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Selection of indicator contaminants of emerging concern when reusing reclaimed water for irrigation — A proposed methodology

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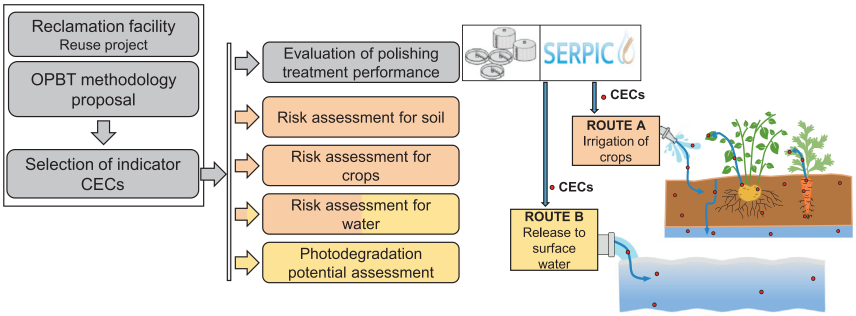
HIGHLIGHTS

- Selection of organic and microbial CECs to assess the polishing treatment performance
- Selection based on CECs occurrence, persistence, bioaccumulation, and toxicity
- Indicator CECs for risk assessment for water, soil and crops
- Indicator CECs to assess their photodegradation potential
- Microbial CECs based on detection occurrence and antibiotic consumption

GRAPHICAL ABSTRACT



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ABSTRACT

Organic and microbial contaminants of emerging concern (CECs), even though not yet regulated, are of great concern in reclaimed water reuse projects. Due to the large number of CECs and their different characteristics, it is useful to include only a limited number of them in monitoring programs. The selection of the most representative CECs is still a current and open question. This study presents a new methodology for this scope, in particular for the evaluation of the performance of a polishing treatment and the assessment of the risk for the environment and the irrigated crops. As to organic CECs, the methodology is based on four criteria (occurrence, persistence, bioaccumulation and toxicity) expressed in terms of surrogates (respectively, concentrations in the secondary effluent, removal achieved in conventional activated sludge systems, Log K_{ow} and predicted-no-effect concentration). It consists of: (*i*) development of a dataset including the CECs found in the secondary effluent, together with the corresponding values of surrogates found in the literature or by in-field investigations; (ii) normalization step with the assignment of a score between 1 (low environmental impact) and 5 (high environmental impact) to the different criteria based on threshold values set according to the literature and experts' judgement; (iii) CEC ranking according to their final score obtained as the sum of the specific scores; and (iv) selection of the representative CECs for the different needs.

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Regarding microbial CECs, the selection is based on their occurrence and their highest detection frequency in the secondary effluent and in the receiving water, the antibiotic consumption patterns, and recommendations by national and international organisations.
The methodology was applied within the ongoing reuse project SERPIC resulting in a list of 30 indicator CECs, including amoxicillin, bisphenol A, ciprofloxacin, diclofenac, erythromycin, ibuprofen, iopromide, perfluorooctane sulfonate (PFOS), sulfamethoxazole, tetracycline, *Escherichia coli,* faecal coliform, 16S rRNA, *sul1,* and *sul2*.

1. Introduction

The reuse of reclaimed water is a timely and current topic of worldwide discussion. In force and ongoing regulations and recommendations at national, European and international level, require that wastewater treatment plants (WWTPs) produce *resources* and *not waste*: reclaimed water, nutrients, bioenergy and biosolids. In addition, increasingly frequent scenarios of drought and water scarcity strongly support the application of water reuse concepts (EC COM (2022) 541 final, 2022). In Europe, the main reasons limiting this practice are the high investment and operation costs of direct reuse of reclaimed water. At the same time, the occurrence of contaminants of emerging concern (CECs) in the water, including organic CECs and microbial CECs, such as antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) may increase the concerns about reclaimed water reuse because of CEC accumulation in the environment.

Due to incomplete removal of the various CECs in conventional WWTPs, measures are necessary to reduce the release of CECs at the source. However, in order to produce an effluent adequate for irrigation, the current municipal and industrial WWTPs require the adoption of an additional end-of-pipe treatment step that is able to improve the quality of the secondary effluent. Additional, quaternary treatment will also contribute to the upcoming revision of the UWWTD (EC COM (2022) 541 final, 2022) and foster implementation of water reuse. The selection of an acceptable technology has to include its technical and economic feasibility as discussed in (Verlicchi and Zanni, 2020), while bearing in mind the minimum requirements set by the recent European Regulation on water reuse (EU Regulation 2020/741, 2020).

Different technologies are available or under research and development. Of these, rapid sand filtration followed by UV irradiation represents a widely applied treatment sequence, which is able to reduce suspended solids, bacteria and viruses. However, it has limited efficiency regarding some CECs and no persistent disinfection effect (Metcalf and Eddy, 2014). The application of chlorination or other chemical agents (such as peracetic acid) is necessary to disinfect, but it has limited efficiency for the removal of CECs in wastewater (Rizzo et al., 2020). Advanced oxidation processes, including ozonation followed by adsorption on activated carbon, have been shown to reduce a wide spectrum of organic CECs in WWTPs in Germany and Switzerland: the adoption of the treatment is not for direct reuse, but for improving the quality of the receiving surface water body, especially if there are drinking water plants withdrawing from it (FOEN, 2012; Rizzo et al., 2019; Sauter et al., 2023).

In addition, membrane processes, commonly applied as a barrier for pathogens, have the potential to reduce organics and microbial CECs. Nanofiltration (NF) and reverse osmosis (RO), in particular, have been reported to reduce ARGs below levels of detection. As NF is less energy intensive than RO, it seems to be more promising for the reduction of CECs (Krzeminski et al., 2020; Rizzo et al., 2019). However, the treatment of NF membrane concentrate, containing the rejected refractory CECs, is still under study (Deng, 2020), and its management may limit the adoption of this technology.

Photo-Fenton, photocatalytic ozonation and electrochemical oxidation are technologies currently being researched (some of them still at pilot plant scale) and seem to be promising (Dewil et al., 2017; Isidro et al., 2018; Lacasa et al., 2019; Rizzo et al., 2020). However, there are still many uncertainties about the formation of CEC intermediate/transformation products from such technologies and whether these products pose a toxic risk whose intensity is similar to that due to their parent compounds (Radjenović et al., 2009; Rodríguez et al., 2013).

The efficiency of all the available technologies is also challenged by the variance in CEC reduction within a specific CEC class due to the different chemical and physical properties of the compounds which affect their behaviour during the specific treatments (Rout et al., 2021; Verlicchi et al., 2015). A multi-barrier treatment approach is a valuable option to face this problem as it is able to promote different removal mechanisms, thus guaranteeing the removal of different types of CECs, as investigated in NEREUS COST Action ES1403 (http://www.nereus-cost.eu) and remarked in (Rizzo et al., 2020).

In this context, a new technology is under study and development within the ERA-NET AquaticPollutants project “SERPIC – Sustainable Electrochemical Reduction of contaminants of emerging concern and Pathogens in WWTP effluent for Irrigation of Crops” (https://www.serpic-project.eu/). It acts as a polishing treatment that aims to reduce the concentrations of organic and microbial CECs from the secondary effluent, producing an effluent adequate for direct reuse for irrigation purposes (see Fig. S1). It combines membrane nanofiltration and disinfection achieved by the electrochemical production of powerful oxidants (peroxosulfate and chlorine dioxide) activated by deep UV (UVC), without generating hazardous by-products. In order to assess its capacity in removing organic and microbial CECs from the feeding, it was necessary to limit the analysis to the most relevant indicator CECs occurring in the water.

In this study, a methodology is developed to identify relevant indicator CECs for the evaluation of the performance of the new end-of-pipe technology in a reuse project for irrigation purposes; for the assessment of the risk for the soil and the crops in the case of reuse of reclaimed water, as well as for the surface and ground water which may be in contact with CECs via surface runoff or percolation due to their mobility once in the soil.

2. Materials and methods

2.1. Organic CECs ranking procedure and selection

The first step of the methodology is the design of a dataset of the CECs and their concentrations detected in secondary effluent of municipal WWTPs in a *reference area*. The *reference area* is defined as the countries and/or regions which may be directly involved in the application of the technology being studied and in the reuse project. A literature overview may provide a large number of concentrations of CECs of the secondary effluent in the reference area. The dataset may also include compounds detected in specific investigations, such as those regarding the WWTP effluent which will represent the feeding to the pilot polishing plant in the case this treatment must be tested.

An accurate control of the quality of the concentration values is required to assess if they may be added to the dataset. Data are included if a description of the analytical methodology used for their detection and the quality assurance programme adopted for sampling, preparation, storage, analysis and elaboration are clearly reported in the specific investigations, in agreement with what remarked in (Verlicchi et al., 2012).

The CEC selection is carried out based on four criteria: *occurrence* (O) in the secondary effluent, *persistence* (P) in the treatment (secondary biological treatment), *bioaccumulation* (B) and *toxicity* (T) towards the aquatic life. The acronym OPBT is thus used to indicate this approach that is described in more detail in Table 1.

Table 1
OPBT criteria for the selection of CECs and the corresponding rationale.

Criteria	Rationale
Occurrence (O)	The higher the concentration of a CEC in the secondary effluent, the higher its expected environmental impact. Occurrence is given by the measured CEC concentration *c*.
Persistence (P)	The persistence of a CEC is related to its resistance to be removed in secondary biological systems. The lower the percentage removal efficiency *R* of a CEC, the higher its persistence P. Persistence is a function of the removal efficiency *R* ($P = 100\text{-}R$).
Bioaccumulation (B)	Bioaccumulation refers to a compound potential to accumulate in the adipose tissue of aquatic organisms and is related to compound lipophilicity. This property may be expressed by the octanol–water partition coefficient (K_{ow}), that is the ratio between the concentration of the CEC in *n*-octanol and the concentration in water. The higher the K_{ow}, the higher the CEC bioaccumulation potential.
Toxicity (T)	Toxicity is expressed by the predicted no-effect concentration in water ($PNEC_{water}$), that is the lowest concentration of CEC below which no toxicity effect on aquatic organisms is measured regarding any trophic level. The lower the $PNEC_{water}$, the higher the toxicity.

Bearing this in mind, the dataset must be completed with:

- the values of the *removal efficiencies* (*R)* in the secondary treatment (mainly a conventional activated sludge system) for the listed CECs, based on the literature, but also on the investigations carried out in the reference area, in order to evaluate persistence (*P*). Also, for these data, quality control must be carried out in order to include only values whose estimation is clearly described according to the considerations on sampling influence, as discussed in (Verlicchi and Ghirardini, 2019);
- the values of LogK_{ow}, from the literature and/or database such as Chemspider (http://www.chemspider.com) and PubChem (https://pubchem.ncbi.nlm.nih.gov) or specific cheminformatics software such as Chemaxon (https://chemaxon.com), Episuite (https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface);
- toxicity data ($PNEC_{water}$). $PNEC_{water}$ values may refer to acute or chronic toxicity to aquatic organisms such as fish, aquatic invertebrates and aquatic plants, and could be determined by experimental investigations or by software using computerized Structure Activity Relationships (SARs) (for instance in the Quantitative structure–activity relationship QSAR). $PNEC_{water}$ values may be included if it is well described how they were estimated and if they refer to acute or chronic effects. Evaluations based on acute $PNEC_{water}$ do not reflect the risks of long-term exposure to subacute levels of compounds. In the environmental risk assessment, chronic values should be preferred (European Chemicals Bureau, 2003), because the effects to aquatic life are related to the dose, which is the product between contaminant concentration and exposure time.

The dataset consists of a list of compounds characterised by ranges of concentrations, removal efficiencies, values of Log $K_{ow,}$ and $PNEC_{water}$.

A distinction is made between criteria and surrogates in accordance with (Pavan and Worth, 2008). The last term corresponds to the measurable attribute related to the specific criterion: concentration for occurrence, removal efficiency for persistence, LogK_{ow} for bioaccumulation potential and $PNEC_{water}$ for toxicity.

The following phase consists of the assignment of a score to the values of each criterion for each CEC. The score may vary in a defined interval, the limits of which are set equal to 1 and 5. A score equal to 1 corresponds to values with an associated or expected low environmental impact and a score equal to 5 is assigned to the highest environmental impact. If no value is available for a specific surrogate, the default score is 5: this is to assume the worst-case scenario of the target CEC where information is missing (in the future, efforts should be done to collect new data and thus assign a scientifically-based score, instead of the default value). The proposed assignment is reported in Table 2 and is in accordance with (Daouk et al., 2015) for criteria P, B and T. However, for O, the score here proposed, was assigned for the first time on the basis of the author's judgement.

For the criteria Occurrence and Persistence where a range of values (concentrations and removal efficiencies) is available for each compound, it is necessary to assume a specific value: for instance, the maximum or the average corresponding surrogate.

Once the four criteria ($j = 1{,}2{,}3{,}4$) are scored for each compound *i* included in the dataset, and assuming the same weight *w* (equal to 1) for each criterion, the final OPBT score ($S_{\text{final,i}}$) is obtained as the sum of the 4 assigned scores S_{i}:

$$S_{final,i} = \sum_{j=1}^{4} S_{i,j} \tag{1}$$

The CECs are ranked according to the descending order of the final OPBT score: compounds with the highest S_{final} are the potential candidates to be selected. The variability range of the final score is between 4 and 20.

2.1.1. Indicator compounds selection

As the dataset may include a large number of compounds, it is necessary to select a subgroup of indicators among them for the scope of the project. A first screening will consider only those compounds with a final OPBT score greater than a defined threshold, leading to a first selection of priority compounds. The selection may be refined on the basis of recommendations by relevant organisations or international reports, such as those by the World Health Organization (WHO), Environmental Protection Agency (EPA) and European Commission, as well as suggestions of surrogate CECs by international research groups (Dickenson et al., 2009). The section can be further refined based on the availability of analytical methods to detect the compounds of potential interest at the relevant concentrations.

The number of indicator compounds should be defined on the basis of the purposes of the ongoing research. Once this list is defined, subgroups of organic CECs may be selected for specific tasks: environmental risk assessment (water and soil) and accumulation in crops.

2.2. Microbial selection of CECs

According to the definition by the NORMAN network (2017) (http://www.norman-network.net/?q=node/9), *emerging pollutants* are substances currently not included in routine environmental monitoring programmes, which may be candidates for future legislation due to their adverse effects

Table 2
Assigned scores for the four OPBT criteria.

Criterion →	Occurrence (O)	Persistence (P)	Bioaccumulation (B)	Toxicity (T)
Surrogates → Score *S* ↓	Concentration *c* (ng/L)	Removal in CAS *R* (%)	Log K_{ow}	$PNEC_{water}$ (μg/L)
1	$c < 50$	$R > 80$	Log $K_{ow} < 1$	$PNEC_{water} > 100$
2	$50 \le c < 100$	$60 < R \le 80$	$1 \le$ Log $K_{ow} < 2$	$10 < PNEC_{water} \le 100$
3	$100 \le c < 500$	$40 < R \le 60$	$2 \le$ Log $K_{ow} < 3$	$1 < PNEC_{water} \le 10$
4	$500 \le c < 1000$	$20 < R \le 40$	$3 \le$ Log $K_{ow} < 4.5$	$0.1 < PNEC_{water} \le 1$
5	$c \ge 1000$ No value is available	$R \le 20$ No value is available	Log $K_{ow} \ge 4.5$ No value is available	$PNEC_{water} \le 0.1$ No value is available

and/or persistency, whose fate, behaviour and (eco)toxicological effects are not well understood. In this context, due to the continuous and ubiquitous release of residues of antibiotics into the environment and the subsequent proliferation of microorganisms resistant to them (EC COM(2017) 339 final, 2017), ARB and the associated ARGs may be considered *microbial emerging contaminants* (microbial CECs) as also remarked by the United Nations Environment Programme Frontiers report (2017) (UNEP, 2017).

Selections should consider the microbial CECs with the highest frequency of detection in the treated effluent and in the receiving water of the area of interest, their occurrence and relevance, the antibiotic consumption patterns in the area of interest (if available), the availability of analytical methods for their detection and quantification, and also recommendations or suggestions by national and international organisations and expert groups. Unluckily it is not possible to adopt a rigorous approach also including thresholds for their selection, similar to that outlined for organic CECs as researches on ARB and ARGs are ongoing and data are still scarce.

3. Results

The described methodology was applied within the SERPIC project to define the list of indicator organic and microbial CECs to monitor in the case of reuse of reclaimed water. In particular, the methodology was applied for the evaluation of the performance of a polishing treatment developed within the SERPIC project with regard to the *reference areas* including Spain, Portugal and Italy (characterised by arid zones and/or scarcity of water resources), and South Africa (where the new technology could be implemented in order to satisfy water demand for agricultural needs).The technology will be tested at the prototype treatment plant built near the Universidad de Castilla-La Mancha University (UCLM) in Ciudad Real, Spain, and in long-term field-tests where the effluent polished by the SERPIC technology will be used to irrigate carrots and potatoes. A brief description of the technology is given in section S1 in the supplementary material and the schematic diagrams of the equipment is provided in Fig. S1.

3.1. Organic CECs

3.1.1. Occurrence in secondary effluent

An in-depth literature survey of occurrence in the secondary effluent (conventional activated sludge system) of the reference areas (Spain, Portugal, Italy and South Africa) was carried out and a specific monitoring campaign was carried out at the Real Ciudad WWTP, the effluent of which will be the feeding of the SERPIC technology investigated at a pilot scale.

Data included in the dataset were taken from peer reviewed research articles, published since 2010, found in Scopus with the keywords: (“compounds of emerging concern” OR “micropollutants” OR “pharmaceuticals”) AND “wastewater” AND (“Italy” OR “Portugal” OR “Spain” OR “South Africa”). Values were included if: (*i*) they refer to conventional activated sludge processes treating urban wastewater; (*ii*) they satisfy the constraints reported in Section 2.1 (quality assurance); and (*iii*) the concentrations in the secondary effluent are provided as measured concentrations in the literature (concentrations estimated starting from influent concentrations and corresponding removal efficiencies are excluded).

In the case of investigations providing many values of the concentration of a compound, all values were included; when minimum, maximum and average concentrations were given, only the minimum and maximum values were considered (in order to define an interval of variability), and, finally, if average values were the only data available, these were considered.

Briefly, 18 studies were found for Spain (64 investigations and 42 studied WWTPs), 9 for Portugal (119 investigations and 23 studied WWTPs), 19 for Italy (47 investigations and 30 studied WWTPs) and 19 for South Africa (43 investigations and 18 studied WWTPs) (see Table S1).

This led to the collection of concentration variability ranges in the secondary effluent for 349 CECs belonging to 39 different classes detected at least once. Tables S2 – S5 show minimum and maximum concentrations, as well as the number *n* of values available from the collected papers and they report for each country (respectively, Spain, Portugal, Italy and South Africa) the CECs in descending order according to their maximum concentration found in the cited literature. It emerges that the highest concentrations were found for different substances in the 4 countries: salicylic acid (236,000 ng/L) and fluconazole (109,480 ng/L) in Spain, metformin (58,000 ng/L) and caffeine (39,200 ng/L) in Portugal, bis(2-ethylexhyl) phthalate (315,000 ng/L) and diethyl phthalate (15,700 ng/L) in Italy (in the largest WWTP in the metropolitan area of Turin), acetylsalicylic acid (118,025 ng/L) and efavirenz (93,100 ng/L) in South Africa.

In addition to the CECs found in the literature in the four show case regions, the results of a dedicated investigation at the Ciudad Real WWTP secondary effluent were included in the dataset, as this will be the feeding to the SERPIC technology investigated at pilot scale. They are reported in the supplementary material Table S6.

The score referring to the Occurrence O criterion (Table 2) is assigned on the basis of the maximum value of the concentrations found for each compound in the literature or in the Ciudad Real WWTP effluent (Table S8 for a global overview, regardless of the country it refers to). The results of this normalization step are reported in Table S9.

3.1.2. Persistence during biological treatment

Persistence P of a CEC is related to its resistance to be removed during the conventional activated sludge system (secondary biological treatment). Removal efficiencies are found directly in the literature and are not evaluated on the basis of the provided influent and effluent concentrations or on new investigations. Details of the collected values for all the listed CECs are available in Table S7. They refer to 29 papers: 6 regarding investigations in Spain, 4 in Portugal, 9 in Italy and 10 in South Africa. In order to assign a score related to the persistence of each CEC to the secondary treatment, the average values of the collected removal efficiencies (see Table S8) were considered. The corresponding assigned scores are reported in Table S9.

3.1.3. Bioaccumulation in aquatic organism tissues

Bioaccumulation is related to the octanol–water partition coefficient (K_{ow}), that is the ratio between the concentration of the CEC in *n*-octanol and the concentration in water (Table 1). These values were found through the software Chemaxon and are reported in Table S8.

3.1.4. Toxicity to aquatic life

$PNEC_{water}$ values were collected from the NORMAN database (https://www.norman-network.com/nds/) which is recommended for prioritization purposes by the NORMAN experts. These values are preferably based on experimental eco-toxicity data, but in the case of lack or insufficient empirical endpoints, QSAR predictions were used to estimate a provisional PNEC value to allow for a first screening. NORMAN $PNEC_{water}$ values refer to long-term exposure to aquatic organisms in freshwater. The selected $PNEC_{water}$ values are reported in Table S8.

3.1.5. OPBT score for the listed compounds

A score is assigned for each of the criteria for the listed CECs, as reported in Table 2, and the final OPBT score is then evaluated by Eq. (1). Table S9 reports the details of each CEC, as well as the corresponding final OPBT scores. Compounds are here grouped into classes which are reported in alphabetic order, whereas in Table S10, they are ranked according to their final OPBT score which varies from 6 to 20.

3.2. Microbial CECs

In order to identify the microbial CECs of interest, an analysis of the ARB and ARGs commonly detected in WWTP effluent was carried out with the support of a literature screening (Amarasiri et al., 2020; Ashbolt et al., 2018; Hong et al., 2013; Leiva et al., 2021; Pazda et al., 2019; Rizzo et al., 2013) and is reported in Tables S11 and S12.

Among the different target bacteria, the following have commonly been utilised and/or proposed for antimicrobial resistance (AMR) monitoring:

Escherichia coli, *Enterococci*, Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Aeromonas* spp. (Berendonk et al., 2015; Davis et al., 2022; Huijbers et al., 2020; Liguori et al., 2022).

For the ARGs, 16S rRNA, intI1, *sul*1, *sul*2, *aad*A, *erm*F, bla_{OXA}, $bla_{\text{CTX-M}}$, *qnr*S, *tet*A, *tet*B, *tet*O, *tet*W, *tet*X, *van*A and *bla*VIM were among the most frequently detected and/or were proposed as indicators to monitor AMR abundance and/or elimination in WWTPs (Goulas et al., 2020; Hiller et al., 2019; Keenum et al., 2022; Liguori et al., 2022; Manaia, 2022; Zheng et al., 2020). Among these, sulfonamide resistance genes *sul*1 and *sul*2 were the two most reported genes across all the environments including water, soil and air (Abramova et al., 2022).

3.3. Selection of the indicators (organic and microbial) CECs according to the defined criteria

For the purpose of projects that need to evaluate CEC removal by a novel polishing technology, a short list of CECs has to be identified and analysed in order to optimise the new treatment processes and to evaluate the spread and transformation in the test fields.

The first provisional selection of organic CECs is made based on Table S10, by setting a threshold value for the final OPBT score equal to 15. This splits the list into a first group of priority 116 organic CECs with a final OPBT score ranging between 20 and 15 and a second group of 234 CECs with a final score between 14 and 6.

A screening of the CECs in the first group is performed on the basis of the following documentation:

- Guidelines to support the application of Regulation 2020/741 on minimum requirements for water reuse (EC Guideline 2022/C 298/01, 2022) which strongly recommend taking into consideration all relevant EU, national and local legislations, as well as the requirements in the legislation on protecting surface and groundwater resources. These include: the Water Framework Directive (EU Directive 2000/60/EC, 2000), the Groundwater Directive (EU Directive 2006/118/EC, 2006), the Environmental Water Quality Directive (EU Directive 2008/105, 2008), the Nitrates Directive (EU Directive 1991/667/EEC, 1991), and also the Bathing Water Directive (EU Directive 2006/7/EC, 2006) and the Drinking Water Directive (EC Directive 2020/2184, 2020).
  In this context, (EU Directive 2008/105, 2008) provides a periodically updated watch list of CECs, candidate to be included in the European priority list. According to (EC Implementing Decision 2020/1161, 2020) and the recent (EC Implementing Decision 2022/1307, 2022) the included pharmaceuticals are: amoxicillin, ciprofloxacin, sulfamethoxazole, trimethoprim, clindamycin, oflaxin, venlafaxine, *O*-desmethylvenlafaxine, metformin and guanylurea, clotrimazole, fluconazole miconazole, butyl methoxydibenzoyl-methane, octocrylene and nemzophenone-3. The Drinking Water Directive sets minimum requirements for parametric values used to assess the quality of water intended for human consumption (Annex 1, Part A and Part B of (EC Directive 2020/2184, 2020)) for *per*- and polyfluoroalkyl substances (PFAS), Bisphenol A and the recent (EC Implementing Decision C(2022) 142 final, 2022) for 17-beta-estradiol and nonylphenol;
- The document (EC COM(2019) 128 final, 2019) which strongly recommends considering cytotoxic pharmaceuticals and X-ray contrast media compounds of priority relevance;
- The document (EC COM(2020) 667 final, 2020) which strongly recommends considering PFAS of priority relevance.

In addition, CECs are included if the corresponding analytical methods are available.

That being said, an inclusion/exclusion analysis is made for all the compounds (Table S13). Table 3 reports the selected organic CECs whereas their main chemical and physical properties are shown in Table S14.

Fig. 1 shows their corresponding final OPBT score and the contribution of the different criteria. It emerges that the maximum score of 5 is assigned to most of the organic CECs for their occurrence, to erythromycin, bisoprolol and venlafaxine for their persistence, to nonylphenol, irbesartan, PFOS and valsartan for their bioaccumulation, and to diclofenac, ibuprofen, azithromycin, amoxicillin, ciprofloxacin, PFOS and tetracycline for their toxicity.

Table 3
Complete list of 30 indicator organic and microbial CECs.

Class	CEC
ARB	*Escherichia coli*
ARB	*Faecal coliforms*
ARG	16S rRNA
ARG	*sul1*
ARG	*sul2*
Antibiotic	Amoxicillin
Antibiotic	Azithromycin
Lipid regulator	Bezafibrate
Beta-blocker	Bisoprolol
Plastic additive	Bisphenol A
Psychiatric drug	Carbamazepine
Psychiatric drug	Carbamazepine 10,11 epoxide (metabolite)
Antibiotic	Ciprofloxacin
Antibiotic	Clarithromycin
Analgesic/anti-inflammatory	Diclofenac
Antibiotic	Erythromycin
Diuretic	Furosemide
Lipid regulator	Gemfibrozil
Analgesic/anti-inflammatory	Ibuprofen
X-ray contrast medium	Iopromide
Antihypertensive	Irbesartan
Surfactant	Nonylphenol
Psychiatric drug	Oxazepam
Surfactant	Perfluorooctane sulfonic acid (PFOS)
Antibiotic	Sulfamethoxazole
Antibiotic	Tetracycline
Analgesic/anti-inflammatory	Tramadol
Antibiotic	Trimethoprim
Antihypertensive	Valsartan
Psychiatric drug	Venlafaxine

Starting from the list reported in Table S11, the selection of the indicator ARB is carried out based on these criteria:

- ARB is clinically relevant and it is identified as a carrier of acquired antibiotic resistance in the aquatic environments,
- ARB is used as an indicator of faecal contamination in the aquatic environments,
- Analytical methods are available for its detection and quantification,
- Recommendations by World Health Organization (World Health Organization, 2017) and by the European Regulation on minimum requirements for water reuse (EU Regulation 2020/741, 2020),
- Suggestions from specific networks or hubs, such as the Nereus COST action (Nereus Cost Action, 2017) and Water JPI Knowledge Hub on

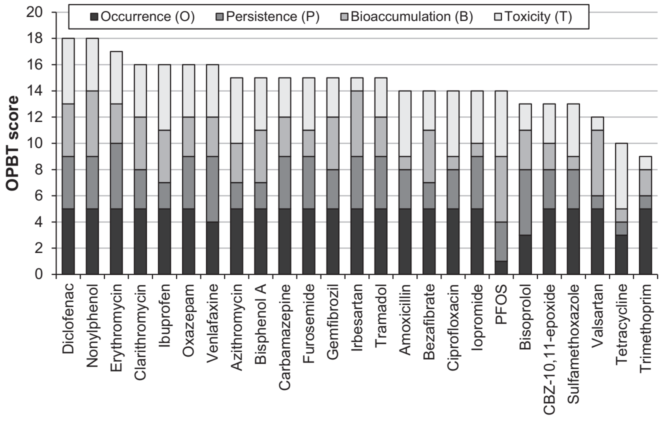

Fig. 1. Final OPBT scores for the indicator organic CECs and contributions by the different criteria.

Contaminants of Emerging Concern (http://www.waterjpi.eu/implementation/thematic-activities/water-jpi-knowledge-hub-1/water-jpi-knowledge-hub-on-contaminants-of-emerging-concern),
- Lessons learned from the literature (Berendonk et al., 2015; Ternes et al., 2017),
- Experts' judgement (authors' acquired experience and knowledge).

Faecal coliforms are selected as they are currently used as indicators of faecal contamination in waters, also for antibiotic-resistant coliforms (Marano et al., 2020). Within this group of bacteria, *Escherichia coli* is included as it is the predominant species and it has a well characterised acquired antibiotic resistance (Berendonk et al., 2015). In addition, in 2017, the World Health Organization included *Escherichia coli* in the global priority pathogens list of ARB and assigned to it the most critical level of priority (World Health Organization, 2017). In 2020, the European Regulation 741/2020 on minimum requirements for water reuse (EU Regulation 2020/741, 2020) set a limit of 10 MPN/100 mL for *Escherichia coli* for the reclaimed water destinated to crop irrigation. Furthermore, *Escherichia coli* has been proposed as an indicator for the surveillance of AMR in the environment (Anjum et al., 2021) and is used in several surveillance systems including Global Tricycle Surveillance (Huijbers et al., 2020; WHO, 2021).

Based on Table S12, the indicator ARGs are selected following these criteria:

- ARG is clinically relevant and has a high detection in wastewater effluent,
- Analytical methods are available for its detection and quantification,
- Suggestions from specific networks or hubs, such as the Nereus COST action (Nereus Cost Action, 2017) and Water JPI Knowledge Hub on Contaminants of Emerging Concern (http://www.waterjpi.eu/implementation/thematic-activities/water-jpi-knowledge-hub-1/water-jpi-knowledge-hub-on-contaminants-of-emerging-concern),
- Lessons learned from the literature (Alygizakis et al., 2020; Berendonk et al., 2015; Cacace et al., 2019; Kampouris et al., 2021; Keenum et al., 2022; Pärnänen et al., 2019; Ternes et al., 2017; Wang et al., 2021),
- Experts' judgement (authors' acquired experience and knowledge).

sul1 and *sul2* are included in the list, as *sul* genes are the most detected (not always the most abundant) ARGs in wastewater effluent in several countries (Amarasiri et al., 2020; Caucci et al., 2016) Manaia, 2022) and in particular *sul*1 and *sul*2 are the most prevalent sulfonamide ARGs in clinical isolates (Keenum et al., 2022). In addition, *sul*1 is strongly correlated with anthropogenic inputs, occurs in abundance in wastewater enabling assessing treatment removal efficiency, is relevant to horizontal gene transfer, and has a high association with multiantibiotic resistance (Liguori et al., 2022). Finally, both *sul* genes are also good indicators of mobile antibiotic resistance which is of importance for AMR spreading and dissemination (Abramova et al., 2022). The 16S rRNA gene is selected as it is often used as an indicator of total bacterial abundance (Alygizakis et al., 2020; Cacace et al., 2019; Wang et al., 2021) and is used to determine the relative abundance of genes (ARG gene copies normalised to 16S rRNA gene copies) (Alygizakis et al., 2020; Keenum et al., 2022).

The final list of the selected microbial CECs (5 microbial) is reported in Table 3.

3.4. Indicator organic CECs for specific needs

3.4.1. Selection of CECs for the risk assessment for the irrigated soil

Reclaimed water intended for crop irrigation may come into contact with terrestrial organisms and the resulting effects are strictly correlated to their concentrations in the soil. According to the Guidelines set by the European Commission (European Chemicals Bureau, 2003), $\text{PNEC}_{\text{soil}}$ is evaluated by means of the equilibrium partition approach (Eq. (2)):

$$\text{PNEC}_{\text{soil}} = \text{PNEC}_{\text{water}} \times K_{\text{d}} \times 10^{-3} \tag{2}$$

where $\text{PNEC}_{\text{soil}}$ is expressed in ng/g, $\text{PNEC}_{\text{water}}$ in ng/L and K_{d} in L/kg.

K_{d} is the solid-water partition coefficient which corresponds to the distribution of the compounds between the soil and the reclaimed water. K_{d} is commonly determined by the carbon-water partition coefficient of the CECs (K_{OC}) and the fraction of organic carbon of the soil (f_{OC}) according to Eq. (3):

$$K_d = K_{OC} \times f_{OC} \tag{3}$$

where K_{d} and K_{oc} are expressed in L/kg.

In this study, the values of K_{oc} for soil are predicted by EPISuite model (https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface) on the basis of Log K_{ow} values. f_{oc} is assumed to be 0.011, which is the average concentration of soil organic carbon obtained in (Calvo de Anta et al., 2020) for arable crops in Castilla-La Mancha, the region of Spain where the field test will be carried out. The estimated K_{d} values for the selected organic CECs are reported in Table S15. In Table S16 the K_{d} values for soil found in the literature are also reported.

According to Eq. (2), the estimated $\text{PNEC}_{\text{soil}}$ values (Table S15) refer to aquatic organisms and not to terrestrial ones, as for the selected compounds only limited toxicological data on CECs in the terrestrial compartment is available in the literature (Table S16).

As for the aquatic compartment, the most critical compounds are those with the lowest values of $\text{PNEC}_{\text{soil}}$. It emerges from Table S15 that $\text{PNEC}_{\text{soil}}$ values vary between 0.033 ng/kg and 9.77×10^5 ng/kg and assuming a threshold equal to 100 ng/kg, the most representative compounds are iopromide, tetracycline, ciprofloxacin, amoxicillin, azithromycin, ibuprofen, clarithromycin, PFOS and erythromycin (see Table S17).

3.4.2. CEC selection for risk assessment for crops

As the SERPIC project aims to produce an effluent adequate for direct reuse for crop irrigation (Route A in Fig. S1), the organic CEC residuals in the effluent might accumulate in the soil or in the plant roots (below ground) or uptake by roots and by translocation mechanisms might accumulate in the above ground (stems, leaves) and edible parts of the plants (Shi et al., 2022). Their fate is influenced by different factors related to: (i) plant properties (percentage of water and lipids, plant health, age at first exposure); (ii) soil properties (pH, soil texture, water content, organic content, cation exchange capacity and nutrient concentrations); (iii) environmental conditions (humidity, temperature, salinity, radiation and exposure duration); (iv) irrigation mode (amount and frequency); and (v) CEC concentration and physical and chemical properties (Bigott et al., 2020; Bueno et al., 2022; Miller et al., 2016).

Plant type has an impact on the potential to uptake and accumulate CECs by the crops, as different crop species have different ability for CEC uptake. Fruit vegetables have the lowest potential for uptake, followed by cereals and fodder crops, root vegetables and, finally, leafy vegetables, which according to current knowledge have the highest potential for uptake (Ben Mordechay et al., 2022b; Christou et al., 2019).

The presence of microorganisms in the soil and in the root surfaces of the plant (rhizobacteria) may promote biodegradation processes and reduce the concentrations of parent compounds, but it may generate (known and unknown) transformation products (Bigott et al., 2020). The CEC residual amount which could potentially be in contact with the plant is strictly correlated to the amount of water, which is species-dependant: those requiring a high amount of water for their development and growth are potentially exposed to a higher CEC quantity.

Intense rain events may generate runoff and thus soil erosion and/or water infiltration leading to tile drainage or percolation. These occasional water streams may transport organic CECs present in the soil towards surface water or groundwater, as discussed in (Ghirardini and Verlicchi, 2019).

Physical and chemical properties of CECs which may affect their translocation within the plants are mainly molecular weight, water solubility, hydrophobicity (related to Log K_{ow}, distribution coefficient Log D_{ow}) and polarity (related to the acid dissociation constant pK_{a}, and charge). Volatile CECs and those with a low molecular weight (<1000 g/mol) tend to be taken up by the roots and translocate in the plant. On the contrary, nonvolatile CECs and those with a high molecular weight (>1000 g/mol) may be only accumulated in the roots (Bigott et al., 2020; Keerthanan et al., 2021). Moreover, CECs with low water solubility have limited translocation and consequently have more tendency to be accumulated in the roots rather than in the other parts of the plant (Bueno et al., 2022).

Neutral CECs present higher membrane penetration in plants than ionised compounds, therefore, they are likely to translocate in the plants. Their fate in plants is related to Log K_{ow} (which is equal to Log D_{ow} see Section S2. *Hydrophobicity and hydrophilicity*). In particular: (*i*) if the compound is characterised by Log $K_{ow} \leq 1$ (highly hydrophilic CEC), it has a low tendency to translocate in the plant; (ii) if $1 <$ Log $K_{ow} < 4$, it may translocate in the plant; and (iii) if Log $K_{ow} \geq 4$ (highly hydrophobic CEC), it has a strong interaction with the soil and the roots and it tends to accumulate in them (Bigott et al., 2020; Keerthanan et al., 2021). Due to the negatively charged cell membrane in the roots (due to the high concentration of uronic acids), ionised CECs may be electrostatic repulsed or attracted. For these compounds, Log D_{ow} more accurately measures their hydrophobicity compared to Log K_{ow}, as it takes into account the pH dependence in an aqueous solution (measured by pk_a). Their behaviour is not completely described by this parameter as it is strongly affected by the interactions with the functional groups on the surface of the plant tissues which could attract and promote the root uptake. Acidic CECs tend to accumulate in roots. Their accumulation is influenced by their partial dissociation in nutrient solutions into the undissociated acid form, which may accumulate in roots via ion trap mechanisms, and the corresponding anion, generally poorly uptaken by plants (due to electrostatic repulsion). Basic CECs are likely to translocate in plants, and on the basis of their dissociation in nutrient solutions in neutral and cationic species, they may be: (i) moderately uptaken by roots due to electrostatic attraction; (ii) accumulated in roots by ion trap mechanisms; and (iii) accumulated in roots if they have high Log D_{ow} (Bigott et al., 2020; Keerthanan et al., 2021; Wu et al., 2015).

On the basis of these considerations, an attempt is carried out to predict the fate of the selected organic CECs once in the soil which will be validated experimentally in the SERPIC project. In particular, attention is paid to the accumulation potential of the CECs in plant roots. Therefore, tuber vegetables, such as potatoes, root vegetables, such as carrots, were selected as species to test in the fields irrigated with the effluent of the SERPIC technology. Details of this analysis are reported in Fig. 2: accumulation in the roots and translocation in the aboveground of the plant of the selected organic CECs are predicted on the basis of their Log D_{ow} and charge (Expected fate) and of a literature survey (Observed fate).

It is important to remark that most of the studies on CEC accumulation and uptake in plants irrigated with reclaimed water are carried out in greenhouses (Blaine et al., 2014; Bueno et al., 2022; Goldstein et al., 2014; Shenker et al., 2011) and, sometimes, reclaimed water used for irrigation was spiked with CECs (Blaine et al., 2014; Bueno et al., 2022; Goldstein et al., 2014; Malchi et al., 2014; Shenker et al., 2011; Wu et al., 2014). This means that the investigational conditions do not correspond to real conditions, but the collected results could be useful in evaluating the fate of CECs and to select the most representative ones.

As reported in Table S17, the most representative organic CECs suggested to evaluate the risk of accumulation in carrots and potatoes are:

- gemfibrozil, PFOS and sulfamethoxazole which, as they are acidic CECs, tend to accumulate in the plant roots, in accordance with the literature investigations,
- nonylphenol, as it is a highly hydrophobic neutral CEC (high Log K_{ow}), that means it has a high potential to accumulate in the plant roots,
- bisphenol A, as it is a neutral CEC with a Log K_{ow} (4.04) slightly higher than the threshold to be a highly hydrophobic CEC and should accumulate in the plant roots,
- erythromycin (a basic CEC) as according to the literature investigations it accumulates in the plant roots.

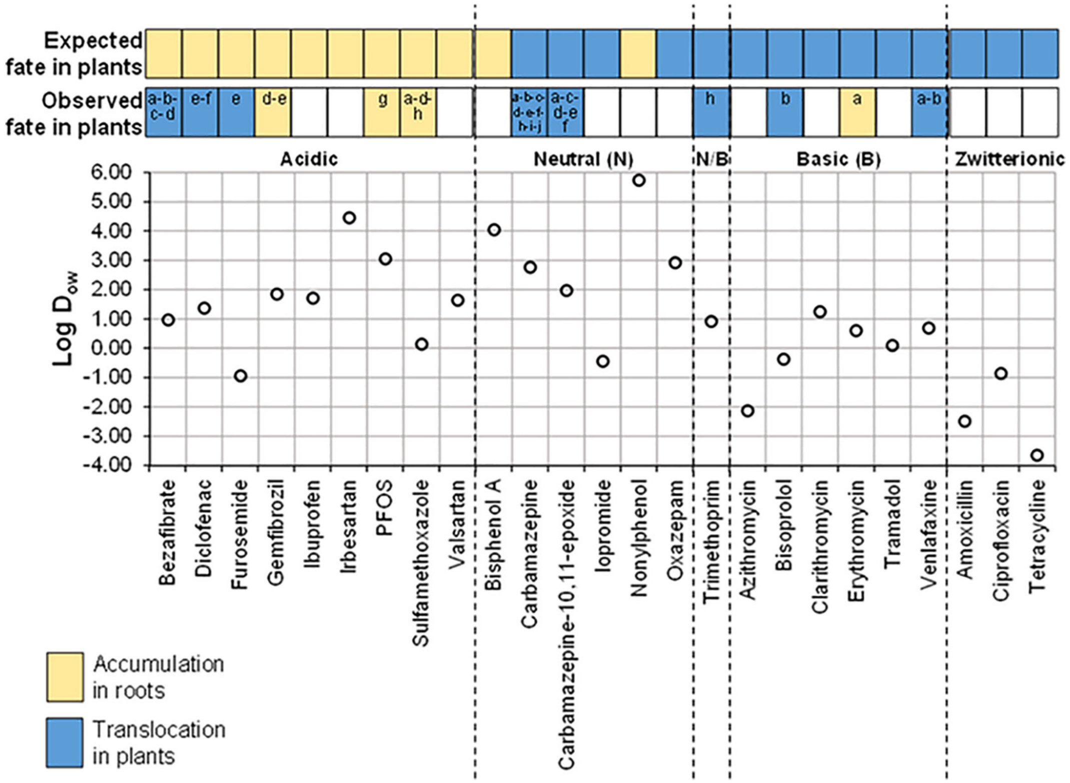

Fig. 2. Fate (accumulation in the roots or translocation in the aboveground parts of the plant) of selected organic CECs in the case of reuse of reclaimed water based on CEC Log Dow and charge (expected behaviour) and on literature experimental investigations (observed fate). Data From: a = (Ben Mordechay et al., 2021), b = (Ben Mordechay et al., 2022a), c = (Goldstein et al., 2014), d = (Malchi et al., 2014), e = (Bueno et al., 2022), f = (Sunyer-Caldú et al., 2022); g = (Blaine et al., 2014), h = (Franklin et al., 2016), i = (Shenker et al., 2011); j = (Wu et al., 2014).

3.4.3. Selection of CEC for the risk assessment for the water compartment

If Route A effluent is not reused for irrigation purposes it is discharged into surface water. Route B effluent released into surface water may still contain small concentrations of CECs which might negatively affect aquatic organisms. The most representative compounds among the 25 organic CECs (Table 3) are selected on the basis of their (chronic) toxicity: the lowest values of $\text{PNEC}_{\text{water}}$, the highest potential environmental risk for aquatic organisms.

The $\text{PNEC}_{\text{water}}$ values vary between 2 ng/L and 7×10^5 ng/L (Table S14). Assuming a threshold value equal to 100 ng/L, the most representative organic CECs of interest for this analysis are: PFOS, ibuprofen, azithromycin, diclofenac, amoxicillin, ciprofloxacin and tetracycline (see Table S17).

3.4.4. Selection of CECs for the evaluation of the performance of SERPIC technology Route B

As shown in Fig. S1b, Route B of the SERPIC technology includes a membrane photoreactor fed by the nanofiltration concentrate generated in Route A and its effluent is released into surface water (rivers). In the membrane photoreactor, CEC removal mechanisms are due to photoelectrochemical reactions, initiated by UV-C lamps. Thus, the CECs to be selected to evaluate the performance of the phototreatment step are those which exhibit a high removal if exposed to the sun. In this context, (Mathon et al., 2016) suggest dividing CECs into three classes according to their corresponding half-lives ($t_{1/2}$) for direct photodegradation: fast-photodegradable compounds when $t_{1/2} < 8$ h, medium-photodegradable compounds when 8 h $\leq t_{1/2} \leq$ 168 h) and slow-photodegradable compounds when $t_{1/2} > 168$ h.

However, the $t_{1/2}$ is not a rigorous comparison parameter, since it widely varies depending on exposure conditions, such as light intensity, exposure time and photoreactor geometry (Challis et al., 2014). Mathon et al. (2021) proposed a method to predict the photodegradability of CECs based on their physical and chemical properties and/or chemical structure characteristics. They also reported that high molecular weights above 700 g/mol, low Log K_{ow} values and high log quantum yield values negatively influence photodegradation. Additionally, this method determined the eight most influential functional groups for the direct photodegradation of CECs, considering the following issues:

- The ether oxide bond (—O—) is the most refractory functional group, followed by chloride (—Cl) and imine (-CH=N-),
- The carboxylic acid bond (OH-C=O) is the most sensitive functional group, followed by nitro (=NO-OH), phosphinate (-O-P=O), alkene (-C=C-) and oxime (-C=N-O-).

Table S18 analyses the physical and chemical properties, and the chemical structure characteristics of the selected organic CECs for the SERPIC project. In this paper, Fig. 3 classifies these CECs as a function of their sensitivity for direct photodegradation. As reported in Table S17, it emerges that the CECs which could be removed to a higher extent in the membrane photoreactor are ciprofloxacin, ibuprofen, amoxicillin, carbamazepine, bisphenol A, tetracycline and sulfamethoxazole. Thus, it is suggested they be considered the most representative compounds to evaluate the performance of Route B of the SERPIC technology.

4. Discussion

4.1. Criteria selection

The selected criteria in this proposed methodology (OPBT) are expressed in terms of the following surrogates: concentration *c*, 100 - removal *R*, octanol-water distribution coefficient K_{ow}, and predicted no effect concentration PNEC. In other studies, they were expressed by means of other variables: regarding occurrence, excreted mass on an annual basis (Daouk et al., 2015), and the predicted environmental concentration (among them: (Golbaz et al., 2021; Ortiz de García et al., 2013; Sui et al., 2012)). As to persistence, some authors refer to biodegradation constant rate (among them (Huang et al., 2022; Li et al., 2020)), degradation half-life in water (Deviller et al., 2020) or organic carbon-water partition coefficient Log K_{oc} (Li et al., 2019; Mansour et al., 2016). Bioaccumulation was also associated with the bioconcentration factor which is a function of Log K_{ow} (for instance (Mansour et al., 2016; Ortiz de García et al., 2013)). Finally, toxicity may be related to ecotoxicological data for aquatic or terrestrial organisms in terms of acute or chronic toxicity or toxicological data for humans or animals in terms of carcinogenicity, mutagenicity, reprotoxicity or endocrine disruption (Deviller et al., 2020; Guo et al., 2021; Kumar and Xagoraraki, 2010).

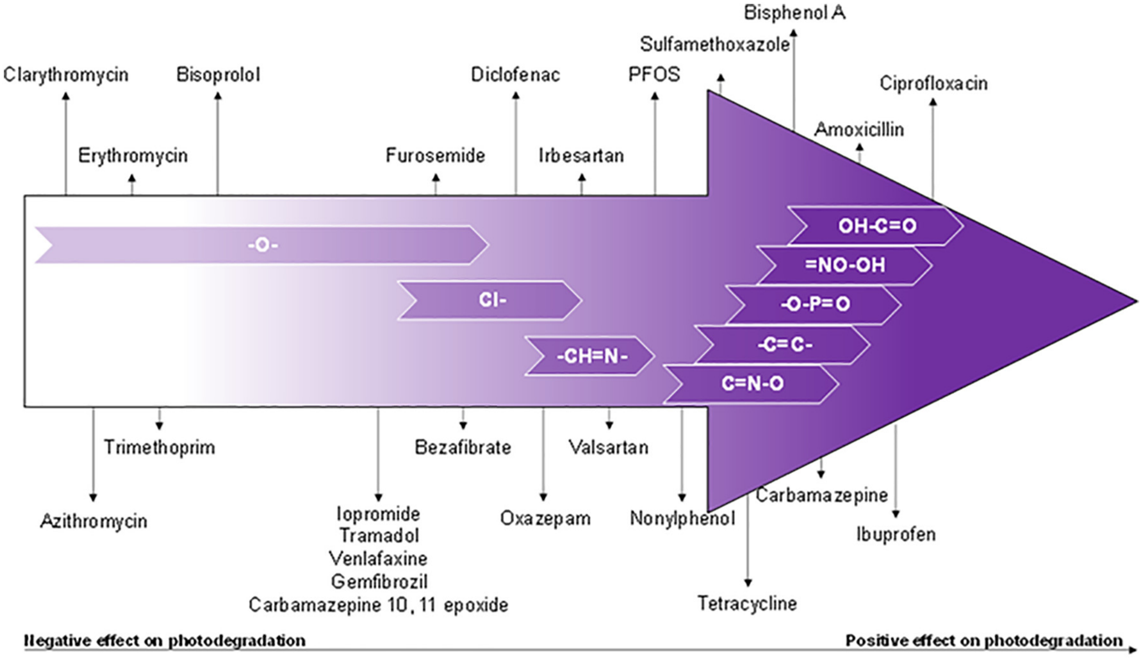

Fig. 3. Classification of selected organic CECs for the SERPIC project as a function of their sensitivity to direct photodegradation.

Biodegradation, bioconcentration and aquatic toxicity may be evaluated by quantitative structure-activity relationships methods (QSAR), quite often with the support of EPISuite (https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface) (for instance (Golbaz et al., 2021; Huang et al., 2022)). The OBPT method may also be combined with prevalence, defined by the number of positive detections of CEC in the aquatic and terrestrial environments, as proposed by (Huang et al., 2022).

4.2. CEC selection by means of the risk quotient approach

The first attempts of CEC prioritization limited the attention to the environmental risk posed by the residual of CECs in the water. The assessment of the specific risk to aquatic life was based on the Risk Quotient RQ, that is the ratio between the CEC measured or predicted environmental concentration and the corresponding PNEC for the specific water compartment (EMEA, 2006; European Chemicals Bureau, 2003). The higher the value of RQ, the higher the risk and the corresponding score to assign to each CEC. A commonly used ranking criterion is that defined by (Hernando et al., 2006): if $RQ < 0.1$ the risk to aquatic organisms is low, if $0.1 \leq RQ \leq 1$ the risk is medium; if $RQ > 1$, the risk is high.

An environmental risk assessment by means of RQ is carried out for all the compounds included in the dataset and the results are reported in Table S19 where all the compounds are ranked according to the descending order of RQ. Table S19 also includes the final OPBT score for all the compounds. It emerges that: (*i*) if RQ is the only criterion considered in the CEC selection, the final list contains 110 compounds characterised by a RQ > 1 for which the level of concern is the same as no score being assigned to this criterion; (ii) in the first 25 CECs of this list there are only 6 compounds selected according to the proposed methodology: ibuprofen, diclofenac, ciprofloxacin, amoxicillin, azithromycin and iopromide.

Table S20 refers to the selected 25 CECs by OPBT methodology. 19 out of the 25 CECs exhibit a RQ >1. The RQ approach gives priority to compounds with an occurrence greater than their PNEC, irrespective of the PNEC value: this is the case for PFOS characterised by an O score equal to 1 (very low concentration), a T score of 5 (very high toxicity) and a final OPBT score equal to 14 (not among the compounds with the highest final score). Its RQ value was instead >1 and for this the compound is considered of high risk.

A similar comparison was carried out in (Daouk et al., 2015) with regard to hospital effluent. If the toxicity T is included among the criteria, CECs with the lowest values of toxic concentrations are more critical: a high score is assigned to a CEC with a very low PNEC value (as shown in Table 2 if PNEC <0.1 μg/L the assigned score is 5). If instead RQ is included, more critical CECs are those with higher RQ values which may be due to a high concentration and not necessarily to a very low PNEC.

In the recent study by (Di Marcantonio et al., 2023) in the RQ evaluation due to release, the dilution effect of the surface water body receiving the treated effluent is considered. The equation thus becomes:

$$RQ_D = \frac{c_i/D}{PNEC_i} \tag{4}$$

where D is the dilution factor (if unknown a default value of 10 is suggested by (European Chemicals Bureau, 2003).

Consequently, the number of compounds with a RQ >1 will reduce depending on the value of the adopted D.

4.3. Weighting the criteria

In the current methodology, each criterion is considered of the same importance. The definition of the weight w is a relatively complex issue and it is generally based on the experts' judgments, according to the relevance of each criterion (Ortiz de García et al., 2013). Sometimes, the same weight was assigned to each criterion to avoid any judgement bias (Kumar and Xagoraraki, 2010; Li et al., 2019; Mansour et al., 2016; Sui et al., 2012). When criteria have a different influence, unequal weight values have to be set for them. Their definition may follow different approaches. For instance, (Guo et al., 2021) assigned $w = 0.5$ to occurrence and detection frequency, $w = 1$ to the environmental fate-related criteria (biodegradation, bioaccumulation and volatilization) and $w = 1.5$ to carcinogenicity, mutagenicity and teratogenicity. (Daouk et al., 2015) arbitrarily set that $w = 1$ if no data are available, 2 if modelled data are available and 3 if the values are from experimental investigations. In another study, (Golbaz et al., 2021) defined weights by means of the entropy function: referring to the values of a specific criterion, the greater their dispersion degree, the greater the differentiation degree, and more information can be derived. As a result, a higher weight has been given to the criterion, and vice versa.

In order to evaluate which criterion most influences the final ranking list, a sensitivity analysis is required. In this context, (Mansour et al., 2016) evaluated the effect of an individual criterion by varying the weights assigned to the different criteria and analysing the resulting final ranking lists. They found that out of the 69 selected compounds, only 9 were common to the different lists.

In (Ortiz de García et al., 2013) the sensitivity analysis was carried out for the weights assigned to the criteria (persistence, bioaccumulation and toxicity) in order to verify the influence and the changes in the resulting compound ranking list. They compared 8 different combinations of weights and only 6 compounds were always included in the different scenarios.

4.4. Uncertainty analysis

(Sui et al., 2012) carried out an uncertainty analysis of the data in assigning a score to each criterion and to the final score. They also provided the overall uncertainty for each compound with regard to any of the three considered criteria (consumption, removal and ecological effects). (Kumar and Xagoraraki, 2010; Li et al., 2019) expressed the uncertainty by assigning for each CEC and each criterion 0.5 if the value was missing and 0 if available. Then, they multiplied the uncertainty factor with the assigned weight to obtain the effective criterion uncertainty for the CEC.

In (Zhong et al., 2022), uncertainty scores were assigned to the occurrence depending on the availability of data and in accordance with the thresholds suggested by (Dulio and Ohe, 2013). As to ecotoxicity and human health effects, they assigned an uncertainty score equal to 0 if they were from experimental evaluation, 0.25 if they were from in silico evaluation; and 0.5 if data were not available. For all the criteria for which chemical data are available, an uncertainty equal to 0 is assigned and where they are not available, a default score (0.5) is assigned. The uncertainty associated to the final score for a compound is evaluated as the arithmetic mean of the individual scores referring to the specific criteria.

5. Suggestions for further research and final considerations

There is a need for studies suggesting short lists of CECs to be included in regular monitoring programs in reuse projects in order to guarantee the use of safe reclaimed water and to safeguard the environment and edible crop.

Future efforts should fill the lack in knowledge still present in the field. In particular, they should include not only pharmaceuticals, but also other categories. Thus, further investigations on a wider spectrum of CECs are expected in order to include measured concentrations and not predicted ones. This is in agreement with the recommendation by the NORMAN Association (Dulio and Ohe, 2013). In this context, it is important to bear in mind that the persistence profiling of selected CECs may vary considerably between treatment types, but also even within the same treatment type, as many biotic and abiotic factors may influence their fate during treatment. For example, considerable seasonal variations in CEC concentrations and removal efficiencies are recorded in WWTPs due to changes in CEC consumption patterns, climatic factors, as well as potential changes in treatment plant operation. For this reason, each study area, where an advanced treatment technology will be applied, should include temporal profiling of the organic and microbial CEC reduction. This would help to establish the best-suited surrogate chemical and microbial markers that can evaluate the treatment performance of the applied technology. It would also take into consideration the defined biotic/abiotic factors of the specified setting that influences the success of the new treatment technology.

Regarding the risk assessment, it is worth noting that establishing a single defined PNEC value for each CEC is challenging since CECs may interact differently with ~~sentinel~~ organisms. Furthermore, the sub-lethal adverse health outcomes should be considered that are more complicated to establish or that are less regulated in water quality policies. This includes adverse outcomes such as endocrine disruption that can present a large range of physiological health and reproductive complications, as well as endpoints such as the behavioural change that impacts predation and predator avoidance in aquatic organisms (eventually having harmful effects at population level). Moreover, the large challenge of evaluating CEC mixture interactions in toxicological outcomes (lethal or sub-lethal) is extremely important for future risk characterisation for the performance of treatment technologies and the fate of treated water used for potable- or non-potable reuse. However, this is something that will only be possible to be done on a site-specific manner, as the CEC "cocktail" will vary considerably between locations. Furthermore, for microbial CECs, the relative health risks associated with ARGs, which may or may not confer resistance, needs to be evaluated (Abramova et al., 2022). This is necessary in order to determine the relevance of each ARG and to rank ARGs by their risk to human health. The risk ranking will also facilitate the selection of suitable indicator ARGs for assessing effectiveness of interventions against AMR spreading and general monitoring of the AMR in the environment.

Researchers should also extend the risk assessment to human health and also to CEC transformation products, by-products and/or metabolites which are currently largely ignored for setting up priority lists due to the limited eco-toxicological information available for such products. Merely reporting on the removal or reduction of parent CECs from treatment technologies may undermine efforts to improve on the evaluation of treatment technologies that aim to produce reclaimed water sources that are safe for potable- and non-potable reuse. Since many pharmaceutical metabolites will rather be excreted after their consumption, along with many pharmaceutical and pesticide metabolites that are shown to have higher physiological properties than their parent compounds, we recommend that future selection criteria should include such CEC transformation products as such information becomes increasingly available.

Routine evaluation of priority CECs in a study area will also allow for the medium- to long-term evaluation of risk quotients over a temporal scale, thus enabling to determine the frequency of risk quotient exceedance for the target CECs (Archer et al., 2023; Liu et al., 2020). Through this estimation, more defined target CECs can be established for a more detailed investigation on the health impacts of their transformation products and/or metabolites.

Finally, the application of wastewater-based epidemiology is recommended in settings where treatment technologies are being evaluated (such as at WWTPs). This would hold an added advantage to gain a higher understanding of community-wide CEC consumption patterns in the defined catchment area that assist with the selection criteria as mentioned in Section 2.2 for microbial CECs (addressing antimicrobial resistance).

CRediT authorship contribution statement

Paola Verlicchi: Conceptualisation, Methodology; Data curation; Writing original draft; Review and editing; Supervision; Project administration; Funding acquisition. **Vittoria Grillini**: Methodology; Data curation; Writing original draft, Review and editing; Visualisation. **Engracia Lacasa**: Data curation; Writing original draft; Review and editing; Visualisation. **Edward Archer**: Writing original draft; Review and editing. **Pawel Krzeminski**: Writing original draft; Review and editing. **Vitor Vilar**: Review and editing. **Ana Gomes**: Review and editing. **Manuel Andrés Rodrigo**: Review and editing. **Jan Gäbler:** Review and editing. **Lothar Schäfer**: Funding acquisition; Methodology; Review and editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162359.

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