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Impact of effluents from wastewater treatments reused for irrigation: Strawberry as case study

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(Article begins on next page)

33 on maximum admitted concentration of pollutants in treated water intended for irrigation, in soils and
34 crops.

35 Even if selected PAHs and PCBs were detected in wastewaters (highest concentrations
36 observed for phenanthrene, 429 $\mu\text{g/L}$, and PCB52, 110 $\mu\text{g/L}$) their presence was not observed in soils
37 and in strawberries above the MQLs. On the contrary, chromium content in strawberries and soils
38 irrigated with TWs suggested a possible transfer of the metal during irrigation, which however does
39 not represent a hazardous situation for consumers since calculated daily intake does not exceed the
40 Tolerable Daily Intake of 300 $\mu\text{g/kg b.w.}$

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42 *Keywords:* food chain; reuse impact; strawberry; treated wastewater

43

44 **1. Introduction**

45 Agriculture is characterized by a high-water demand; one third of the water use in Europe is
46 addressed to the agricultural sector, most of it for crop irrigation (European Environment Agency,
47 2012). Italy is one of the Countries with the highest water footprint in Europe, 25% above the
48 European Union average, and, at a global level, 66% above the world average (Sartori et al., 2014).
49 It is worth to be mentioned that agriculture in Italy accounts for about 85-89% of the water footprint
50 of consumption.

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

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

65 Among chemicals of high environmental concern, still potentially present in TWs, Cr(VI) and
66 organic micropollutants arouse a great attention. Cr(VI) is a hazardous compound for human health,

67 frequently used for chrome plating, dyes and pigments, leather tanning, and wood preserving. Cr(VI)
68 is mobile in the environment and can easily penetrate the cell wall, exerting its noxious influence in
69 the cell itself, being also a source of various cancer diseases [IARC Group 1] (Straif et al., 2009).
70 Also polycyclic aromatic hydrocarbons (PAHs) are a frequently detected class of water pollutants
71 (Bruzzone et al., 2010); they are mainly formed by the incomplete/inefficient combustion of organic
72 material (anthropogenic source), by diagenesis and biosynthesis. (Boehm, 1964). It is well known
73 that PAHs are recalcitrant and that they are mutagenic/carcinogenic pollutants. Hence there is serious
74 concern about their presence in the environment, especially for their tendency for bioaccumulation in
75 food chains (Yan et al., 2004), particularly due to their lipophilicity (Balducci, 2008). Similar
76 behaviour can be pointed out for polychlorinated biphenyls (PCBs). PCBs were manufactured and
77 used in industry as heat transfer fluids, hydraulic lubricants, dielectric fluids for transformers and
78 capacitors, plasticizers, pesticide extenders and were detected in the environment since 1966 (Jensen,
79 1972). This class of pollutants is divided into dioxin-like (polychlorinated non-ortho and mono-ortho
80 biphenyls) and non-dioxin-like PCBs. The higher toxicity of dioxin-like PCB compounds is ascribed
81 to their role in the activation of the Ah receptor (AhR), responsible for gene expression (Giesy and
82 Kannan, 1998).

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

95 For this purpose, suitable extraction protocols and analytical methods were appositively
96 developed. To the best of our knowledge, the use of industrial or mixed municipal/industrial TWs for
97 crop irrigation or plant nursery is under-investigated (Gori and Caretti, 2008, Hashem et al., 2013,
98 Lin et al., 2000, Sou et al., 2013, Vergine et al., 2017). In fact, in no case, studies about the fate and
99 the accumulation of pollutants in the agricultural and food chain are as comprehensive as the one
100 presented in this manuscript, since only a partial uptake evaluation (i.e. water-soil or water-crop

101 transfer) is presented. Moreover, organic and inorganic pollutants fate was also not considered
102 simultaneously (Amin et al., 2013, Arora et al., 2008, Khan et al., 2008, Kipopoulou et al., 1999,
103 Song et al., 2006).

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105 **2. Materials and methods**

106 *2.1 Treated waters and sampling campaign*

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

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

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121 *2.2 Experimental cultivation plant*

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

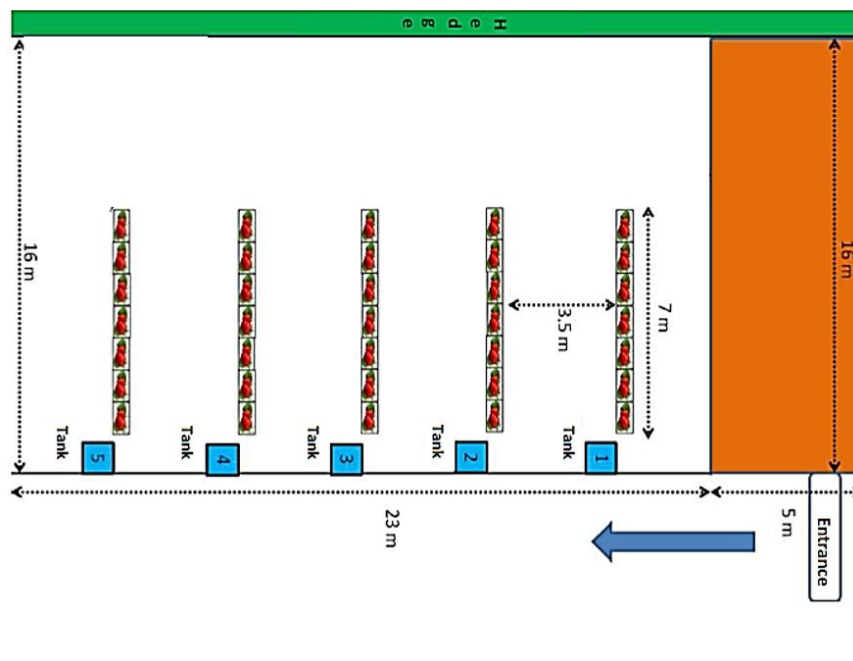


Fig. 1. The experimental site for strawberry cultivation

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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

148 compounds (marked with an asterisk in Table 1). Labelled isotope compounds from both categories,
 149 (Wellington Laboratories, Ontario, Canada), were used as internal standards and surrogates in order
 150 to obtain calibration curves and extraction recoveries, respectively. From chromium analysis, Cr(VI)
 151 standard solution was prepared from K₂CrO₄ (Alfa Aesar, Haverhill, USA). Reagent grade
 152 dichloromethane, 2-propanol, cyclohexane, acetone, Na₃PO₄ were from Sigma Aldrich-Merck
 153 (Darmstadt, Germany). High-purity water (18.2 MΩ cm resistivity at 25 °C), produced by an Elix-
 154 Milli Q Academic system (Millipore-Merck, Vimodrone, MI, Italy) was used.

156 **Table 1.** Target PAHs and PCBs studied in waters, soils and strawberries, together with labelled compounds
 157 used as internal standards (IS) and surrogates
 158

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

159 (*): dioxin like PCBs

160 2.4. Instrumentation

161 For PAHs and PCBs analysis, a gas chromatographic-mass spectrometric (GC-MS) method
 162 was optimized, moving from both EPA 8275A procedure (Environmental Protection Agency, 1996)
 163 and Zhang et al. results (Zhang et al., 2007). A (5%-Phenyl)-methylpolysiloxane column (HP 5ms,
 164 30 m x 0.25 mm x 25 μm, Agilent) was used. Analysis was performed in Single Ion Monitoring (SIM)
 165 mode, selecting for each analyte its proper m/z ratio (m/z ratio available upon request). 2 μL of each
 166 sample were injected using the Pulsed Splitless mode (pressure at 40 psi for 2.5 minutes). The oven
 167 ramp was set as follows: starting temperature: 40°C, hold for 2 min; ramp to 176 °C, 12 °C/min rate;
 168 ramp to 196°C, 5 °C/min rate, hold for 3 mins; ramp to 224°C, 12 °C/min rate; ramp to 244 °C,

169 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,
170 5°C/min, hold for 10 min to completely clean and restore the GC column. The total run time for the
171 complete separation of PAHs and PCBs is 52 min.

172 The determination of Cr(VI) was performed by ion chromatography (IC) with post-column
173 derivatization and spectrophotometric detection, as developed by our research group (Bruzzoniti et
174 al., 2017).

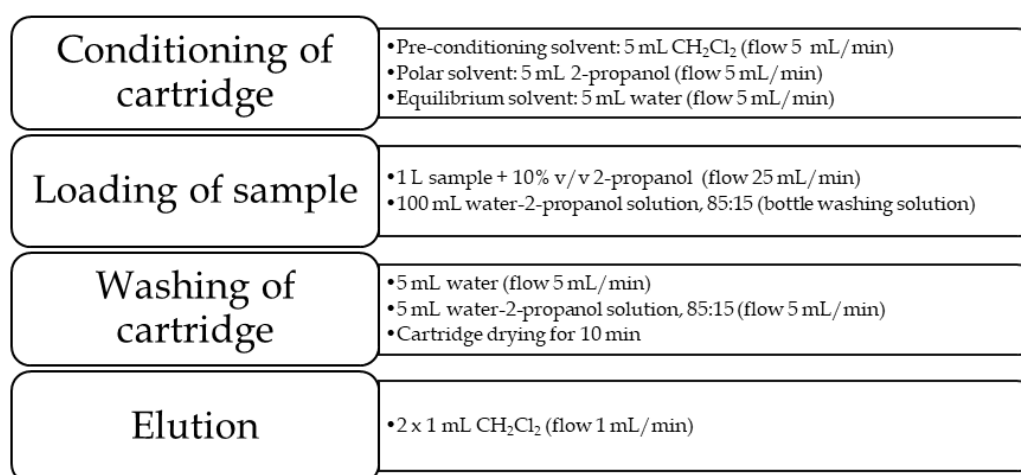
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176 2.5. Extraction Procedures

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

180 **Water samples.** PAHs and PCBs were extracted from TWs and FW by solid-phase extraction
181 (SPE) on a polymeric reversed-phase cartridge (STRATA XL, Phenomenex, Torrance, USA), as
182 schematized in Fig. 2, and injected for GC-MS analysis.

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185

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

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187 Cr(VI) was analysed by direct injection IC of filtered samples (nylon filters, 0.45 µm).

188 **Soil samples.** PAHs and PCBs were extracted by microwave assisted extraction (MAE). The
189 procedure, developed for PCB extraction (Bruzzoniti et al., 2012), was here tested for the extraction
190 of PAHs as well. Briefly, 0.4 g of soil, previously sieved at 2 mm, were put in a disposable Pyrex
191 vessel with 5 ml of a 3:2 acetone-cyclohexane solution. After a temperature ramp (0-10 min up to
192 130°C, 10-15 min T=130°C, 15-25 min decrease to 60°C) in microwave oven, the vessel was
193 centrifuged at 3850 rpm for 5 min, acetone was evaporated by heating at 60°C and the solution made

194 up at 5 mL with cyclohexane. Finally, 2 mL of H₂SO₄ were added, as a clean-up step, and 1 mL of
195 supernatant was withdrawn and injected for GC/MS analysis.

196 Cr(VI) was extracted using a Na₃PO₄ solution (Bruzzoniti et al., 2017). In detail, two aliquots
197 of the same soil were extracted at the same time. The first aliquot (0.5 g) was extracted with 50 mL
198 Na₃PO₄ (10 minutes, 100°C), filtered with a 0.45-µm nylon syringe filter and injected in IC system.
199 To evaluate the extraction yield, the second aliquot (0.5g) was spiked with Cr(VI) to obtain 1 µg/L
200 in the final extract and then extracted as previously described.

201 **Strawberry samples.** PAHs and PCBs were extracted using the QuEChERS approach
202 (Bruzzoniti et al., 2014, De Carlo et al., 2015). Briefly, 5 g of strawberries were put in a vial
203 containing 10 mL dichloromethane, 400 mg MgSO₄ and 1 g NaCl. The tube was vigorously shaken
204 and centrifuged at 1507 xg for 5 minutes. The supernatant was then transferred for clean-up in a new
205 vial containing 50 mg Primary and Secondary Amine (PSA) sorbent and 150 mg MgSO₄. Again, the
206 tube was shaken and centrifuged (7871 xg, 10 minutes). 1 mL of the supernatant was directly analysed
207 by GC-MS.

208 On the opposite, the determination of Cr(VI) on strawberry was not successfully achieved by
209 transferring the soil extraction procedure to crops, due to a residual colour interference of the matrix.
210 Hence, the determination was performed measuring total Cr by means of an acid MAE digestion
211 (HNO₃/H₂O₂ mixture) followed by ICP-MS detection.

212

213 **3. Results and discussion**

214 *3.1 Performances of analytical methods*

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

217 For PAHs and PCBs extraction recoveries for each matrix were in the following ranges,
218 waters: 60% (13BP)- 99% (13PCB52); soils: 45% (13BP) – 95% (13PCB52); strawberries: 62%
219 (13Chr) – 102% (13PCB118). For Cr(VI), recoveries were 85% (waters) and 30% (soils).

220 For PAHs and PCBs, MQLs for each matrix were in the following ranges, waters: 1.3 (Phe)-
221 11.0 ng/L (PCB169); soils: 3 (PCB180) - 10 µg/Kg (BbFl); strawberries: 1.0 (Phe) - 10.9 µg/Kg
222 (PCB169). For Cr(VI), MQLs were 0.15 µg/L (waters) and 0.018 mg/kg (soils). These performances
223 fully satisfy limits fixed by Italian or European regulations on maximum admitted concentration of
224 pollutants in i) treated waters to be reused for irrigation (D. Lgs 185/2003); ii) private and commercial
225 soils (D.Lgs 152/2006), since limits are not present for soils intended for agricultural aims; iii) fruit
226 crops (CE Regulation 1881/2006). These limits will be fully discussed in the subsequent sections.

227

228 3.2 Chemical characterization of TWs

229 **PAHs and PCBs.** Data for PAHs found in TWs and FWs are summarized in Table 3.

230

231 **Table 3.** Concentration (ng/L) and standard deviations in brackets of PAHs in TWs and FW,
 232 for each sampling period (see Materials and Methods section). Average data for the whole
 233 campaign is also shown.

	<i>AcPy</i>	<i>Flu</i>	<i>Phe</i>	<i>Ant</i>	<i>Pyr</i>	<i>Chr</i>	<i>BaA</i>
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)

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

244 Some concentration trends could be highlighted. PAH concentrations in all TWs reached a
245 maximum in the summer months (July and August), for decreasing in September and October. This
246 trend could be explained by summer storms phenomena affecting Mediterranean countries, included
247 Italy, where concentration level of PAHs in wastewater could greatly increase up to 10–100 fold
248 (Blanchard et al., 2001).

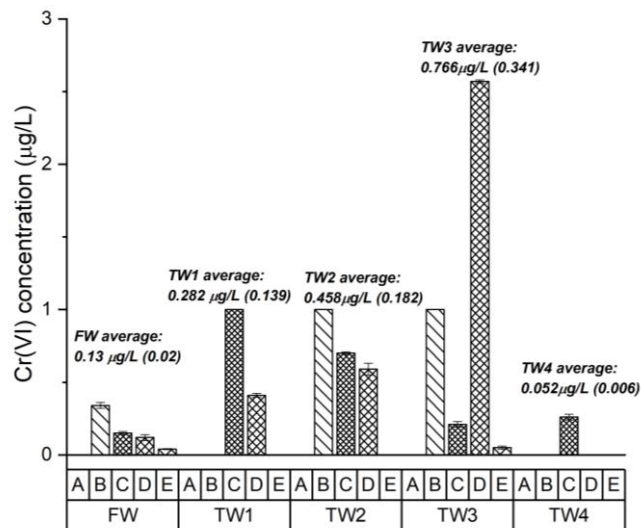
249 Another trend is highlighted for AcPY, Flu, Phe and Ant concentrations which are higher in
250 TW1 and TW4. This behaviour can be correlated with the Total Suspended Solid (TSS) parameter
251 that for TW1 and TW4 was 1.5 order of magnitude higher than the other TWs (21.1 mg/L and 19.8
252 mg/L against an average of 1.2 mg/L). These PAHs with low aromatic ring number are known to
253 slightly interact with particulates (Li et al., 2010, Rodenburg, 2006): therefore, for high TSS contents
254 in the water, these compounds are expected to be present in higher concentrations (Sangster, 1989).

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

264 PCB concentrations (data not shown but available upon request) for all the samples ranged
265 from below detection limits (1-2 ng/L) to about 200 ng/L (PCB52) with concentrations in FW lower
266 in respect to TWs. PCBs are not included in the D.M. 185/2003 and, therefore, no comparison with
267 maximum admitted concentrations could be discussed. As for PAHs, the removal of PCBs in WWTPs

268 is strongly dependent on their sorptive behaviours, affected by their octanol-water partition
 269 coefficients. However, contrary to what evidenced for PAHs, no seasonal trend could be observed. It
 270 is important to underline that most PCB presence is expressed by PCB11 and PCB52; in particular,
 271 the predominance of the PCB11 species (which however is not a dioxin-like PCB) is in good
 272 agreement with data on WWTPs effluents, (Balasubramani et al., 2014, Yao et al., 2014). Its presence
 273 can be explained with the wastewater treatment processes, as a result of dechlorination of heavier
 274 PCBs into lighter PCBs (Balasubramani et al., 2014). Furthermore, the presence of PCB11 can be
 275 ascribed also to the peculiarity of the influent (domestic/textile waters). In fact, PCB11 is known to
 276 be produced in the manufacture of diarylide yellow pigments, used in textile industries (Grossman,
 277 2013). Pigments are a point source of other dioxin-like congeners, such PCB167, thus justifying its
 278 detection even if sporadic and at trace levels, in the October sampling.

279 **Chromium(VI).** Hexavalent chromium concentrations found in irrigating waters are
 280 summarized in Fig. 3.



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282

283 **Fig. 3.** Concentration of Cr(VI) in TWs and FW, for each sampling period (A-E); average
 284 value for the whole campaign is also indicated, together with standard deviation in brackets
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286 Samples withdrawn in September and October (F and G) are not presented in figure, due to
 287 fail of storage conditions during transportation to our laboratory. The colour of some TW samples
 288 interfered in Cr(VI) determination by IC at concentrations lower than 1 µg/L, hence for these samples,
 289 1 µg/L was assigned as a precautionary value.

290 The presence of Cr(VI) in the TWs effluents is not surprising, since it is well known how
 291 traditional removal treatments could be affected by incomplete removal of heavy metals (Barakat,
 292 2011), especially in textile districts. It should be remarked, however, that for all the tested samples,

293 Cr(VI) concentration is under the limits set by Italian decree 185/2003 on the reuse of treated waters
 294 for irrigation (5 µg/L).

295 TW3 sample exhibits a higher Cr(VI) contamination than TW1, although it derives from a
 296 further refinement of TW1 (see Material and Methods section). This behaviour could be ascribed to
 297 the treatment process that relies on dilution of TW1 with an adjacent river. Indeed, it is not unusual
 298 to detect hexavalent chromium in natural waters basins (Hemmatkhah et al., 2009, Vasilatos et al.,
 299 2008).

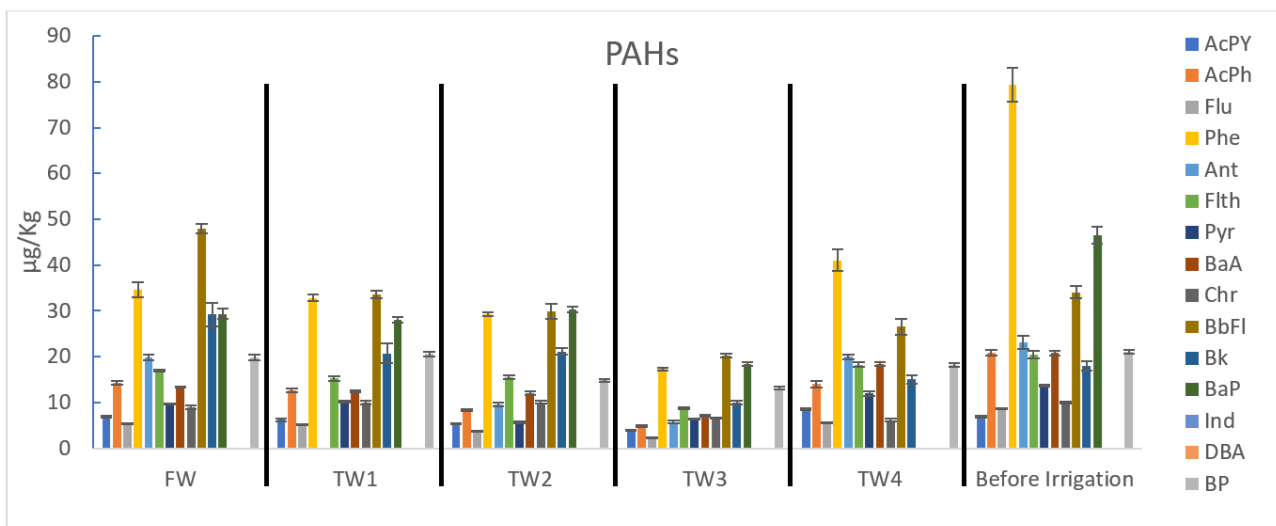
300

301 3.3 Soil chemical characterization

302 **PAHs and PCBs.** The presence of PAHs, PCBs and Cr(VI) was evidenced in TW samples.
 303 Therefore, the analysis of soils irrigated with TWs is fully justified to evaluate whether a
 304 contamination took place or not from waters. Soils used for the cultivation of strawberry plants were
 305 analysed before the irrigation period started (to obtain a “zero level” characterization) and at its end
 306 (Fig. 4).

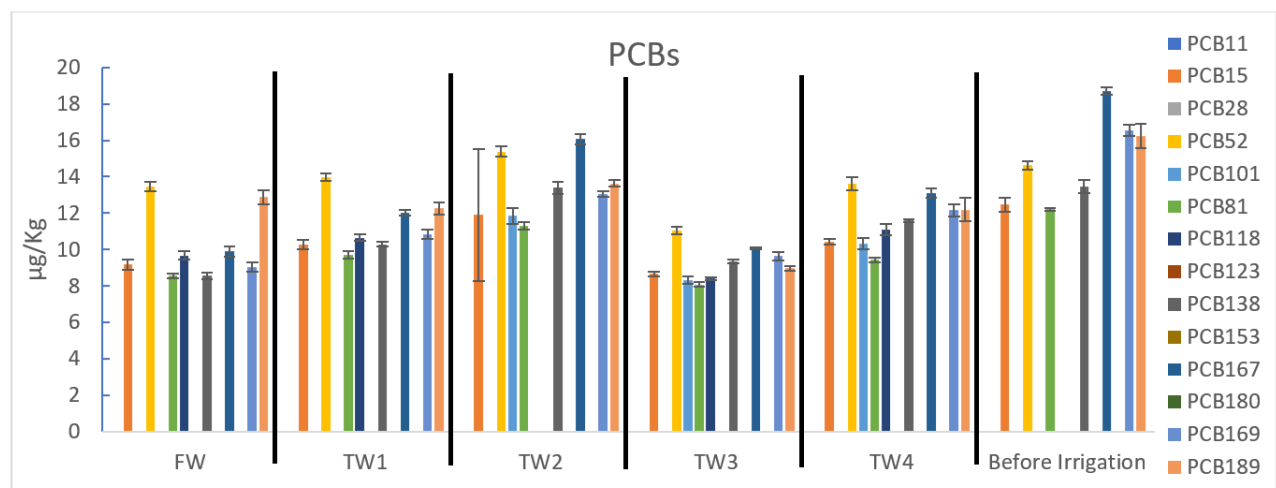
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(b)

Fig. 4. Concentration ($\mu\text{g}/\text{kg}$) 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 $320 \mu\text{g}/\text{Kg}$ (data range: $6.85 \mu\text{g}/\text{Kg}$ AcPY - $80 \mu\text{g}/\text{Kg}$ Phe). Toxic equivalency (TEQ) value was also evaluated as follows (Eq. 1):

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

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 $54.5 \mu\text{g}/\text{Kg}$. 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 $125 \mu\text{g}/\text{Kg}$ (TW3) to $320 \mu\text{g}/\text{Kg}$ (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 $10 \text{ mg}/\text{Kg}$ 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 $100 \text{ mg}/\text{Kg}$ for soils intended for commercial aims.

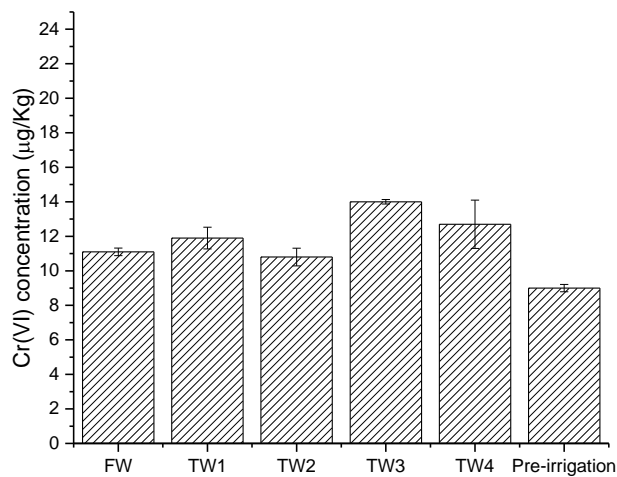
According to the data obtained, a slightly higher contamination of soils by PAHs before irrigation was observed in respect to the end of the experimental trial. This behaviour could be explained by the contribution of perlite (present in the soil substrate) to partially retain PAHs, as demonstrated by 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.). 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 $60 \mu\text{g}/\text{kg}$ 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

344 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
345 commercial aims, respectively.

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

351 Data obtained fully satisfied the limits declared by the Italian decree D.Lgs 152/2006 (2
352 mg/Kg) and are in good agreement with previous studies on urban soils (Jankiewicz and Ptaszynski,
353 2005).



354
355 **Fig. 5.** Concentration of Cr(VI) in soils before and after irrigation by TWs and FW.

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357 *3.4 Strawberries*

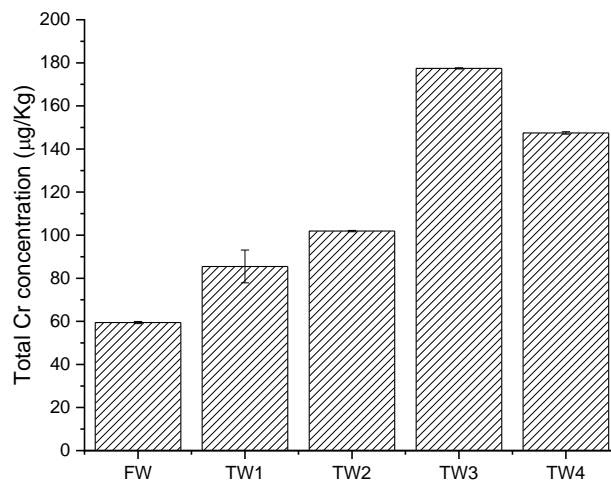
358 **PAHs and PCBs.** Strawberry crop grown up under irrigation by TWs represents the final part
359 of the agricultural chain. Several studies demonstrate that PAHs and PCBs (Lovett et al., 1997, Paris
360 et al., 2018), as well as metal ions (Khan et al., 2015) could be detected in fruit and vegetables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

362 To what concern PAHs and PCBs, none of the above-mentioned molecules was detected at a
363 quantifiable level, apart from BaA in strawberries irrigated with TW1. In fact, BaA was observed at
364 1.14 µg/Kg (MQL 1.17 µg/Kg); this value agrees with PAHs found in fruit and vegetable cultivated
365 in rural areas (Camargo and Toledo, 2003). EU regulation on food contamination (CE) N. 1881/2006
366 (European Council, 2006) does not set a limit for PAHs and PCBs in fruits. BaP, which however was

367 not detected in strawberries, is the only PAH for which a limit ranging from 2 to 10 $\mu\text{g}/\text{kg}$ is set
368 according to the type of food considered (infant food excluded).

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

376 **Chromium.** The IC analysis of Cr(VI) in strawberries suffered for residual colour
377 interference. In addition, even after the direct spike of Cr(VI) in the fruit at concentrations as high as
378 500 $\mu\text{g}/\text{L}$, the method was not able to quantify Cr(VI), presumably due to the high content of anti-
379 oxidant species in strawberries (Doumsett et al., 2011) which reduced Cr(VI) to Cr(III). Hence, total
380 chromium determination was performed (Fig. 6).



381

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

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

388 Cr concentrations are from two to five times higher than those detected in other strawberries,
389 as presented in the EFSA Scientific Opinion of 2014 (EFSA Panel on Contaminants in the Food Chain
390 (CONTAM), 2014). Nevertheless, assuming the same premises previously presented for PAHs (100
391 g/die consumption for an adult over 50 kg bw), Cr content in crop irrigated with TW3 results in a
392 daily intake of 0.4 $\mu\text{g}/\text{kg}$ bw, which summed with the estimated daily intake expressed as Cr(III) (0.6-

393 5.9 µg/kg bw) does not exceed the TDI of 300 µg/kg b.w (EFSA Panel on Contaminants in the Food
394 Chain (CONTAM), 2014).

395

396 **4. Conclusions**

397 For the first time, the possible chemical contamination by organic (PAHs, PCBs) and
398 inorganic compounds (chromium) in the agricultural chain of strawberries irrigated with different
399 kind of reclaimed waters was assessed. All the treatments considered are capable of guaranteeing the
400 levels set for PAHs and Cr(VI) by Italian legislation for wastewater reuse for irrigation. Although
401 PAHs, PCBs were detected in waters, their presence was not observed in strawberries, except for BaA
402 at amounts comparable with quantitation limit of the method. Irrigation with these TWs does not
403 impact the quality of the soil that exhibits similar PAHs and PCBs content before and after irrigation.
404 On the contrary, chromium content in one of the strawberry crops (which however does not represent
405 a risk for consumer) presumably derives from the original residual contamination of treated water.
406 The results observed within this study seems in agreement with a negligible impact of lipophilic
407 compounds and a possible transfer of inorganic water-soluble compounds (metals) in fruits of high-
408 water content such as strawberries.

409

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415

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