Development of analytical methods for the determination of organic micropollutants in rocket and olive.

Water scarcity is a growing issue on a global scale. In the last few decades, this problem has been exacerbated by global warming which has led to the appearance of extreme phenomena, such as droughts. In addition, there is an increase in the demand for freshwater for agriculture, leading to further water resources deployment. To mitigate this problem, the European Union released the 2020/741 Regulation for the safe reuse of treated wastewater in agriculture (Figure 1). However, the reuse of treated wastewater must be performed under strict control because of the possible residual microbiological and chemical contamination that can be transferred to crops. Within the chemical pollutants, polycyclic aromatic hydrocarbons (PAHs), the nitrogen containing analogues (NPAHs) and polychlorinated biphenyls (PCBs) may persist in treated wastewater. PAHs are formed by incomplete organic matter combustion. PAHs are recalcitrant, and some of them mutagenic and carcinogenic; moreover, due to their lipophilicity they tend to bioaccumulate in organisms. PCBs have a similar toxicological behavior; and their presence is due to the use in the past as heat transfer fluids, hydraulic lubricants, dielectric fluids and plasticizers. PCBs are classified as non-dioxin-like and dioxin-like congeners that have higher toxicity. Some nitro-PAHs can be more mutagenic and carcinogenic than their parent PAHs due to the presence of nitro groups (NO_2). On these premises, UNITO was in charge of developing analytical protocols to determine possible residual concentrations of PAHs, PCBs and NPAHs in the fruits (rocket and olive) grown up after irrigation with treated wastewater.

Figure 1 – Pictorial scheme of reuse of treated wastewaters.

➤ ROCKET

Analytical methods were optimized in Rocket bought in a local supermarket, dried for 48 h at 60°C in oven, and ground with a mortar.

Before the chromatographic analysis of micropollutants, QuEChERS procedure was applied (Figure 2) to evaluate the effect of the main parameters (extraction solvent and dispersive-solid phases (*d*-SPE) in the purification steps) on the extraction yields.

Five extraction solvents of different polarity, and some of their mixtures, were tested: dichloromethane, cyclohexane, acetone, acetonitrile and hexane.

For the purification step, five *d*-SPE were evaluated: PSA, Z-Sep, Florisil, C18 and GCB (Graphitized Carbon Black). Qualitative and quantitative analysis was performed to check the removal of the co-extracted interferents and determine extraction recoveries of the target compounds (PAHs, N-PAHs, PCBs).
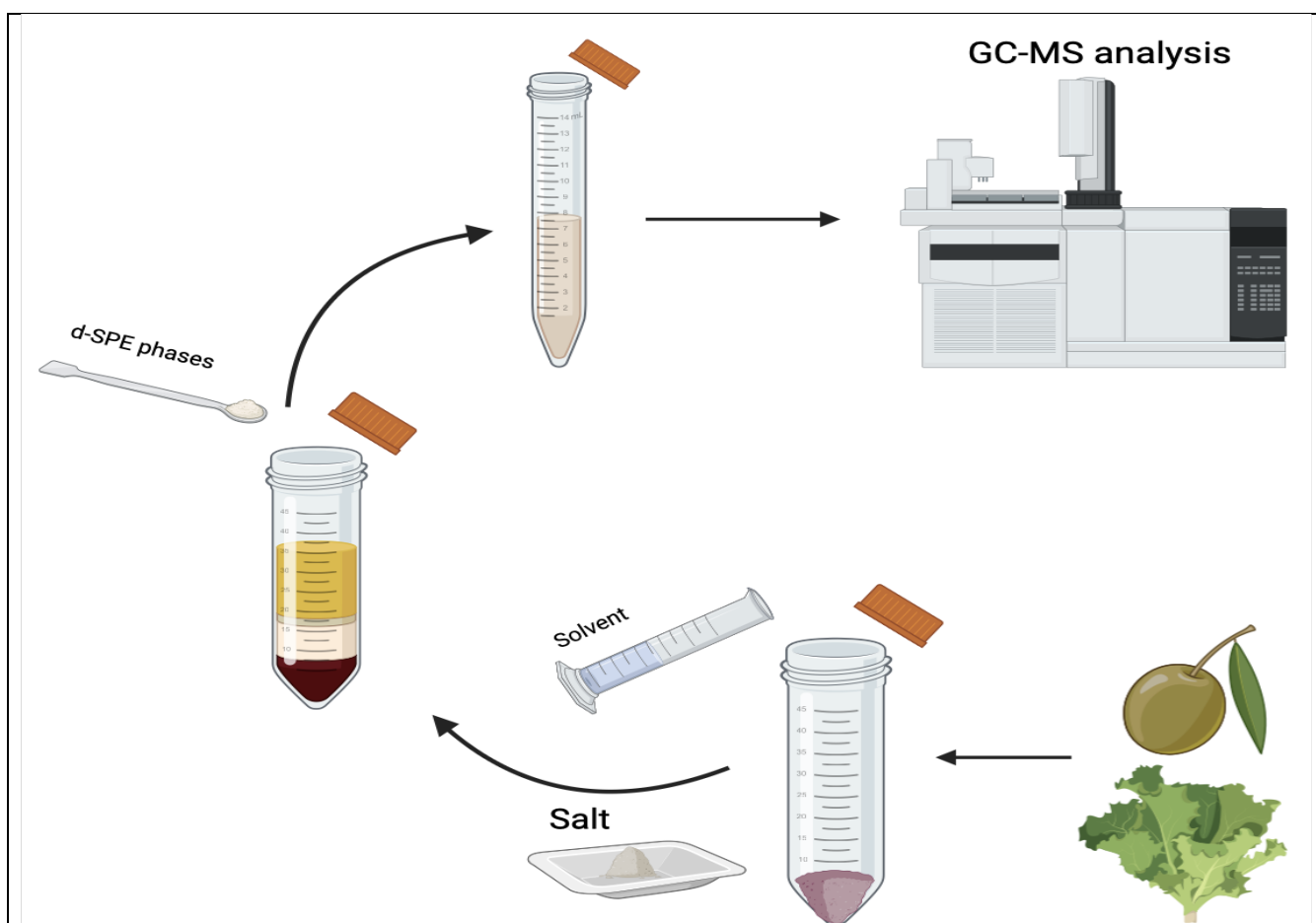

Figure 2 – Pictorial scheme of the optimized protocols

The best extraction recoveries (Figure 3) were obtained using cyclohexane as extraction solvent followed by purification with PSA and Z-Sep. Lastly, a purification step with H_2SO_4 was used. The extraction yields are satisfactory (**Figure 3**) with recovery percentages higher than 63.0%. For PAHs, the minimum recovery is 63.0% of BP-d_{12} and the maximum recovery is 139.1% of BaA-d_{12} and Chr-d_{12}. Whereas for PCBs the minimum recovery is 91.9% of $^{13}C_{12}$-PCB52 and the maximum is 102.4% of $^{13}C_{12}$-PCB28.

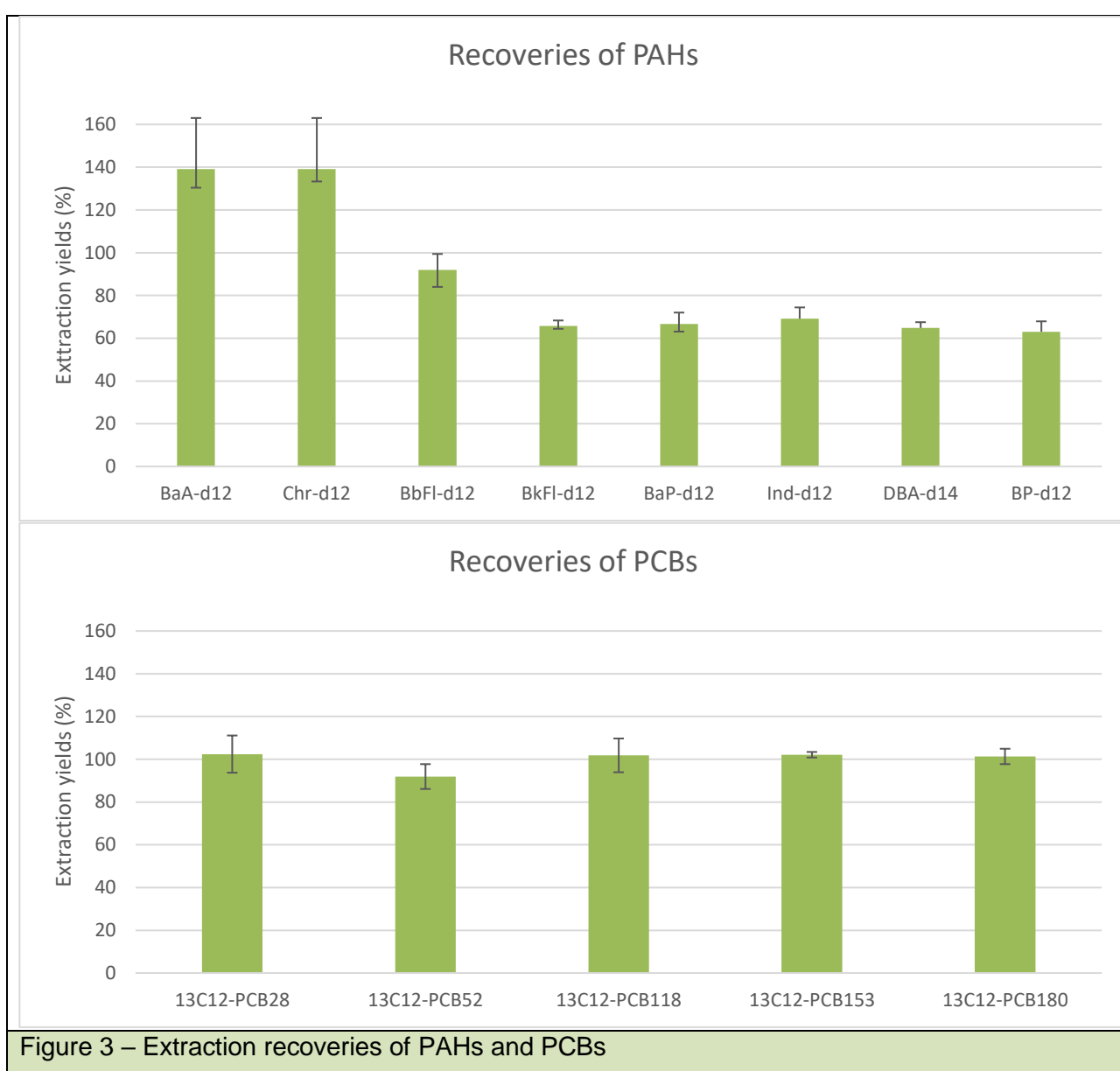Analysis of the extract was performed by GC-MS.

Figure 3 – Extraction recoveries of PAHs and PCBs

➢ OLIVE

The analytical method was developed directly on fruits (cultivar "Frantoio") grown under irrigation with reclaimed wastewater. Olives were freeze-dried and stored at -20°C immersed in liquid nitrogen. Before analysis, samples were defrosted, dried in the oven at 60°C, and homogenized in a mortar.

Determination of PAHs and PCBs in olives is challenging because of the lipophilic nature of olives. In fact, the extraction of these pollutants leads to the co-extraction of matrix components which can interfere with the overall GC-MS analysis.

First, we evaluated apparent extraction recovery by olive samples spiked with surrogate solutions of analytes, comparing the GC-MS response with a solution of the same surrogates prepared in pure solvent.

QuEChERS protocols were applied to optimize, also in this case (Figure 2), the choice of extraction solvent and d-SPE sorbent for the clean-up phase. The tested protocols are summarized in Table 1 below.

QuEChERS trial	Conditions	
	Extraction	Clean up
#1		100 mg PSA
#2		300 mg PSA
#3	10 mL dichloromethane:water [a]	300 mg PSA [b], 2 mL 96% H_2SO_4
#4		300 mg PSA, 50 mg C18
#5		100 mg PSA, 50 mg Florisil
#6		100 mg PSA
#7	10 mL cyclohexane:water [a]	100 mg Z-Sep, 50 mg Florisil
#8		100 mg Z-Sep [b], 2 mL 96% H_2SO_4
#9		100 mg PSA, 50 mg Florisil
#10	10 mL acetonitrile:water [a]	100 mg PSA, 50 mg Florisil , 100 mg PSA, 50 mg Florisil
#11		100 mg Z-Sep, 50 mg Florisil , 100 mg Z-Sep, 50 mg Florisil

Table 1 - Solvents mixture and d-SPE sorbent tested.

The experimental conditions #11 are considered optimal for the extraction of micropollutants: extraction in acetonitrile:water 1:1, and double d-SPE purification with Z-Sep and Florisil. Among the three solvents tested, acetonitrile is the most polar one, thus ensuring the extraction of compounds with wide range of polarity. The presence of salts promotes the separation of the two phases (acetonitrile and water), and the most polar and medium polar compounds can be partitioned in water, obtaining an acetonitrile phase rich in non-polar compounds. The presence of non-polar compounds in acetonitrile is expected to be lower than in cyclohexane due to the difference in the polarity index. PSA and Z-Sep were tested as d-SPE phases coupled with Florisil, which appeared selective from removing color from the extract. Apparent recoveries with these experimental conditions are included within 94% and 122% for PAHs and within 83% and 100% for PCBs.

The method underwent additional validation, including assessment of inter and intraday precision, limits of detection, and matrix effect. The matrix effect describes how the sample matrix can impact the accuracy and precision of the analytical method. The validated method was utilized to analyze, with GC-MS, PAHs and PCBs in olive crops irrigated with treated and freshwater. Results showed that only Fluorene and Phenantrene among the tested PAHs were detected in olives. Fluorene was also found in olives irrigated with freshwater, suggesting atmospheric deposition as an additional contamination source. Investigated PCBs were not detected. PAH concentrations detected were within the contamination levels of commercially available olive oils from various European countries, and about 50 times lower than olive oil consumed in India. These residual concentration levels comply with the maximum permitted levels set by EU regulations (CE 1881/2006), as none of the regulated compounds, including benzo[a]pyrene, were detected.

The importance of fulfilling safety regulation requirements