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Abstract

European and national waste directives prioritize recycling of wastes, as well as material and energy recovery from wastes themselves. Bio-waste fraction can be converted into new resources whose quality is strictly dependent upon that of waste feedstock. Methods to evaluate the contamination from organic micropollutants in bio-waste are rarely investigated. The aim of this work was to develop an innovative analytical method for the extraction and quantification of 16 polycyclic aromatic hydrocarbons (PAHs) and 14 polychlorinated biphenyls (PCBs, including dioxin-like compounds) in bio-waste.

Through a full-factorial experimental design, a microwave-assisted extraction technique was optimized to extract the thirty targeted micropollutants, studying the effect of cyclohexane and dichloromethane as extraction solvents with or without acetone, and of extraction temperature. Purification of the extract was obtained by a silica-based solid-phase extraction cartridge, followed by a sulfuric acid treatment. The analysis was carried out by gas chromatography coupled with mass spectrometry. The optimized method, validated directly in the bio-waste matrix fortified with isotopically marked surrogates, is characterized by good extraction recoveries, included within 47 and 106% (relative standard deviations < 10%), by satisfactory intra-day (<1.1 %) and inter-day (<9.3%) precision, and by low matrix effect (<17%), despite the complexity of the matrix.

The optimized procedure, applied to the analysis of PAHs and PCBs in a bio-waste sample collected from a local anaerobic digestion and composting plant, showed a total PAHs content of 562 µg/kg. As regards PCBs, the dioxin-like congener PCB 118 was the only compound quantified (25±6 µg kg^{-1}).

Keywords: MAE, GC-MS, Polycyclic Aromatic Hydrocarbons, Polychlorinated biphenyls, bio-waste, municipal solid waste.

1 Introduction

Disposal of waste has become one of the hot topics in current European policies and its management represents one of the greatest challenges faced by society [1].

In 2018, the total waste generated in the EU-27 by all economic activities and households amounted to 2.3 billion tonnes with municipal solid waste (MSW) accounting for about 8.2 % of the total [2]. MSW is also the less homogeneous waste due to its composition, origin and relationship with consumption patterns and citizen attitude to the differentiated waste collection [3,4].

The characterization of the amount and of the composition of municipal waste is a fundamental step necessary for adequate decision making within the integrated solid waste management system [5,6].

Bio-waste represents a significant share, up to 50 % w/w of the whole MSW [7,8]. According to the European Directive on Waste 2008/98/EC [9], bio-waste includes *"biodegradable garden and park waste, food and kitchen waste from households, restaurants, caterers and retail premises and comparable waste from food processing plants*". Hence, this kind of waste is very heterogeneous and it changes in composition and source according to geographical provenience and seasonality [10]. However, as a general feature, bio-waste is characterized by high water content (around 70-80 %) [11] and by a high putrescibility which both determine a rapid decomposition.

If not properly managed, bio-waste is one of the most environmentally problematic waste in terms of both the large volume generated and of the potential pollutant emissions and leachate generation, when deposited in landfills [7].

In Europe great efforts to recycle organic waste have been undertaken. The Landfill Directive 1999/31/EC [12] and the Waste Framework Directive 2008/98/EC [9] require European Union Member States to adopt a hierarchy of actions for a correct management of MSW including biodegradable municipal waste to limit the amount disposed in landfills. Diverting bio-waste fraction from the MSW stream has many environmental benefits, such as the reduction of greenhouse gas

emissions [8,13], the decrease of the amount of landfill leachate [14,15], as well as the production of a good quality compost and of biogas enhancing material and energy recovery, in a circular economy perspective [16].

Compost can be produced via aerobic treatment of the bio-waste or via aerobic treatment of the digestate derived from the anaerobic digestion of the bio-waste itself [17].

Across the European Union, about 125 million tons of bio-waste arise annually, of which currently about 40% is effectively recycled into high-quality compost and digestate [18]. Although compost can improve chemical, physical and biological soil properties [19], its use in agriculture may present environmental and healthy criticalities related to the quality of the input feedstocks, which, if not adequate, can produce contamination of the soil propagating along the food chain [20–22], as well as polluting emissions into the atmosphere [23]. Hence, it becomes important to implement knowledge on the actual level of contamination of bio-waste destined for composting [24].

Bio-waste may contain a wide range of toxic organic contaminants [25], such as pesticides, priority pollutants like polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCBs) [24], and inorganic compounds, i.e. heavy metals [26]. These contaminants may be originally present in the bio-waste fraction, enter via aerial deposition, via accidental (e.g. improper separation of input materials), or deliberate input (e.g. pesticide application to fruits and vegetables) or via release by the increasingly ubiquitous plastic debris which are recognized to adsorb contaminants and to transfer contamination [27,28].

Despite the possible contamination of bio-waste, the poor degradation efficiency of biological methods for organic micropollutants like PAHs and PCBs [26], the proved increase of PCBs concentration after composting [29], there is a lack of legislative requirements for the quality of the bio-waste fraction to be accepted as input feedstock for both aerobic and anaerobic conversion processes. To the best of our knowledge the only regulation in force within this topic is represented by the regional Resolution of the Veneto (Italy) Region 235/2009 [30] which sets the limits for PAHs

(<6 mg/Kg DM) and PCBs (<0.8 mg/Kg DM) in bio-wastes to be employed for the production of quality compost, as well as for metals, and polychlorinated dibenzodioxins/furans (not included in this study). In addition, no target detection limits for analytical methods for the determination of PAHs and PCBs in the same matrix were fixed by European or National regulations.

The determination of organic micropollutants in bio-waste has not been thoroughly investigated yet and no standardized analytical method has acquired international acceptance for the analysis of organic micropollutants in composting feedstocks. On the contrary, some literature for the determination of organic micropollutants in compost is available, where the most commonly techniques used for extracting hydrophobic contaminants from compost are based on Soxhlet extraction [31], liquid-liquid extraction assisted with sonication [32], which are characterized by the consumption of relatively high volumes of extracting solvents, and accelerated solvent extraction (ASE) [33]; under a variety of experimental conditions based on the use of organic solvents such as e.g. hexane, toluene, dichloromethane, acetone or mixtures of them.

In view of possible control actions on the quality of the input bio-waste feedstock before the thermal conversion process, already in force for the acceptance of sludges deriving from wastewater treatment plants, the aim of this study has been the development of a green protocol for the extraction and analysis of organic priority micropollutants present in the bio-waste fraction derived from the MSW. PAHs and PCBs have been chosen as model organic micropollutants since they are representative of punctual and diffuse contamination and because of the risk to be propagated along the food chain via agricultural practices [34,35]. As previously mentioned, these classes of compounds are included in Resolution 235/2009 of the Regional Council of Veneto [30] and therefore have been considered of interest for this study.

For this purpose, an extraction method based on microwave assisted extraction, which ensures many advantages over conventional extraction techniques like low solvent consumption, high extraction rate and process automation, was developed through a chemometric experimental design

for the extraction of PAHs and PCBs from bio-waste. The optimization of a purification step of the extract allowed the analysis of the target pollutants by gas chromatographic analysis at μg kg^{-1} levels. To the best of our knowledge, this study represents the first attempt of the determination of organic micropollutants in bio-waste samples.

2 Materials and Methods

2.1 Reagents

All reagents used throughout this work were of analytical grade. Dichloromethane and cyclohexane were from VWR Chemicals (Radnor, PA, USA). Acetone and sulfuric acid (95-97 %) were from Honeywell Research Chemicals (Charlotte, NC, USA). High-purity water (18.2 MΩ cm resistivity at 25 °C), produced by an Elix-Milli Q Academic system (Millipore-Billerica, MA, USA) was used.

The 16 PAHs, i.e., naphthalene (Naph), acenaphthylene (AcPY), acenaphthene (AcPh), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flth), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbFl), benzo[k]fluoranthene (BkFl), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (Ind), dibenz[a,h]anthracene (DBA) and benzo[ghi]perylene (BP), studied were the compounds listed by the United States Environmental Protection Agency (US-EPA) and were purchased from Wellington Laboratories (Ontario, Canada). The 14 PCBs studied were purchased from Chemical Research 2000 (Rome, Italy).

They were chosen according to the results of the main monitoring campaigns and included 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), and 2,3,3′,4,4′,5,5′-heptachlorobiphenyl (PCB 189). Furthermore, the following dioxin-like PCBs were included 3,4,4′,5-

tetrachlorobiphenyl (PCB 81), 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118), 2′,3,4,4′, 5-pentachlorobiphenyl (PCB 123), and 2,3′,4,4′,5, 5′-hexachlorobiphenyl (PCB 167).

Isotope labelled compounds for PAHs (5 mg L^{-1}) and for PCBs (2 mg L^{-1}), both purchased from Wellington Laboratories, were used as internal standards and surrogates in order to obtain calibration curves and to calculate extraction recoveries. The deuterated PAH surrogate solution included the following compounds: benzo[a]anthracene-d_{12} (BaA-d_{12}), chrisene-d_{12} (Chr-d_{12}), benzo[b]fluoranthene-d_{12} (BbFl-d_{12}), benzo[k]fluoranthene-d_{12} (BkFl-d_{12}), benzo[a]pyrene-d_{12} (BaP-d_{12}), indeno[1,2,3-cd]pyrene-d_{12} (Ind-d_{12}), dibenzoanthracene-d_{14} (DBA-d_{14}), benzoperylene-d_{12} (BP-d_{12}). The ^{13}C-PCB surrogate solutions included the following congeners: $^{13}C_{12}$-PCB28, $^{13}C_{12}$-PCB52, $^{13}C_{12}$-PCB118, $^{13}C_{12}$-PCB153, and $^{13}C_{12}$-PCB180.

2.2 Extraction of PAHs and PCBs from bio-waste and extract purification

PAHs and PCBs were extracted through a Microwave-Assisted Extraction (MAE), using a Discover® SP-D microwave (CEM, Cologno al Serio, BG, Italy). The instrument is operated with a software that allows for the setting of Ramp Time (the default time defined for the instrument to reach the temperature or pressure), temperature, pressure, microwave power and sample stirring speed.

After extraction, the extract was treated with Sep-Pak Silica cartridges (690 mg of adsorbent, particles size 55-105 μm, Waters SpA, Sesto San Giovanni, MI, Italy). For centrifugation, a Jouan B4 Centrifuge (ThermoFisher, Waltham, MA USA) was used.

2.3 Chromatographic analysis

PAHs and PCBs extracted from bio-waste were analysed by gas chromatography coupled with mass spectrometry (GC-MS) using an Agilent 6980 series gas chromatograph and an Agilent 5973 Network MS detector controlled by Agilent ChemStation software. The gas chromatograph was provided with an autosampler of the Agilent 7683 Series.

The GC column was a (5%-Phenyl)-methylpolysiloxane column (DB-5 ms, 30 m × 0.25 mm × 25 μm; Agilent). Helium was used as gas carrier (1 mL/min). MS detection was performed in Single

Ion Monitoring (SIM) mode at proper m/z ratio (m/z ratio available upon request). 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: 80 °C, hold for 2 min; ramp to 176 °C, 12°C min^{-1} rate; ramp to 196 °C, 5 °C min^{-1} rate, hold for 3 mins; ramp to 224 °C, 12°C min^{-1} rate; ramp to 244 °C, 12 °C min^{-1} rate, hold for 3 min; ramp to 270 °C, 7 °C min^{-1} rate, hold for 3 min; final ramp to 300 °C, 5 °C min^{-1}, hold for 10 min to completely clean and restore the GC column. The complete separation of the 16 PAHs and 14 PCBs was obtained within 49 min.

2.4 Bio-waste sample and pre-treatment

Bio-waste was provided by a local plant (Piedmont, Italy) treating bio-waste, derived from MSW, by anaerobic digestion and composting. The biowaste sampled before entering the anaerobic digestor is characterized by 9.5 % fixed solids, 74 % volatile solids, pH 6.2 and chemical oxygen demand of 100.6 mg kg^{-1}.

The sampled bio-waste was put in Pyrex containers (pre-cleaned with acetone and high-purity water) hermetically closed and transported to the laboratory where they were treated by autoclave (120 °C) to eliminate the presence of any pathogens. An aliquot of about 300 g (pH ~ 8) was dried in oven for 48 hours at 60 °C, and subsequently grounded and homogenized. Residues of plastic bags and other visible fragments, such as small pieces of aluminum sheets, were manually removed by tweezers. Afterward, the sample was stored in a glass holder and refrigerated until the analysis.

2.5 Analytical protocol

2.5.1 Extraction of PAHs and PCBs

Approximately 0.5 g of sample was accurately weighed into a 50 ml disposable Pyrex vessel with 11 mL 10:1 cyclohexane-acetone solution and PAHs and PCBs were extracted at the following microwave digestor conditions (350 psi, 300 W): 0 - 10 min up to 110°C. Afterward, the solution was centrifuged at 1970 × g for 5 min. The extract was then cleaned-up by solid phase extraction (SPE) method using a Sep-Pack Silica cartridge, previously conditioned with 10 mL of cyclohexane, to

remove organic compounds possibly co-extracted by MAE. An aliquot of 5 mL of eluted extract was cleaned up with 2 mL of concentrated sulfuric acid for 30 min. This treatment is intended to remove co-extracted polar compounds. Also, since sulfuric acid is a dehydrating agent, the treatment removes residual water as well.

2.5.2 Analysis of PAHs and PCBs

Before injection in GC-MS, 1 mL extract was spiked with the internal standard solution of PAHs and PCBs to achieve a final concentration of 5 μg L^{-1}. The whole analytical protocol developed for the extraction and the analysis of PAHs and PCBs is depicted in **Fig. 1**.

To evaluate the extraction recoveries of PAHs and PCBs, before MAE extraction, the sample was spiked with surrogate solutions of PAHs and PCBs to achieve a final concentration of 1 μg L^{-1} (C_S).

After extraction, the concentrations were calculated by using an external standard calibration curve.

The extraction yield (E %) was calculated according to the equation (1).

$$E\ \% = \frac{Ce}{Cs} * 100 \quad (1)$$

where C_e is the calculated concentration of the surrogate after extraction expressed as μg L^{-1}.

Matrix effect (ME) was evaluated by comparing the concentration corresponding to the surrogates spiked into the bio-waste, extracted and purified according to the optimized procedure, with the concentration corresponding to the surrogates spiked in the extraction mixture and subjected to the same extraction and clean-up procedure.

Method detection limits (MDL) and method quantitation limits (MQL) for the 30 target compounds were calculated by means of the response error and the slope of the calibration curve, according to the expression MDL= 3.3 S_y/m, and MQL= 10 S_y/m, where S_y = response error; m = slope of the calibration [36,37].

The accuracy was determined on replicated extractions (n=3) of 1 μg L^{-1} surrogates spiked on bio-waste.

The intra-day precision was determined using replicate (n=10) determinations for bio-waste spiked with 1 μg L^{-1} surrogate standards, on a single day of analysis. Inter-day precision was calculated using replicate (n=30) determinations of the same spiked biowaste on three separate days.

3 Results and discussion

The analytical protocol was developed by optimizing the microwave assisted extraction procedure and the purification step prior to GC-MS analysis, in order to achieve high extraction recoveries with the least interference from the matrix components.

3.1 Optimization of MAE parameters through experimental design

The parameters affecting the performance of the MAE approach are basically the type and the amount of extraction solvent and temperature. The tests for the optimization of MAE conditions were performed on the bio-waste fraction as received spiked with known amounts (1 μg L^{-1}) of surrogate standards for PAHs and PCBs. After MAE extraction, the extracts were treated with H_2SO_4 before GC-MS analysis.

As regards the extraction solvents, we studied the efficacy of two organic solvents, i.e. cyclohexane and dichloromethane. Cyclohexane was selected since it proved efficient in the extraction of several Aroclor mixture from soil, as elsewhere described [38]. Dichloromethane was chosen because of its suitability to extract several organic micropollutants including PAHs [39,40] and because of its full compatibility with the subsequent instrumental GC-MS analysis, without requiring any solvent exchange prior to GC analysis [40]. The presence of a solvent polar solvent, i.e. acetone, was also tested in order to investigate any effect on extraction recovery.

The effect of temperature was also studied. This parameter can affect the overall performance of the MAE method, since its increase can provide higher extraction yields for certain analytes but in such cases also for matrix components.

A chemometric approach based on the two level, full factorial (2^3) design was used to optimize MAE conditions, varying the previously described factors: (i) extraction temperature (X_1), (ii) extraction solvent (X_2) and (iii) acetone as a co-solvent (X_3). The parameters were investigated at two levels (**Table 1**), hence, the factorial design results in eight tests with all possible combinations of X_1, X_2, X_3. The extraction yield was considered as dependent factor (response) and was evaluated for each test for all the surrogate standards. The whole experimental design matrix is shown in **Table 2**, whereas the extraction recoveries observed for each surrogate standard are reported in **Table 3**. MAE extraction tests using only solvents transparent to microwave (i.e. 1, 2, 5, 6) were possible due also to the intrinsic humidity of the bio-waste fraction.

Data show that the extraction recovery varies remarkably in the experimental domain considered and are included within 16 and 260%. In agreement with the fact that at this stage purification is not yet optimized, extraction recoveries indicate that high matrix effects are present and, de facto, a deep investigation with Pareto diagrams [41] and or Yates algorithm [42–44] to highlight main and interaction effects within the experimental factors is not possible. Nevertheless, the experimental design allows to derive many considerations for the main effects of the factors studied and to get optimal extraction conditions.

The solvent type seems to influence the extraction selectively. In fact, MAE extracts in dichloromethane had an intense red-brown colour, suggesting a greater extraction of interfering compounds, which therefore could lead to a more marked matrix effect. This is clearly identified also by comparing data obtained within couples of experiments in which the type of the extracting solvent is the only variable changing, e.g. 1 vs 2, 4 vs 3, 5 vs 6 and 8 vs 7.

The presence of acetone generally provides higher extraction recoveries, both using cyclohexane and dichloromethane. The interpretation of acetone effect on the extraction yields is ascribed to the fact that, as polar solvent, acetone allows the absorption of microwave energy and hence a rapid heating of the non-polar solvent, favouring the extraction of organic compounds.

As concerns the temperature, all the experiments performed at 150 °C led to a considerable increase in pressure and vent during the extraction. The same phenomenon was observed even at lower extraction temperatures but only in the presence of dichloromethane (tests 5 and 8) due to the lower boiling point of dichloromethane (39.6 °C) in respect to the one of cyclohexane (80.7 °C).

According to the experimental design, conditions of tests 6 and 7 (110°C with cyclohexane, without and with the presence of acetone, respectively) represented the best extraction conditions that could avoid loss of analytes, especially PAHs which are characterized by a significant volatility [45].

In order to maximize extraction recoveries, experimental conditions of test 7 (10 mL of cyclohexane, 1 mL of acetone and extraction temperature 110 °C) were chosen for the optimization of the further analytical step, i.e. the purification of the extract.

3.2 Effect of extract clean-up

As shown by the experimental design, the MAE conditions (10 mL of cyclohexane, 1 mL of acetone and extraction temperature 110 °C) adopted for the extraction of PAHs and PCBs from bio-waste, followed by a treatment based on sulfuric acid only, provided extraction yields included within 44 and 197%, with E higher than 100% for six out of twelve surrogates (i.e. chrisene-d_{12}, benzo[b]fluoranthene-d_{12}, benzo[k]fluoranthene-d_{12}, $^{13}C_{12}$-PCB118, $^{13}C_{12}$-PCB153, $^{13}C_{12}$-PCB180), indicating that some co-extracted compounds interfere with PAHs and PCBs GC-MS determination. Worth to be mentioned that a relevant background noise was also observed in the gaschromatogram of the treated extract injected.

In order to remove interferents co-extracted during MAE procedure, a solid-phase extraction (SPE) step was used after the MAE extraction. A normal-phase SPE with silica gel cartridge was used, for its capabilities to remove polar compounds [46] and for the low affinity with target analytes [47].

After MAE extraction, the extract was loaded on the direct-phase SPE cartridge, previously conditioned with cyclohexane and the purified extract was subsequently cleaned with concentrated sulfuric acid, according to the procedure depicted in **Fig. 1**.

The extraction recoveries for each labelled surrogate, expressed as the average of three analysed samples, are compared in **Fig. 2** for the two purification methods.

The normal-phase SPE purification in addition to sulfuric acid provided extraction recoveries included within 47 and 106 % (relative standard deviations, RSD, lower than 10.5 %), thus allowing to reduce the interfering compounds co-extracted by MAE, which caused an enhancement or a suppression of the signal of the analytes studied.

3.3 Matrix effect

The presence of any matrix effect (ME) was evaluated. Results, depicted in **Fig. 3**, indicate that $|ME|$ is on average 17 % and spans within 2 and 38 %, with higher percentages slightly higher than 20 % only for BaP, Ind, DBA, BP. The method is not systematically influenced by either enhancement or suppression effects, since average ME is -1.6 %.

3.4 Figures of merit

The figures of merit, i.e. linearity, method determination limit (MDL), method quantification limit (MQL) and precision intra-day and inter-day, of the optimised analytical method were determined.

3.4.1 Linearity

The method linearity was verified for PAHs in the range 0.16 – 3.5 µg L^{-1} and for PCBs in the range 0.3 – 6.5 µg L^{-1} with R^2 included within 0,997 and 0,999 for both class of compounds.

3.4.2 Method detection and method quantitation limits

Method detection and method quantitation limits (MDL and MQL, respectively) were calculated and shown in **Table 4**. MDL varied between 0.9 and 3.1 µg kg^{-1} for PAHs and between

1.3 and 7.5 μg kg^{-1} for PCBs. MQL for PAHs ranged from 2.6 to 9.4 μg kg^{-1} and from 3.9 to 23 μg kg^{-1} for PBCs. The method allows to obtain MQL values which are much lower than the limits fixed by the Resolution of the Veneto Regional Council n. 235/2009 [30] on maximum admitted concentration in bio-waste as feedstock for composting processes, which is set at 6000 μg kg^{-1} for PAHs and 800 μg kg^{-1} for PCBs. Worth mentioning that the MDL of the method developed are compatible with the average contamination of bio-waste in organic household waste reported by EPA [48].

3.4.3 Method precision

The intra-day and inter-day the precision expressed as relative standard deviation (RSD) ranged from 0.3 to 1.1 %, and from 0.4 to 9.3 %, respectively (**Table 5**). These data confirm the repeatability of the method developed.

3.4.4 Comparison with literature methods

It is worth to be mentioned that the literature dealing with the analysis of organic pollutants in bio-waste is not abundant and it focuses on the evaluation of pollutants abatement during biological stages of biomass conversion [33,47,49] rather than on the performance of the analytical methods used. Therefore, a comparison of the performances of the method developed for bio-waste with those of other literature methods for the same feedstock is not possible. However, albeit in limited quantities, data of method performance are available for PAHs and PCBs determination in the conversion product of organic waste (i.e. in compost) using accelerated solvent extraction and multi-stage clean-up steps [33].

The MAE approach and the extraction conditions developed, which allows for the simultaneous extraction of PCBs and PAHs, are highly encouraging in respect to ASE approach which, on the contrary requires two different extraction steps. In addition, extraction recoveries, determined at similar fortification levels (tens of μg L^{-1}), as well as repeatability of the MAE method

developed are improved in respect to the ASE approach (**Table 6**), especially for PAHs due to the simplicity of the clean-up step of the extracts that minimize loss of analytes.

3.5 PAHs and PCBs in bio-waste

The optimized procedure (**Fig. 1**) was applied to the analysis of PAHs and PCBs in a bio-waste sample collected from a local anaerobic digestion and composting plant. The sample was analysed in triplicates, together with two blanks to exclude laboratory contamination.

As regards PAHs, they are present in bio-waste at a total concentration of 562 μg kg^{-1}, ranging between 10 μg kg^{-1} for anthracene to 108 μg kg^{-1} for benzoperylene (**Fig. 4**), with Naph, AcPY, AcPh, Ind and DBA below the MDL. The relative standard deviation values did not exceed 15 %.

As regards PCBs, *PCB 118 was the only congener quantified (25±6 μg kg^{-1}), being the other investigated congeners below the MDL.

The above-mentioned results are well below the limits established by the Resolution of the Veneto Region. Furthermore, data obtained well compared with those presented by Bråndli [29] for biowaste, which were 410 μg kg^{-1} for fifteen PAHs and 16 μg kg^{-1} for eleven PCBs, seven of which in common with those here investigated.

4 Conclusions

Bio-waste management, via anaerobic digestion and composting, plays a key role in bioeconomy and contributes to the European Union circular economy objectives.

The evaluation of the original contamination of bio-waste fractions before conversion is poorly investigated, even if conversion processes can even increase the concentration of certain pollutants.

With the aim to provide a reliable determination of organic micropollutants PAHs and PCBs in bio-waste we propose an analytical protocol based on MAE extraction and GC-MS analysis. The accurate determination of micropollutants was ensured by a careful optimization of the extraction conditions through a 2^3 full factorial design and by using a SPE clean-up stage of the extract. The

method provides minimum sample handling, short extraction times and easy purification procedures in comparison to existing procedures developed for matrices different from bio-waste. Notably, the method uses a single extraction step and a unique chromatographic run for the simultaneous determination of PAHs and PCBs with extraction recoveries ranging from 47 % to 85 % for PAHs and from 92 % to 106 % for PCBs. The method optimized and tested directly on bio-waste from an anaerobic digestion and composting plant proved to be rugged and appliable as a routine monitoring tool for the presence of PAHs and PCBs in the bio-waste feedstock before entering the plant for the conversion.

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Table 1 – Factors and levels used in the 2^3 factorial design study.

Description factor	Coded value	Level	
		(-)	(+)
Temperature	X_1	110	150
Extraction solvent (type)	X_2	Dichloromethane[a]	Cyclohexane[a]
Co-solvent (mL acetone)	X_3	0	1

[a] 10 mL

Table 2 – Experimental design matrix in coded and actual factors.

Experiment	Coded factor			Factor		
	X_1	X_2	X_3	Temperature (°C)	Extraction solvent[a]	Acetone (mL)
1	+	-	-	150	Dichloromethane	0
2	+	+	-	150	Cyclohexane	0
3	+	+	+	150	Cyclohexane	1
4	+	-	+	150	Dichloromethane	1
5	-	-	-	110	Dichloromethane	0
6	-	+	-	110	Cyclohexane	0
7	-	+	+	110	Cyclohexane	1
8	-	+	-	110	Dichloromethane	1

[a] 10 mL

Table 3 – Experimental response expressed as the percentage of extraction achieved for each PAH and PCB surrogate at each experimental run of the 2^3 design.

Surrogate	**Experimental run**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
BaA-d_{12}	160	48	50	171	99	62	63	130
Chr-d_{12}	98	35	55	91	136	114	197	200
BbFl-d_{12}	200	52	60	230	157	110	152	166
BkFl-d_{12}	210	65	70	260	134	75	119	150
BaP-d_{12}	185	54	62	196	144	48	63	164
Ind-d_{12}	173	30	50	182	105	62	80	119
DBA-d_{14}	178	34	35	178	125	48	68	138
BP-d_{12}	98	16	19	100	101	38	44	106
^{13} PCB28	123	96	102	124	102	90	95	109
^{13} PCB52	112	87	98	98	107	80	88	120
^{13} PCB118	117	125	138	162	114	111	121	120
^{13} PCB153	136	132	149	164	153	115	130	134
^{13} PCB180	173	122	129	182	128	114	121	136

Table 4 – Method detection and method quantitation limits for the analysis of 16 PAHs and the 14 PCBs in bio-waste.

Analyte	MDL (µg kg^{-1})	MQL (µg kg^{-1})	Surrogate	MDL (µg kg^{-1})	MQL (µg kg^{-1})
Naph	1.7	5.1	**PCB11**	5.8	18
AcPY	1.8	5.5	**PCB15**	6.7	20
AcPh	2.2	6.6	**PCB28**	1.3	3.9
Flu	1.9	5.7	**PCB52**	5.8	17
Phe	3.1	9.4	**PCB101**	3.6	11
Ant	1.4	4.3	***PCB81**	6.7	20
Flth	2.4	7.2	***PCB118**	7.5	23
Pyr	2.1	6.3	***PCB123**	4.0	12
BaA	1.2	3.7	**PCB138**	3.7	11
Chr	1.3	4.0	**PCB153**	4.5	14
BbFl	2.3	7.0	***PCB167**	4.0	12
BkFl	1.6	4.9	**PCB180**	3.8	11
BaP	2.1	6.5	***PCB169**	4.0	12
Ind	2.2	6.7	***PCB189**	2.9	8.7
DBA	0.9	2.6			
BP	1.9	5.7			

Table 5 – Intra-day (n=10, one day) and inter-day (n=30, three days) precision on the analysis of surrogates spiked in bio-waste. Standard deviations in brackets.

Surrogate	Extraction recovery (%)	
	Intra-day (n=10)	Inter-day (n=30)
BaA	89.2 (0.4)	88.0 (2.0)
Chr	83.4 (0.3)	83.0 (0.7)
BbFl	79.6 (0.6)	78.5 (1.6)
BkFl	71.3 (0.2)	70.6 (1.0)
BaP	78.2 (0.5)	78.0 (0.6)
Ind	78.0 (0.4)	77.4 (0.9)
DBA	47.1 (0.6)	44.2 (4.1)
BP	58.4 (0.2)	57.6 (1.2)
PCB28	97.5 (0.6)	97.2 (0.3)
PCB52	89.3 (0.5)	88.6 (0.9)
PCB118	102 (1)	102 (1)
PCB153	99.6 (0.2)	99.4 (0.3)
PCB180	101 (1)	101 (1)

Table 6 – Comparison of extraction recoveries of surrogates obtained within this work (expressed as average of n=3 repetitions, relative standard deviation in brackets) and within [33] (expressed as minimum and maximum value of n=18 repetitions).

Surrogate	**Extraction recovery, ER %**	
	This work[a]	**Ref** [33] **(n=18)**[b]
BaA	85 (2)	44-65
Chr	83 (2)	42-76
BbFl	80 (1)	37-72
BkFl	71 (1)	51-78
BaP	77 (2)	53-75
Ind	78 (4)	55-76
DBA	47 (1)	n.a.
BP	58 (1)	51-71
PCB28	97 (4)	89-125
PCB52	92 (8)	57-70
PCB118	106 (10)	88-104
PCB153	101 (4)	78-99
PCB180	104 (8)	79-92

Fortification level: [a] 20 μg kg^{-1} for PAHs and PCBs surrogates; [b] 7-10 μg kg^{-1} for PCBs and 30-40 μg kg^{-1} for PAHs (min, max). n.a.: not available.

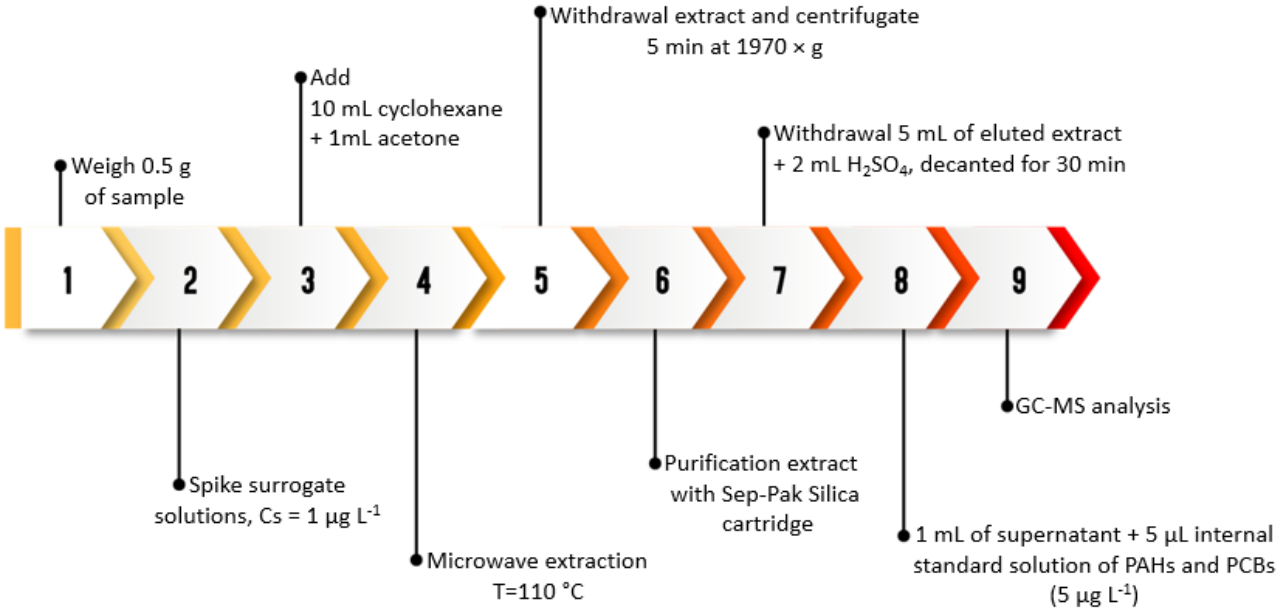

Fig. 1. Analytical protocol (MAE, purification and GC-MS analysis) optimized for the analysis of PAHs and PCBs in bio-waste.

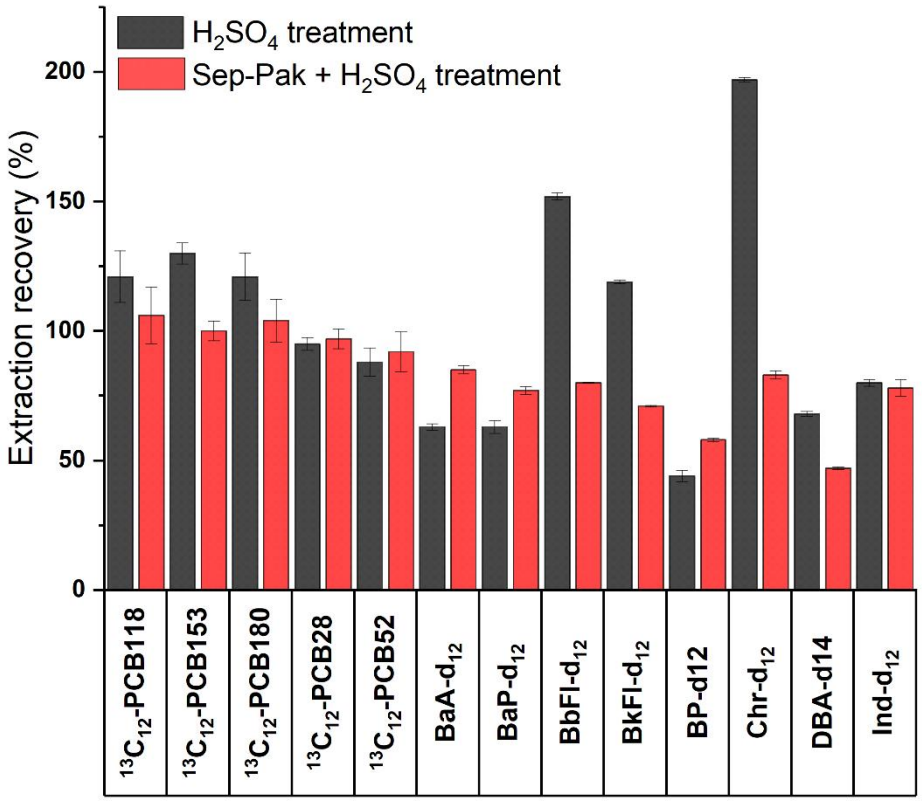

Fig. 2 – Comparison of MAE extraction recoveries for PAHs and PCBs surrogates in bio-waste after treatment of the extract by sulfuric acid and normal-phase SPE + sulfuric acid. Standard deviations are referred to n=3 replicated extractions.

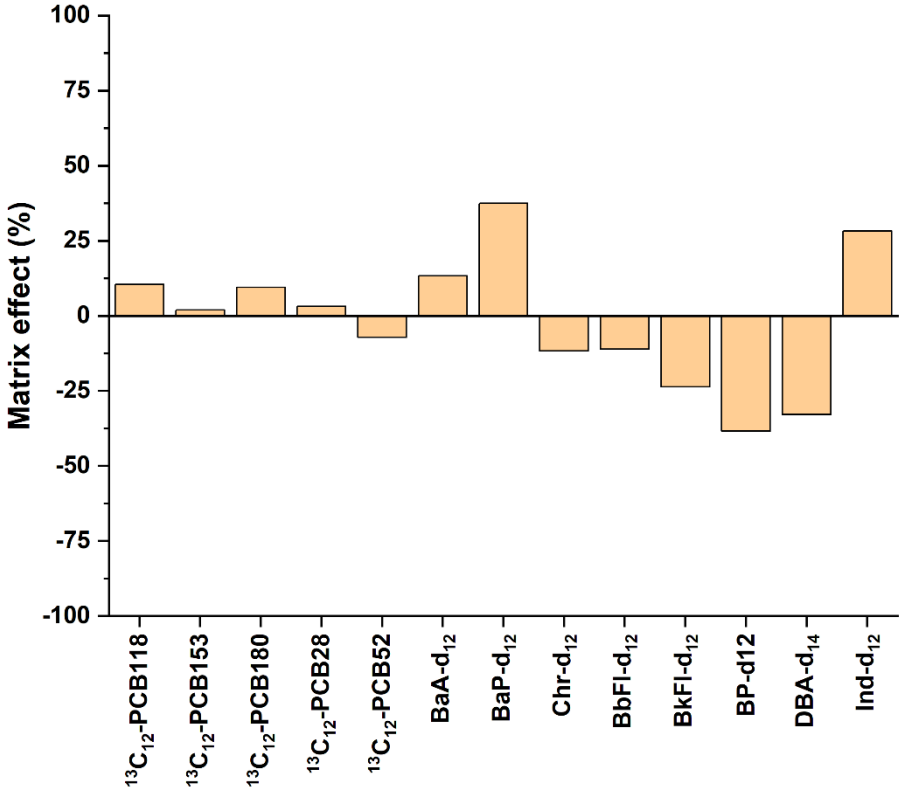

Fig. 3. Matrix effect of the analytical protocol (MAE, purification and GC-MS analysis) optimized for the analysis of PAHs and PCBs in bio-waste.

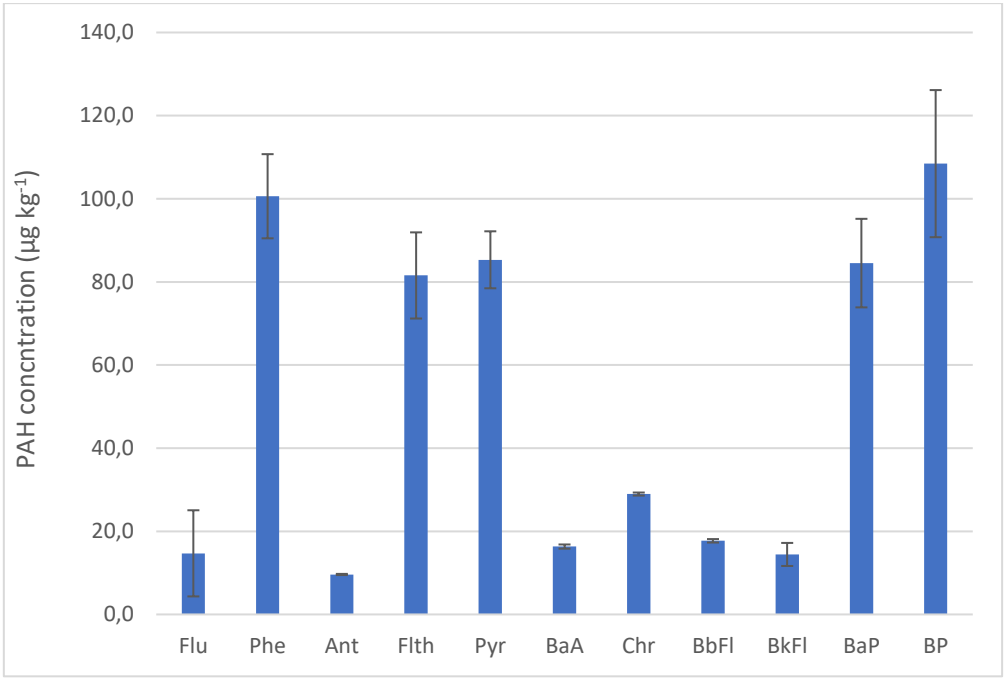

Fig. 4. PAHs content in bio-waste determined by the analytical protocol (MAE, purification and GC-MS analysis) optimized.