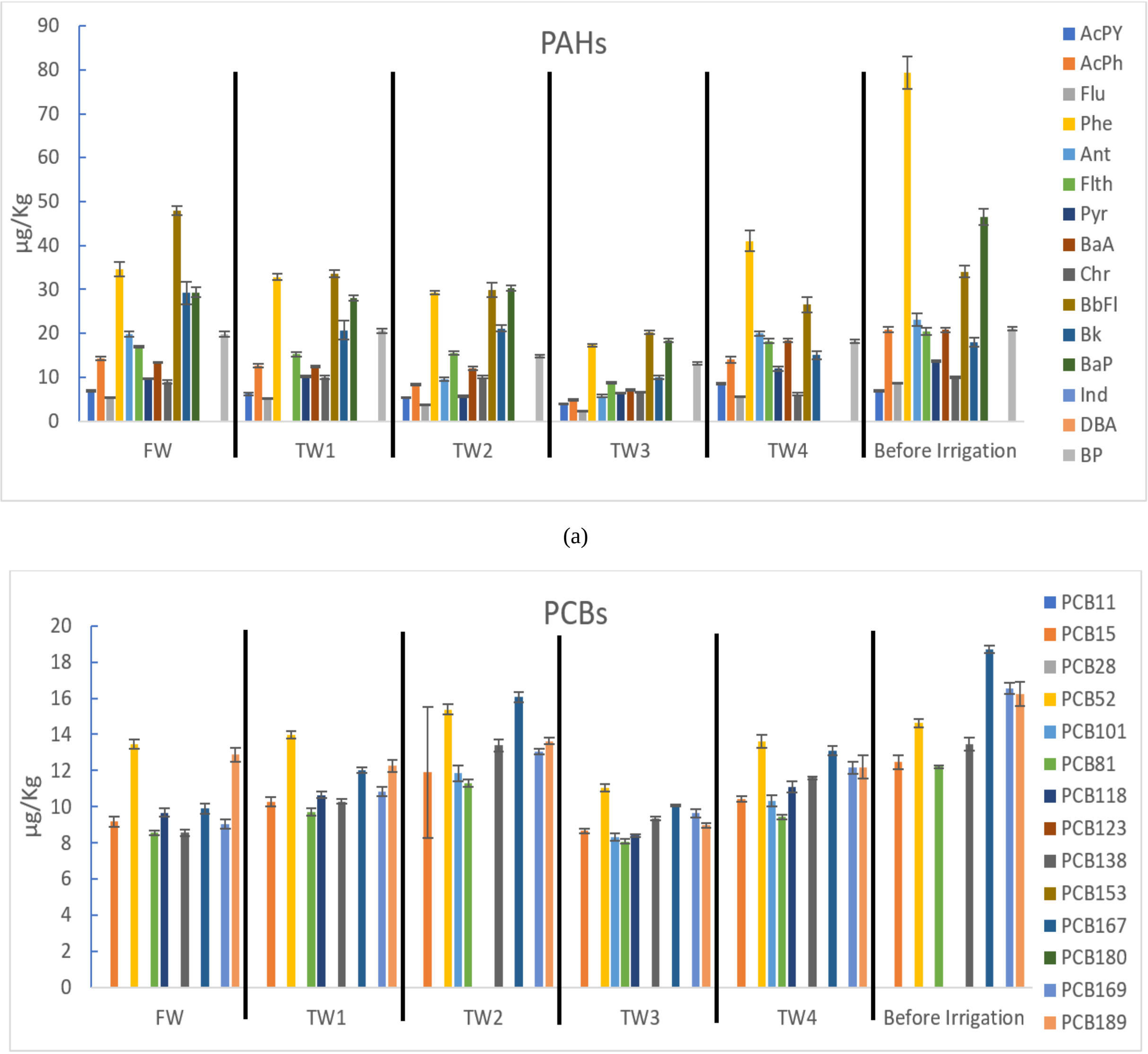


Fig. 3. Concentration of Cr(VI) in TWs and FW, for each sampling period (A-E); average value for the whole campaign is also indicated, together with standard deviation in brackets



(a)

(b)

Fig. 4. Concentration (μg/kg) of (a) PAHs and (b) PCBs in soils, before and after the irrigation period

Both for PAHs and for PCBs, concentration levels before and after the irrigation period are very similar. The total amount of PAHs in the pre-irrigated soils is 320 µg/Kg (data range: 6.85 µg/Kg AcPY - 80 µg/Kg Phe). Toxic equivalency (TEQ) value was also evaluated as follows (Eq. 1):

$$TEQ = \sum (TEF_{PAHi} \cdot [PAH_i]) \tag{1}$$

where: *[PAHi]* is the concentration of the i-th PAH congener and TEF_{PAHi} is the toxicity equivalent factor of the i-PAH congener (Jimenez et al., 2014). TEQ value is 54.5 µg/Kg. Based on literature data, the sum of PAHs matches values observed for agricultural soil (Zheng et al., 2014); however, TEQ value suggests that soil used for strawberry cultivation exhibits toxicity properties more similar to urban soils, due to the high contribution of BaP in the TEQ calculation (85%) (Soukarieh et al., 2018).

The total amount of PAHs in soils irrigated with TWs ranged from 125 µg/Kg (TW3) to 320 µg/Kg (FW). These concentrations are more than one order of magnitude lower than the limit imposed by Italian regulations (D.Lgs 152/2006), which is set at 10 mg/kg for the sum of specific PAHs (BaA, BaP, BbFl, BkFl, Chr, DBA, together with Benzo(g,h,i,)terylene, Dibenzo(a,e)-, Dibenzo(a,l)-, Dibenzo(a,i)pyrene, not investigated in this work) for soils in public-green, private and residential areas, and at 100 mg/kg for soils intended for commercial aims.

According to the data obtained, a slightly higher contamination of soils by PAHs before irrigation was observed in respect to the end of the experimental trial. This behaviour could be explained by the contribution of perlite (present in the soil substrate) to partially retain PAHs, as demonstrated by other authors (Bjorklund and Li, 2015, Turan et al., 2009). Additionally, contribution of microbial PAH degradation in soil should not be excluded (Cardak et al., 2007). It should be remarked that, even if possible retention of PAHs by the strawberry plant could occur, the evaluation of the uptake by different parts of the plant (excluded the fruit) is out of the scope of this work.

Also, for PCBs, concentration in soils before and after irrigation indicated the absence of impact from TWs. Their presence in the original soil substrate can derive from atmospheric deposition (Glüge et al., 2016). PCB concentrations reaching about 60 µg/kg were observed in rural soils (Meggo and Schnoor, 2013), confirming the ubiquitous presence of these compounds. Despite the fact that, to the best of our knowledge, PCBs in soil are not regulated at a EU level, in Italy concentration limits for PCBs are set by the D.Lgs 152/2006, at 0.06 mg/kg and 5 mg/kg for soil intended for private or commercial aims, respectively.

Cr(VI). As done for organic compounds, the presence of hexavalent chromium was monitored both before and after irrigation. Results (Fig. 5), showed that only a marginal increment in Cr(VI) concentration occurred after irrigation for all samples. The highest increment observed for soil irrigated with TW3 is in agreement with the higher Cr(VI) content observed for this treated water (see previous paragraph).

Data obtained fully satisfied the limits declared by the Italian decree D.Lgs 152/2006 (2 mg/kg) and are in good agreement with previous studies on urban soils (Jankiewicz and Ptaszynski, 2005)**.**

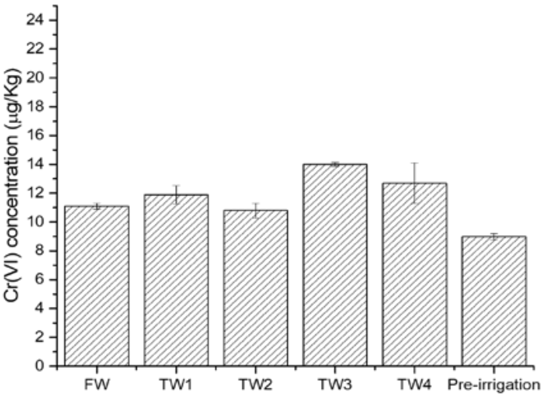


Fig. 5. Concentration of Cr(VI) in soils before and after irrigation by TWs and FW

3.4. Strawberries

3.4.1. PAHs and PCBs

Strawberry crop grown up under irrigation by TWs represents the final part of the agricultural chain. Several studies demonstrate that PAHs and PCBs (Lovett et al., 1997, Paris et al., 2018), as well as metal ions (Khan et al., 2015) could be detected in fruit and vegetables depending on the contamination of crop area.

To what concern PAHs and PCBs, none of the above-mentioned molecules was detected at a quantifiable level, apart from BaA in strawberries irrigated with TW1. In fact, BaA was observed at 1.14 µg/Kg (MQL 1.17 µg/Kg); this value agrees with PAHs found in fruit and vegetable cultivated in rural areas (Camargo and Toledo, 2003). EU regulation on food contamination (CE) N. 1881/2006 (European Council, 2006) does not set a limit for PAHs and PCBs in fruits. BaP, which however was not detected in strawberries, is the only PAH for which a limit ranging from 2 to 10 µg/kg is set according to the type of food considered (infant food excluded).

For strawberry irrigated with TW1, TEQ is 0.114 µg/kg. This value is comparable to those estimated for other Italian food products, such as cheese, bread and eggs (Lodovici et al., 1995). If a precautionary intake of strawberry is fixed to 100 g/day for an adult person, the Bench Mark Dose Lower Confidence Limit of BaP (100 µg BaP/kg bw/day, concentration producing a predetermined change in the response rate of an adverse effect, established as carcinogenicity in mice orally dosed with a mixture of representative genotoxic and carcinogenic PAH present in food) is fully respected (FSANZ, 2005).

3.4.2. Chromium

The IC analysis of Cr(VI) in strawberries suffered for residual colour interference. In addition, even after the direct spike of Cr(VI) in the fruit at concentrations as high as 500 µg/L, the method was not able to quantify Cr(VI), presumably due to the high content of anti-oxidant species in strawberries (Doumett et al., 2011) which reduced Cr(VI) to Cr(III). Hence, total chromium determination was performed (Fig. 6).

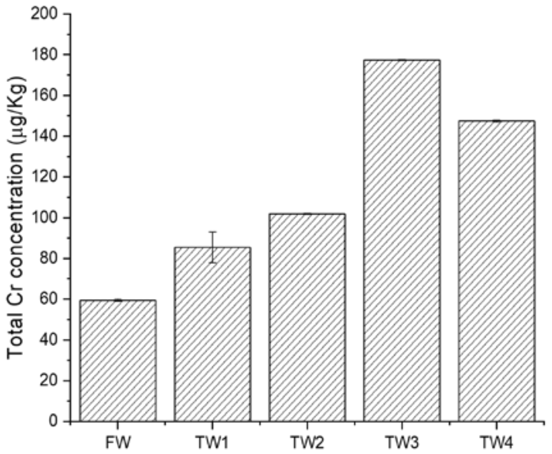


Fig. 6. Total Cr determination in strawberries irrigated with TWs and FW

Differently from what observed for soils, strawberries irrigated with TWs have a higher Cr content, in respect to the control, thus suggesting the transfer of the metal during irrigation. The highest concentration of Cr in strawberries grown up under irrigation with TW3 are in agreement with the highest concentration of Cr(VI) detected in the TW3 sample.

Cr concentrations are from two to five times higher than those detected in other strawberries, as presented in the EFSA Scientific Opinion of 2014, EFSA Panel on Contaminants in the Food Chain (CONTAM, 2014). Nevertheless, assuming the same premises previously presented for PAHs (100 g/die consumption for an adult over 50 kg bw), Cr content in crop irrigated with TW3 results in a daily intake of 0.4 µg/kg bw, which summed with the estimated daily intake expressed as Cr(III) (0.6-5.9 µg/kg bw) does not exceed the TDI of 300 µg/kg b.w-EFSA Panel on Contaminants in the Food Chain (CONTAM, 2014)

4. Conclusions

For the first time, the possible chemical contamination by organic (PAHs, PCBs) and inorganic compounds (chromium) in the agricultural chain of strawberries irrigated with different kind of reclaimed waters was assessed. All the treatments considered are capable of guaranteeing the levels set for PAHs and Cr(VI) by Italian legislation for wastewater reuse for irrigation.

Although PAHs, PCBs were detected in waters, their presence was not observed in strawberries, except for BaA at amounts comparable with quantitation limit of the method. Irrigation with these TWs does not impact the quality of the soil that exhibits similar PAHs and PCBs content before and after irrigation. On the contrary, chromium content in one of the strawberry crops (which however does not represent a risk for consumer) presumably derives from the original residual contamination of treated water.

The results observed within this study seems in agreement with a negligible impact of lipophilic compounds and a possible transfer of inorganic water-soluble compounds (metals) in fruits of high-water content such as strawberries.

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