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Multi-target assessment of advanced oxidation processes-based strategies for indirect potable reuse of tertiary wastewater: Fate of compounds of emerging concerns, microbial and ecotoxicological parameters

S. Murgolo [a], O. De Giglio [b], C. De Ceglie [a], F. Triggiano [b], F. Apollonio [b], C. Calia [c], C. Pousis [c], A. Marzella [c], F. Fasano [b], M.E. Giordano [d], M.G. Lionetto [d,e], D. Santoro [f], O. Santoro [g], S. Mancini [g], C. Di Iaconi [a], M. De Sanctis [a], M.T. Montagna [b], G. Mascolo [a,h,*]

[a] Consiglio Nazionale delle Ricerche (CNR), Istituto di Ricerca Sulle Acque (IRSA), Via F. De Blasio 5, Bari, 70132, Italy
[b] Interdisciplinary Department of Medicine, Hygiene Section, University of Bari Aldo Moro, Piazza G. Cesare 11, 70124, Bari, Italy
[c] Department of Biomedical Science and Human Oncology, University of Bari Aldo Moro, Piazza G. Cesare 11, 70124, Bari, Italy
[d] Department of Biological and Environmental Sciences and Technologies, University of Salento, Via Provinciale Lecce-Monteroni, 73100, Lecce, Italy
[e] National Biodiversity Future Center (NBFC), Palermo, 90133, Italy
[f] Department of Chemical and Biochemical Engineering, Western University, London, N6A 5B9, Ontario, Canada
[g] AquaSoil S.r.l., Via del Calvario 35, 72015, Fasano, Brindisi, Italy
[h] Consiglio Nazionale delle Ricerche (CNR), Istituto di Ricerca per La Protezione Idrogeologica (IRPI), Via Amendola 122 I, Bari, 70126, Italy

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ABSTRACT

Two advanced oxidation processes (AOPs), namely ozone/H_2O_2 and UV/H_2O_2, were tested at pilot scale as zero-liquid-discharge alternative treatments for the removal of microbiological (bacteria and viruses), chemical (compounds of emerging concern (CECs)) and genotoxic responses from tertiary municipal wastewater for indirect potable reuse (IPR). The AOP treated effluents were further subjected to granular activated carbon (GAC) adsorption and UV disinfection, following the concept of multiple treatment barriers. As a reference, a consolidated advanced wastewater treatment train consisting of ultrafiltration, UV disinfection, and reverse osmosis (RO) was also employed.

The results showed that, for the same electrical energy applied, the ozone/H_2O_2 treatment was more effective than the UV/H_2O_2 treatment in removing CECs. Specifically, the ozone/H_2O_2 treatment, intensified by high pressure and high mixing, achieved an average CECs removal efficiency higher than UV/H_2O_2 (66.8% with respect to 18.4%). The subsequent GAC adsorption step, applied downstream the AOPs, further improved the removal efficiency of the whole treatment trains, achieving rates of 98.5% and 96.8% for the ozone/H_2O_2 and UV/H_2O_2 treatments, respectively. In contrast, the ultrafiltration step of the reference treatment train only achieved a removal percentage of 22.5%, which increased to 99% when reverse osmosis was used as the final step.

Microbiological investigations showed that all three wastewater treatment lines displayed good performance in the complete removal of regulated and optional parameters according to both national and the European Directive 2020/2184. Only *P. aeruginosa* resulted resistant to all treatments with a higher removal by UV/H_2O_2 when higher UV dose was applied. In addition, *E. coli* STEC/VTEC and enteric viruses, were found to be completely removed in all tested treatments and no genotoxic activity was detected even after a 1000-fold concentration.

The obtained results suggest that the investigated treatments are suitable for groundwater recharge to be used as a potable water source being such a procedure an IPR. The intensified ozone/H_2O_2 or UV/H_2O_2 treatments can be conveniently incorporated into a multi-barrier zero-liquid-discharge scheme, thus avoiding the management issues associated with the retentate of the conventional scheme that uses reverse osmosis. By including the chemical cost associated with using 11–12 mg/L of H_2O_2 in the cost calculations, the overall operational cost (energy plus chemical) required to achieve 50% average CECs removal in tertiary effluent for an hypothetical

* Corresponding author. Consiglio Nazionale delle Ricerche (CNR), Istituto di Ricerca Sulle Acque (IRSA), Via F. De Blasio 5, Bari, 70132, Italy.
E-mail address: giuseppe.mascolo@cnr.it (G. Mascolo).

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full-scale plant of 250 m^3/h (or 25,000 inhabitants) was 0.183 €/m^3 and 0.425 €/m^3 for ozone/H_2O_2 and UV/H_2O_2 treatment train, respectively.

CRediT statements

Sapia Murgolo: Conceptualization, Methodology, Investigation, Data Curation, Writing. **Osvalda De Giglio**: Conceptualization, Methodology, Investigation, Data Curation, Supervision, Project administration, Writing, Reviewing and Editing. **Cristina De Ceglie**: Conceptualization, Methodology, Investigation, Data Curation. **Francesco Triggiano**: Methodology, Investigation, Formal analysis. **Francesca Apollonio**: Methodology, Investigation. **Carla Calia**: Methodology, Investigation. **Chrysovalentinos Pousis**: Methodology, Investigation. **Angelo Marzella**: Methodology, Investigation. **Fabrizio Fasano**: Methodology, Investigation. **Maria Elena Giordano**: Methodology, Investigation, Data Curation. **Maria Giulia Lionetto**: Methodology, Data Curation, Writing. **Domenico Santoro**: Conceptualization, Methodology, Investigation, Data Curation, Validation, Formal Analysis, Resources, Writing, Reviewing and Editing, Supervision, Project administration. **Oronzo Santoro**: Funding acquisition, Project administration. **Sergio Mancini**: Methodology, Investigation, Data Curation, Formal analysis, Resources. **Claudio Di Iaconi**: Conceptualization, Methodology, Supervision, Project administration. **Marco De Sanctis**: Methodology, Investigation. **Maria Teresa Montagna**: Conceptualization, Methodology, Writing, Reviewing and Editing Supervision, Project administration. **Giuseppe Mascolo**: Conceptualization, Methodology, Investigation, Data Curation, Validation, Formal Analysis, Resources, Writing, Reviewing and Editing, Supervision, Project administration.

1. Introduction

Freshwater resources are essential for human life and sanitation. However, as episodes of droughts and water scarcity become increasingly frequent, assessment of alternative water supply options for drinking water purposes is deemed essential due to the increased water demand associated with population growth and climate change (Jeffrey et al., 2022). Furthermore, overexploitation of water resources can lead to severe drawbacks, especially in regions lacking large freshwater bodies such as those of the Mediterranean basin. Even when accessible, freshwater resources are affected by a wide range of contaminants with presence of arrays of compounds of emerging concern (CECs) including pharmaceuticals, personal care and household chemicals, antibiotic-resistant bacteria/genes, that are currently discharged by wastewater treatment plants (WWTPs) into receiving water bodies (Rapp-Wright et al., 2023). As a result, water reuse has been regarded as an effective strategy to mitigate issues related to water scarcity and droughts. Advanced wastewater treatments (prior to reuse) plays a crucial role in this strategy, as it represents a barrier to remove a wide range of contaminants prior to wastewater reclamation. Among the most common treatment options, ozone-based and UV-based advanced oxidation processes are reported as the most cost-effective ones (Rizzo et al., 2019). Therefore, as AOPs are useful to minimize the presence of organic contaminants it follows that when the water would be reused the final disinfection often performed by chlorination would also lead to a lower content of by-products that are formed during the chlorination step (Lopez et al., 1997).

Up to recently, the only form of planned wastewater reuse was for non-potable applications, such as municipal and agricultural irrigation and industrial reuse including cooling water, car wash facilities, fire-fighting, public fountains, stream flow augmentation to control seawater intrusion (Meneses et al., 2010; Vivaldi et al., 2022). However, with the continuous improvement of wastewater effluent quality due to tertiary treatments for nutrients and pathogens control to protect sensitive receiving bodies, the possibility of indirect potable reuse (IPR) and direct potable reuse (DPR) applications using tertiary treated effluents (alone or blended with surface water or groundwater) as drinking water intake waters has been opened (Drewes and Horstmeyer, 2016; Haarhoff and Van der Merwe, 1996). As a result, planned potable water reuse has been increasingly recognized as an important supply option in regional water resource management plans, with a growing number of IPR and DPR projects initiated worldwide (Gerrity et al., 2013; Jeffrey et al., 2022). IPR (planned, or de-facto) has widespread more than DPR, with tertiary effluents first introduced into an environmental buffer of suitable size and retention time, and then blended with conventional drinking water resources prior to potabilization. (Drewes et al., 2012; Fajnorová et al., 2021). In Europe, including Italy, it is not yet applied due to the lack of coherent legislation and in favor of this practice (De Giglio et al., 2018).

Indeed, both IPR and DPR applications require a rigorous assessment of the reliability of the treatment trains employed prior to potable reuse together with a comprehensive assessment of water quality produced, to minimize public health risks associated with the presence of CECs, pathogens, and genotoxic compounds in drinking water for human consumption. This is especially important in consideration of unintended IPR practices (often referred as “de facto reuse”) which, while unavoidable (Council, 2012; Paul, R.J.A.-R.J.A.-W.A.A.-W., 2013) and favored by natural attenuation mechanisms able to reduce CECs and pathogens in aquifers and groundwaters, can still lead to unintended consequences. As a result, redundancy offered by multi-barrier treatment trains in wastewater treatment plants has been recommended (Haas and Trussell, 1998).

While protection of human health is typically guaranteed by compliance with water quality standards set for drinking water produced from conventional surface water, the use of reclaimed wastewater raises new issues associated with the presence of trace level of CECs and other wastewater-specific emerging issues including resistant viruses, antibiotic resistance bacteria/genes and genotoxicity (Cacace et al., 2019; Krzeminski et al., 2019). Thus, simplified monitoring strategies are not adequate for DPR and IPR applications, as they do not consider a comprehensive assessment of the above-mentioned issues.

Although several studies have been carried out about advanced wastewater treatment systems and the assessment of the risks connected with their employment for water reuse in drinking water supply (Di Guardo et al., 2024; Kim et al., 2023), there are no regulations on water reuse at EU level other than the Urban Wastewater Directive (European Commission, 2005) which states that “treated wastewater should be reused whenever appropriate”.

In this paper, a pilot-based comprehensive study was carried out to assess ozone-based and UV-based advanced oxidation processes (AOPs), followed by GAC adsorption, as strategies for advanced treatment of tertiary wastewater effluents for IPR. The study was carried out at the municipal water reuse reclamation facility (WRRF) of Fasano (Brindisi, Italy). Specifically, a pilot-scale set up consisting of three parallel treatment lines, namely ozone/H_2O_2, UV/H_2O_2 and ultrafiltration/reverse osmosis (UF/RO), was operated with tertiary treated effluent as shown in Fig. 1. The goal of our study was to assess the efficacy of ozone-based and UV-based AOPs as pre-treatment prior to GAC adsorption, to determine if such zero-liquid-discharge solutions were able to produce an effluent with chemical and microbiological characteristics in compliance with IPR requirements.

The assessment was not limited to drinking water regulated parameters, but also extended to a wide range of chemical, microbiological, genotoxic compounds. As for chemical quality, CECs concentrations were monitored in line with the Commission Implementing Decision (EU) 2022/1307 (European Commission, 2022). Specifically, the

microbiological quality was assessed against drinking water standards coming from both the Italian regulation for drinking water (Legislative Decree, 2001) awaiting implementation of the new EU Directive 2020/2184 on the quality of water intended for human consumption (Dettori et al., 2022). An array of bioassays was also deployed to check the response of the treated water against the potential presence of mutagenicity and genotoxicity compounds.

2. Experimental section

2.1. The pilot plant, sampling strategy and energy cost calculation

The pilot plant was installed and operated at the WRRF of Fasano, Brindisi, Italy. The influent wastewater feeding the pilot plant was obtained from the tertiary stage of the adjacent full-scale municipal WWTP treating an average flow rate of 6000 m^3/d and consisting of a conventional primary settler followed by an activated sludge system and a tertiary step by integrated coagulation (aluminum polychloride), sedimentation in lamella clarifiers and disinfection by sodium hypochlorite for unrestricted agricultural reuse purposes. The tertiary treated wastewater is then stabilized into a large environmental buffer (lagoon) being also the reservoir used for directing the excess resource to experimental groundwater recharge studies. The average composition of the tertiary treated wastewater is listed in Table 1.

The pilot plant (Fig. 1) included three parallel lines, namely two initial AOPs by ozone/H_2O_2 (Line A) and UV/H_2O_2 (Line B), and one reference line (Line C) employed as a baseline using the more conventional and widely adopted membrane-based concept of ultrafiltration followed by reverse osmosis (with UV disinfection in between to reduce the fouling potential of the RO unit). More specifically, Line A employed the intensified ozone/H_2O_2, operated by the patented MITO3X® technology (AquaSoil, Fasano, Italy) whose details are illustrated elsewhere (Piras et al., 2020). Such technology, which uses a 400 W ozone plasma generator (Primozone, Sweden), allows the simultaneous dosage of O_3 and H_2O_2 under high mixing (mixing speed = 60 Hz or 3600 rpm, velocity gradient G > 100,000 s^{-1}) and high pressure (>2 bar) to enhance mass transfer and reaction rates. After the simultaneous dosage of O_3 and H_2O_2 under high mixing a settler of 0.9 m^3 was placed. Line B employed a more classical AOP-based solution based on the use of a UV 254 nm photoreactor (VIQUA model SHF-140/2, Guelph, Canada) of 0.019 m^3 volume equipped with four low-pressure mercury lamps for a total power 440 W. The employed experimental conditions for UV/H_2O_2 were chosen based on the electrical energy reported in the literature for achiving CECs removal in water matrix having similar UV 254 transmittance of that employed in the present investigation (Sgroi et al., 2021). Then, the power of the ozone generator was chosen in order to operate at the same electrical power of the UV lamps.

The GAC employed in lines A and B was SV50 carbon (SICAV Spa, Gissi-Chieti, Italy) that is characterized by cylindrical shape, 4 mm grain size, apparent density 500 kg/m_3, iodine index >1000 mg/g and specific surface >1050 m^2/g. The identical GAC adsorption steps of lines A and B were designed in order to have a modular system were different empty

Table 1
Average Influent and effluent concentrations of the main gross parameters of the employed wastewater, in terms of mean values and standard deviations.

Parameter	Unit	Sampling point		
		1 (influent)	A1 (effluent ozone/H_2O_2)	B1 (effluent UV/H_2O_2)
COD	mg L^{-1}	56.5 ± 11	54 ± 11	52 ± 11
TC	mg L^{-1}	85 ± 5	80 ± 6	79 ± 5
IC	mg L^{-1}	60 ± 3	59 ± 3	60 ± 3
NH_4–N	mg L^{-1}	27 ± 2	28 ± 2	27 ± 2
NO_3–N	mg L^{-1}	1.7 ± 0.2	6 ± 0.5	3 ± 0.4
NO_2–N	mg L^{-1}	1.1 ± 0.1	0.2 ± 0.05	0.4 ± 0.05
TN	mg L^{-1}	62 ± 7	38 ± 4	38 ± 4

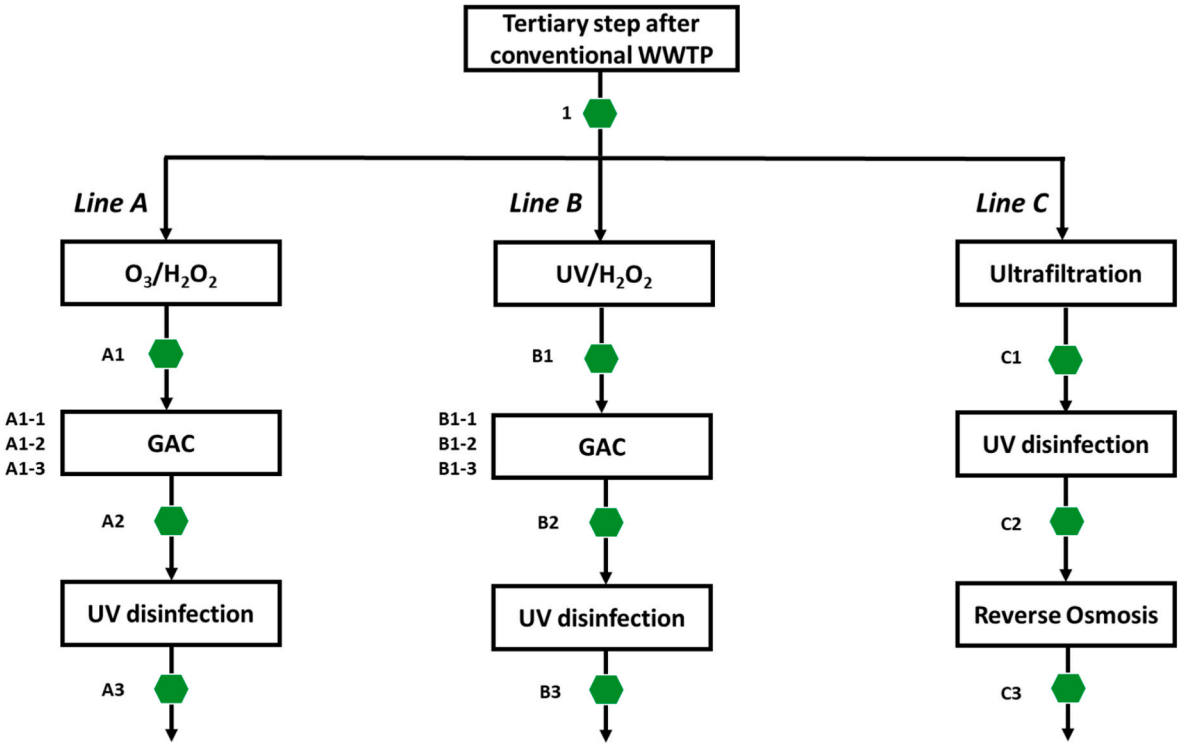


Fig. 1. Experimental design of advanced (lines A and B) and conventional (line C) wastewater treatment combinations investigated in this study.

bed contact times (EBCT) could be tested. Specifically, the GAC step consisted of seven consecutive GAC cells each one having an EBCT of 16 min at a flow rate of 1.2 m^3/h.

The rationale for selecting these specific treatment trains stems from their zero liquid discharge capability, absence of brine or waste production, and suitability for both coastal and inland implementation of IPR. These treatment trains leverage the combined effects of multiple treatment processes, thereby enhancing the multi-barrier approach necessary for wastewater reuse applications. In this study, two lines based on AOP were employed, followed by a modular GAC filtration system equipped with multiple sampling ports between modules. UV 254 disinfection, operating in disinfection mode, was also included in each line. Both lines were supplied with the same tertiary treated pilot influent, exhibiting an average UV 254 transmittance of 68.8%. The operational stability and performance of these treatment trains were evaluated over an extended period at a constant flow rate, with three key sampling points (A1-3 and B1-3) being assessed. Additionally, the GAC filtration system was characterized along its length, incorporating three additional sampling points located between the GAC modules. These comprehensive evaluations provide valuable insights into the efficiency and reliability of the treatment trains, highlighting their suitability for sustainable water reuse initiatives.

The investigation consisted of two consecutive experimental phases whose operative conditions are listed in Table 2. The first experimental phase lasted five months during summer-autumn seasons whereas the second one lasted two months during winter season. The rationale of having the second experimental phase was to investigate at least two operating conditions for each line, in an attempt to verify with two different UV doses the performance of UV-based AOP pre-treatment installed in Line B (while keeping the ozone and peroxide dosage in the Line A nearly unchanged). In both experimental phases, composite 4-h samples were collected from all sampling points. It was also verified that average composite samples of 4 and 24 h yielded nearly identical results. Where present, residual oxidants (H_2O_2, O_3) were quenched by 10 mg/L of sodium bisulfite. It was found that residual concentration of H_2O_2, O_3 measured at the exit of both UV and ozonation was always lower than 1 mg/L. For microbiological and toxicological parameters, instantaneus samples were collected.

The assessment of energy cost of the two multi-barrier, zero-liquid discharge treatment trains was carried out by projecting the dose required to achieve 50% average removal of CECs as AOP pre-treatment to a hypothetical, full-scale installations of 250 m^3/h which corresponds to an average wastewater treatment plant in Apulia (IT) of approximatively 25,000 inhabitants.

The components exerting energy demand of ozone/H_2O_2 step are two centrifugal pumps required by the MITO3X® system, a peristaltic pump delivering the required H_2O_2 dose, and a 400 W ozone generator with the ozone chiller. In aggregate, these components (operated to deliver an ozone dose of 7.5 mg/L required for 50 % CECs removal) are expected to exert an electrical energy demand of 0.21 kWh/m^3 corresponding to an electrical energy per order of 0.7 kWh/m^3/log. Similarly, the treatment train employing UV/H_2O_2 includes one centrifugal pump required by the VIQUA® photoreactor system, four 110 W low-pressure monochromatic UV lamps used to deliver the UV dose at 254 nm, and a peristaltic pump to deliver the required H_2O_2 dose. In aggregate, these components (operated to deliver a UV dose ranging from 275 to 366 mJ/cm^2) when projected to the dose required for 50 % CECs removal, are expected to exert an electrical energy demand of 1.18 kWh/m^3 corresponding to an electrical energy per order of 3.90 kWh/m^3/log.

Table 2
Operative conditions employed in the three treatment lines during the two consecutive experimental phases. During the first phase the ozone generator was operated at the same electrical power of the UV lamps.

	unit	Line A MITOX® O_3/H_2O_2	Line B UV/H_2O_2	Line C Ultrafiltration
First phase				
Flow rate	m^3/h	1.2	1.2	0.7
Ozone dose	mg/L	13	–	–
H_2O_2 dose	mg/L	12	12	–
UV dose	mJ/cm^2	–	275	–
Second phase				
Flow rate	m^3/h	0.6	0.6	0.7
Ozone dose	mg/L	12	–	–
H_2O_2 dose	mg/L	12	12	–
UV dose	mJ/cm^2	–	366	–

2.2. Chemical determinations

Analytical standards of CECs were purchased from Labservice Analytica and Sigma-Aldrich (Italy). Solvents used for diluting the stock solutions of the analytical standards as well as for preparing the mobile phases of liquid chromatography were HPLC-MS grade. Ultrapure water used for preparing both the eluents of ultra-high pressure liquid chromatography (UPLC) and the analytical standard solutions (18.2 MΩcm, organic carbon <4 μg/L) was delivered by a MilliQ-ultra Gradient A-10 system (Merck-Millipore). Analytical working standard solutions of the selected CECs, employed for building the calibration curves, were prepared by properly diluting the relative stock solutions in ultrapure water. All other chemicals employed were analytical grade and used without further purification.

Bulk parameters analyses were carried out according to well assessed methods and details of the procedures are reported in Text SI-1. CECs determinations were performed by on-line solid phase extraction coupled with ultra-high resolution liquid chromatography/high resolution tandem mass spectrometry (SPE-UPLC-HR/MS/MS). Details also reported elsewhere (Montagna et al., 2020) as well as in Text SI-1.

The raw data obtained by the online SPE-UPLC-HR/MS/MS analyses of the influent wastewater feeding the pilot plant were processed for a suspect screening analysis using CECs list of the SusDat database (20 suspect lists comprising ~58,000 compounds) compiled by the Norman network (Alygizakis et al., 2019; Mohammed Taha et al., 2022). The screening was mainly based on exact mass, isotopic pattern and retention time index. The selected compounds were further screened comparing MS/MS fragmentation pattern of the suspect identified compounds with those of relative standard compounds, where available, analyzed employing the same analytical method. Finally, the CECs detected at concentration lower than 0.5 ng/L were discharged because not suitable to be followed during the treatment steps being the average limit of detection of the analytical method 0.05 ng/L which was strongly compound dependent. Accordingly, based on the aforementioned screening procedure, 29 CECs were selected to be followed throughout the present experimental investigation that are listed in Table 3.

The MS data processing using principal component analysis (PCA) was performed by MarkerView 1.3.1 software (AB-Sciex). PCA allows to reduce the overall data dimensionality based on a projection into a new orthogonal space (the principal components' one) retaining only two or three components accounting for the most substantial variance in a dataset. It is worth pointing out that the PCA processing results shown in the present work are a particular PCA known as cluster analysis. Specifically, a clustering method focuses on the analysis of (dis)similarities, whereas the general PCA attempts to explain as much variation as possible in as few components as possible. In addition, it is worth noting that the PCA is a simple method with a straightforward application, but the drawn conclusions are based only on the graphical evidence.

2.3. Microbiological determinations

Mandatory (total coliforms at 37 °C, *Escherichia coli*, Enterococci) and optional parameters (*Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococcus aureus*, *Clostridium perfringens, Enterovirus)* were detected

Table 3
List of CECs selected for the present investigation after suspect screening search performed on the influent wastewater, calculated LODs and average concentrations measured in the influent wastewater employed for the two experimental phases.

Category	CEC name	LOD of the employed method (ng L^{-1})	Average initial concentration (ng L^{-1})	
			First phase	Second phase
Analgesics	Lidocaine	1	70 ± 19	82 ± 13
	Tramadol	2	214 ± 48	111 ± 48
Antibiotics	Clarithromycin	5	39 ± 29	286 ± 190
	Levofloxacine	0.5	846 ± 346	3328 ± 374
	Trimethoprim	2	737 ± 674	950 ± 398
Anti-diabetic agents	Sitagliptin	1	867 ± 369	737 ± 55
Antiepileptic agents	Lamotrigine	1	188 ± 60	347 ± 121
Antifungal agents	Climbazole	0.5	119 ± 33	184 ± 87
	Fluconazole	1	126 ± 41	166 ± 67
Antihistamines	Cetirizine	1	113 ± 57	149 ± 17
Antiplatelets	Clopidrogel	0.5	24 ± 9	33 ± 13
Antipsychotics	Amisulpride	1	165 ± 82	136 ± 21
	Sulpride	1	346 ± 141	200 ± 16
Fungicide	Carbendazim	2	25 ± 19	40 ± 4
Heart-related disorders	Atenolol	0.5	211 ± 82	277 ± 57
	Bisoprolol	1	121 ± 55	138 ± 19
	Flecainide	0.5	1066 ± 583	1256 ± 123
	Losartan	1	476 ± 77	339 ± 52
	Metoprolol	1	118 ± 47	148 ± 25
	Sotalol	2	158 ± 78	205 ± 49
	Telmisartan	2	1157 ± 337	2575 ± 823
	Valsartan	5	2704 ± 1485	1573 ± 566
Hypertension treatments	Irbesartan	1	2222 ± 470	1463 ± 164
Lipid regulators	Atorvastatin	2	192 ± 122	126 ± 39
	Fenofibric acid	2	544 ± 270	193 ± 76
Psychiatric drugs	Carbamazepine	1	440 ± 87	659 ± 76
	Venlafaxine	1	193 ± 68	180 ± 66
Stimulant	Caffeine	5	564 ± 467	1917 ± 780
Transformation product	Carbamazepine 10,11-epoxide	1	326 ± 70	106 ± 43

in the treated samples according to Italian regulation (Legislative Decree, 2001). It should be mentioned that italian law is being reviewed to align its requirements with the new European Directive 2020/2184 on the quality of water intended for human consumption. *E. coli* producers of Shiga-like toxin or verocytotoxin (STEC/VTEC) were also monitored. During each sampling, 2 L water samples were collected in accordance with ISO 19458:2006 in sterile bottles with sodium thiosulphate pentahydrate (0.01%, w/v), to neutralize disinfectants present in water samples. All samples were transported at 4 °C and processed within 5 h. Specific aliquots of each sample were filtered through a cellulose ester membrane of 0.45 μm pore size (Millipore, Italy).

Determinations were carried out according to available methods, namely ISO 9308–1:2017 for E. coli/coliform, ISO 7899–2:2003 for Enterococchi, ISO 14189/2013 for *C. perfringens*, APAT and IRSA-CNR, 2003 for Salmonella, Rapporti Istisan 07/5 for *S. aureus*, UNI EN ISO 16266:2008 for *P. aeruginosa*. Details of the procedures are reported in Text SI-2.

The detection of *E. coli* STEC/VTEC was performed by molecular methods. One liter of sample was filtered, enriched in 100 mL of buffered peptone water (BPW) and incubated overnight at 36 ± 2 °C. The DNA was extracted from the enrichment and tested by real time polymerase chain reaction (PCR) PCR-iQ-Check STEC PCR Detection Kits (BioRad, Hercules, USA) for the Shiga-toxin genes (stx1 and stx2) and for the eae gene (intimin), an additional virulence marker (Bonetta et al., 2016). If the sample was positive for stx1/2 and eae followed a Real Time PCR with the iQ-Check® STEC SerO kit (BioRad, Hercules, USA) to allow the detection of the seven main serotypes: O156:H7, O26, O45, O103, O111, O121 and O145. At the same time, cultural investigation was performed (Morris et al., 2016).

As for virus detection, during the two experimental phases, molecular methods were used to detect enterovirus (EV), according to Italian regulation (Legislative Decree, 2001), and other non-regulated viruses, namely adenovirus (AdV), norovirus genogroups I and II (NoV-I and NoV-II), hepatitis A and E (HAV and HEV), rotavirus (RoV), and pepper mild mottle virus (PMMoV). Treated wastewater samples (100 L) were filtered by Nanoceram electropositive cartridges (Argonide Corporation, Sanford, USA) following the virus adsorption-elution technique (VIRADEL) (Iaconelli et al., 2015). For each sample, before concentration, a murine norovirus (MNV-1) was added to all samples as a sample process control to measure the recovery efficiency and to detect the presence of inhibitors (1 mL, 8.81×10^4 genome copies). After filtration, each cartridge was eluated with 400 mL of 3% beef extract (pH 9.5) by direct contact in the housing and stirred for 20 min. The cartridge was then removed, and the eluate neutralized with 1.2 M NaOH. The secondary concentration step was performed by precipitation with polyethylene glycol PEG. In particular, PEG 6000 and NaCl were added at final concentrations of 10 % and 1.6 % w/v, respectively. The suspension was incubated at 4 °C overnight and then centrifuged 8650*g* at 4 °C. The final pellet was dissolved in 10 mL of phosphate buffered saline (PBS) pH 7.4.5 mL was used for nucleic acid extraction, another 5 mL stored at −20 °C. Nucleic acids were extracted using the NucliSENS miniMAG semi-automatic extraction system with magnetic silica, according to the manufacturer's instructions (bioMerieux, Marcy-l'Etoile, France). The RNA was resuspended in 100 μL of elution buffer, and the extracts were kept at −80 °C until use.

These viruses were detected by reverse transcription-nested PCR, following the method described by Bonanno Ferraro et al. (Bonanno Ferraro et al., 2021).

Nested PCR amplicons were visualized by gel electrophoresis on 2 % agarose gel containing GelRed stain (Biotium, CA, USA), then purified using a Montage PCRm96 Microwell Filter Plate (Millipore, MA, USA) and sequenced on both filaments by Bio-Fab Research (Rome, Italy). The sequences were compared with the sequences available in the GenBank database using BLAST (Suparyanto dan Rosad (2015, 2020).

2.4. Genotoxicity determinations

Genotoxicity was assessed by SOS chromotest, based on the determination of the SOS repair response activated by bacterial cells for repairing DNA damages induced by genotoxic contaminants (Quillardet and Hofnung, 1985). The test was carried out using EBPI SOS ChromotestTM kit (Environmental Bio-Detection Products, EBPI, Missisauga,

Ontario, Canada) based on an engineered *Escherichia coli* strain (strain PQ37) (EBPI, 2008), in which the β-galactosidase (β-gal) gene (lacZ) is fused to the bacterial sfiA SOS operon. Therefore, the β-gal induction, which can be quantified photometrically using a chromogenic substrate, is thus representative of SOS induction and, in turn, indicative of genotoxicity. The parallel measurement of the activity of alkaline phosphatase (AP) was used for the assessment of bacterial vitality. 4-nitroquinoline-N-oxide was used as a positive genotoxic standard to test the validity of the assay. The ratio between β-gal and alkaline phosphatase activities was defined as the induction factor (IF), and it was used to express the extent of SOS induction for the tested sample. IF values > 1.5 are considered genotoxic according to (Quillardet and Hofnung, 1985). More specifically, IF values included in the range 1.5 > IF > 2 are referred to as slightly genotoxic, while IF values > 2 are considered strongly genotoxic (Mersch-Sundermann et al., 1996).

Prior to the test, the samples underwent solid phase extraction (SPE) according to already published procedures in order to further increase the sensitivity of the bioassay (Piras et al., 2020). Each sample (1 L) was pre-filtered at 0.45 μm and then passed through a LiChrolut EN® cartridge (200 mg, Merck, Darmstadt, Germany) at a flow rate of 8 mL min-1 using a 12-port vacuum manifold (LiChrolut® extraction unit, Merk). The cartridge was pre-conditioned with methanol, followed by acetone, and then ultrapure water. The cartridge was finally eluted with 5 mL of acetone which was evaporated under a nitrogen stream. The extract was resuspended in 100 μL DMSO and then used for the analysis. Three preconcentration factors were applied to each sample, namely 10X, 100X, and 1000X.

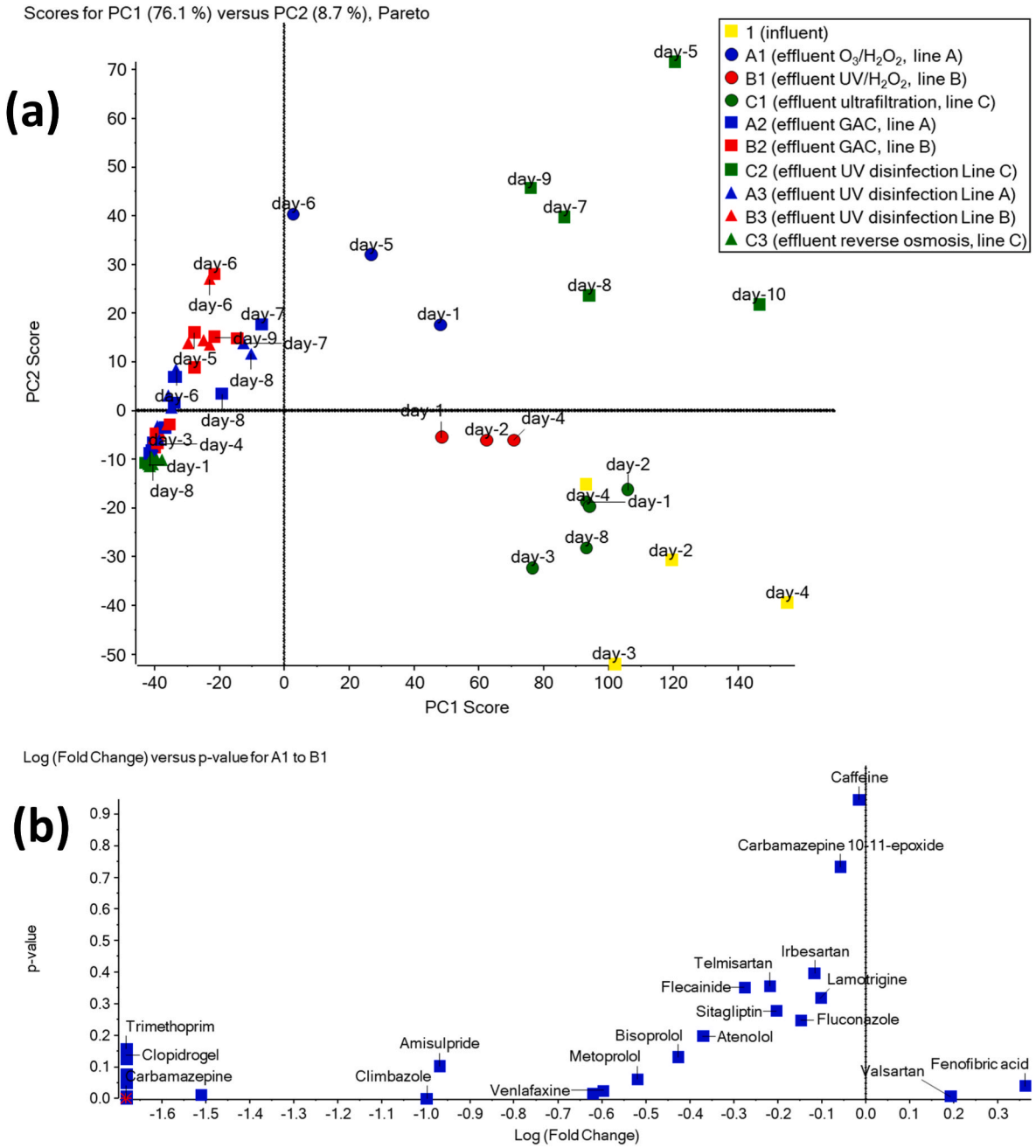


Fig. 2. (a): PCA score plot resulting from processing of the 29 monitored CECs in all sampling points of the first experimental phase. In the score plot selected replicate samples, taken at different sampling days, are shown. Sampling points are those displayed in Fig. 1. (b): *t*-test obtained by comparing samples A1 (effluent of ozone/H_2O_2) and B1 (effluent of UV/H_2O_2) showing the CECs mostly present in each effluent.

3. Results and discussion

3.1. Degradation of CECs

Initial CECs screening in the influent (tertiary) wastewater revealed the presence of a large number of compounds. Among those detected, the compounds list was restricted in order to have a consistent number of CECs to be monitored throughout the whole experimental campaign that fulfilled the criteria of (i) quantification based on the availability of authentic standard, (ii) initial concentration in the influent wastewater high enough in order to be able to quantify a removal factor across the treatment train of 50 times (i.e., a concentration of 50 times lower than in the pilot influent wastewater). Accordingly, 29 CECs were selected to be monitored in the two phases of the experimental campaign.

First experimental phase. The presence of a large number of CECs suggests the use of data reduction techniques, such as PCA that, as described in the experimental section, allows to reduce the overall data dimensionality based on a projection into a new orthogonal space (the principal components' one) retaining only two or three components accounting for the most substantial variance in a dataset. Fig. 2a depicts the PCA score plot resulting from processing the concentration values of the 29 monitored CECs obtained in all sampling points for a selected number of replicates. To improve clarity, the intermediate sampling points within the GAC adsorption of both ozone/H_2O_2 and UV/H_2O_2 treatment lines (between A1 and A2, and between B1 and B2) were excluded from data processing.

The score plot of Fig. 2a shows that after the PCA execution, columns were reduced to two major components (PC1 and PC2) which captured 84.8% of the variance of the original dataset (76.1% for PC1 and 8.7% for PC2). It follows that the main dissimilarities are located along the PC1 axis that holds most of the variance. A closer look at Fig. 2a reveals that C1 samples (the first step of conventional treatment, i.e., ultrafiltration) are very close to influent samples (sampling point n. 1). Conversely, the separation from sampling point 1 is evident for B1 samples (UV/H_2O_2 step) and even more for A1 samples (ozone/H_2O_2). Therefore, the dissimilarities against the influent samples (1) are increasingly evident in the order A1 > B1 > C1.

Fig. 2a also shows that A2 and B2 samples are all clustered in the same zone that is rather far from where influent samples (1) are located. C2 samples (UV disinfection) are clustered in another zone further separated from influent samples along the PC2 axis holding much lower variance. The samples of the final step, namely A3, B3 and C3, are all clustered together in the score plot of Fig. 2a. Overall, Fig. 2a shows that the first two steps of conventional treatment line lead to effluents being rather similar to influent samples. Conversely, first two steps of both ozone/H_2O_2 and UV/H_2O_2 lead to effluents A1 and B1, respectively, whose CECs composition is much different from influent samples. However, the final effluents of all treatment lines show to be similar in terms of CECs composition, namely all having very low residual CECs concentration whose little differences are depicted in Fig. 3 and S1. As expected, the final step (UV disinfection) of treatment lines A and B does not lead to significant differences in terms of CECs composition while the final step of treatment line C (reverse osmosis) allows the effluent to be very similar to the final ones of treatment lines A and B. Thus, reverse osmosis has a very similar effect as AOPs followed by GAC adsorption in terms of CECs effluent composition.

It is also possible to get further insights of PCA processing by comparing coupled samples using the *t*-test to determine which CECs are responsible for similarities and dissimilarities. In Fig. 2b, samples A1 (ozone/H_2O_2) and B1 (UV/H_2O_2) are compared using the *t*-test. In the *t*-test plot, negative data points located on the left-hand side (lowest log (fold change) and lowest p-value) belong to CECs that are mostly abundant in B1 samples. Conversely, positive data points located on the right-hand side (highest log (fold change) and lowest p-value) belong to CECs that are mostly abundant in A1 samples. Data points located in the middle of the plot belong to CECs that are abundant in both samples, namely they are responsible for the similarity of the two samples. Fig. 2b show that most CECs have negative values of Log (fold change) meaning that the relative concentrations in the B1 samples are higher than those of the A1 sample. Examples of such a finding are trimethoprim, sotalol, climbazole, tramadol, carbamazepine but most of the 29 monitored CECs showed higher abundance in B1 samples with respect to A1 ones. Only sitagliptin, carbamazepine 10,11-epoxide and fluconazole had similar abundance in A1 and B1 samples while fenofibric acid was the only CEC more abundant in A1 samples. These findings are quantitatively displayed in Fig. 3, which depicts the residual effluent concentrations of all sampling points for trimethoprim, sotalol, climbazole, venlafaxine (better removed by ozone/H_2O_2) and fenofibric acid (better removed by UV/H_2O_2) as well as for the total CECs. The specific plots of the remaining CECs are displayed in Fig. S1 of the Supplementary Material. The better performance of the ozone/H_2O_2 process is consistent with results already published showing that the required electrical energy to reduce the concentration by around 80 % for most of CECs is much lower for ozonation (0.33 kWh/m^3) with respect to UV/H_2O_2 (0.6–0.9 kWh/m^3) (Rizzo et al., 2019). The low removal efficiency of sitagliptin by ozone/H_2O_2 is likely due to its pH-dependent removal kinetics leading to incomplete removal even at high ozone doses of 1.7 and 0.9 mg O_3/mg_{DOC} (Hermes et al., 2020). As for the fenofibric acid (better removed by UV/H_2O_2), it has been reported that the presence of a carboxylic acid group in its chemical structure is responsible of its inertness to ozonation (Mathon et al., 2021).

Overall, plots in Fig. 3 show that ozone/H_2O_2 performs better in terms of CECs removal (64 ± 18 % removal) than UV/H_2O_2 (25 ± 12 % removal), and that the final effluent of the parallel treatment lines is practically identical. Specifically, the reverse osmosis of the third treatment line (conventional set-up) allows to remove most of CECs thus achieving what the previous two steps were not able to do.

Second experimental phase. The first experimental phase, in which the ozone generator was operated at the same electrical power of the UV lamps, demonstrated that ozone/H_2O_2 outperformed UV/H_2O_2 in removing most CECs. Accordingly, a second experimental phase was planned to increase the UV dose, namely from 275 to 366 mJ/cm^2, while maintaining the original concentrations of both ozone and H_2O_2. This was accomplished by halving the water flow rate as well as both ozone and H_2O_2 dosage. In this way the dosage ratio ozone/H_2O_2 remained identical to the first phase. After the pilot plant reached the steady state, three samplings were performed during a two-weeks operating period.

Fig. 4a presents the PCA score plot obtained by processing the concentration values of the 29 monitored CECs obtained in all sampling points for the three replicates. PC1 and PC2 components represented 90.6% and 3.5% of the variance, respectively. Inspection of the score plot of Fig. 4a reveals a similar situation to that obtained in the previous experimental phase, namely the ozone/H_2O_2 and UV/H_2O_2 still show significant dissimilarities even though the UV dose was increased by 33 %. Once again, the *t*-test (Fig. 4b) provides further insights into similarities and dissimilarities between these two set of samples. Specifically, several CECs (trimethoprim, sotalol, climbazole, tramadol, carbamazepine, venlafaxine, clarithromicin, atorvastatin, sulpride, cetrizine, losartan, clopidrogel, lidocaine) were more abundant in the B1 sample while only fenofibric acid was better removed by UV/H_2O_2. These findings are quantitatively displayed in Fig. 5 for a selected number of CECs, while all the other plots are displayed in Fig. S2 of the Supplementary Material. Comparison of plots of Fig. 5 with those of Fig. 3 show that the 33% increase of the UV dose almost doubled the removal of total CECs (46 ± 13 % with respect to 25 ± 12 %). However, such a better performance of CECs removal was still lower than that obtained by the intensified ozone/H_2O_2 treatment, being 88 ± 4 % and 64 ± 18 % for the second and first experimental phases, respectively. It is worth noting that these two removal percentage values were both obtained with the same ozone and H_2O_2 doses, being the only difference the water flow rate that in the second experimental phase was halved (Table 2).

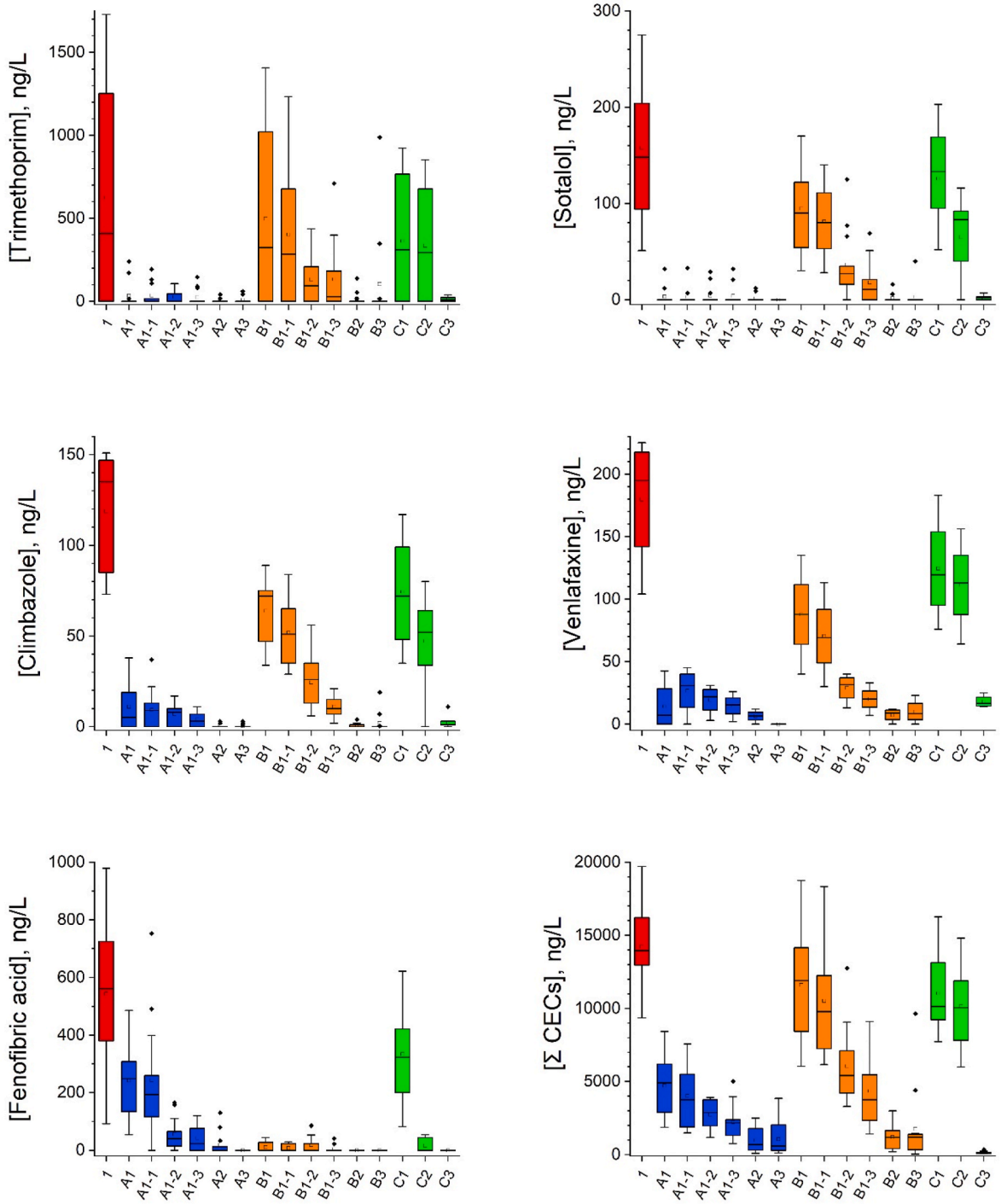


Fig. 3. Box-whisker plots of removal of selected CECs obtained by the three parallel treatment lines, namely ozone/H_2O_2 (line A), UV/H_2O_2 (line B) and the conventional treatment (line C) during the first experimental phase. Sampling points are as follows: 1: influent; A1: effluent O_3/H_2O_2, line A, A2: effluent GAC, line A; A3: effluent UV disinfection, Line A; B1: effluent UV/H_2O_2, line B; B2: effluent GAC, line B; B3: effluent UV disinfection, Line B; C1: effluent ultrafiltration, line C; C2: effluent UV disinfection, Line C; C3: effluent reverse osmosis, line C.

3.2. Removal of bacteria and viruses and genotoxicity tests

Fig. 6 shows the concentration trends of mandatory and optional parameters according to national and new European legislation. Specifically, the microbial concentrations in the three different treatment lines were reported, both for experimental phase 1 and 2.

First experimental phase. The influent wastewater feeding the pilot plant (point 1) showed the presence of coliforms (median load = 4.5 cfu/100 mL, range <1–1000 cfu/100 mL), *E. coli* (median load = <1 cfu/100 mL, range <1–100 cfu/100 mL), Enterococci (median load = <1 cfu/100 mL, range <1–500 cfu/100 mL), *P. aeruginosa* (median load = <1 cfu/250 mL, range <1–30 cfu/100 mL) and *C. perfringens* (median load = <1 cfu/100 mL, range <1–6 cfu/100 mL). *Salmonella* spp and *S. aureus* were never present. In particular, the median load of *E. coli* was always below the limit (100 cfu/100 mL) allowed by Italian Ministerial Decree 185/03 and by Regulation (EU) 2020/741 for reclaimed water quality classes B-D for irrigation purposes refering to the water reuse (European Commission, 2020).

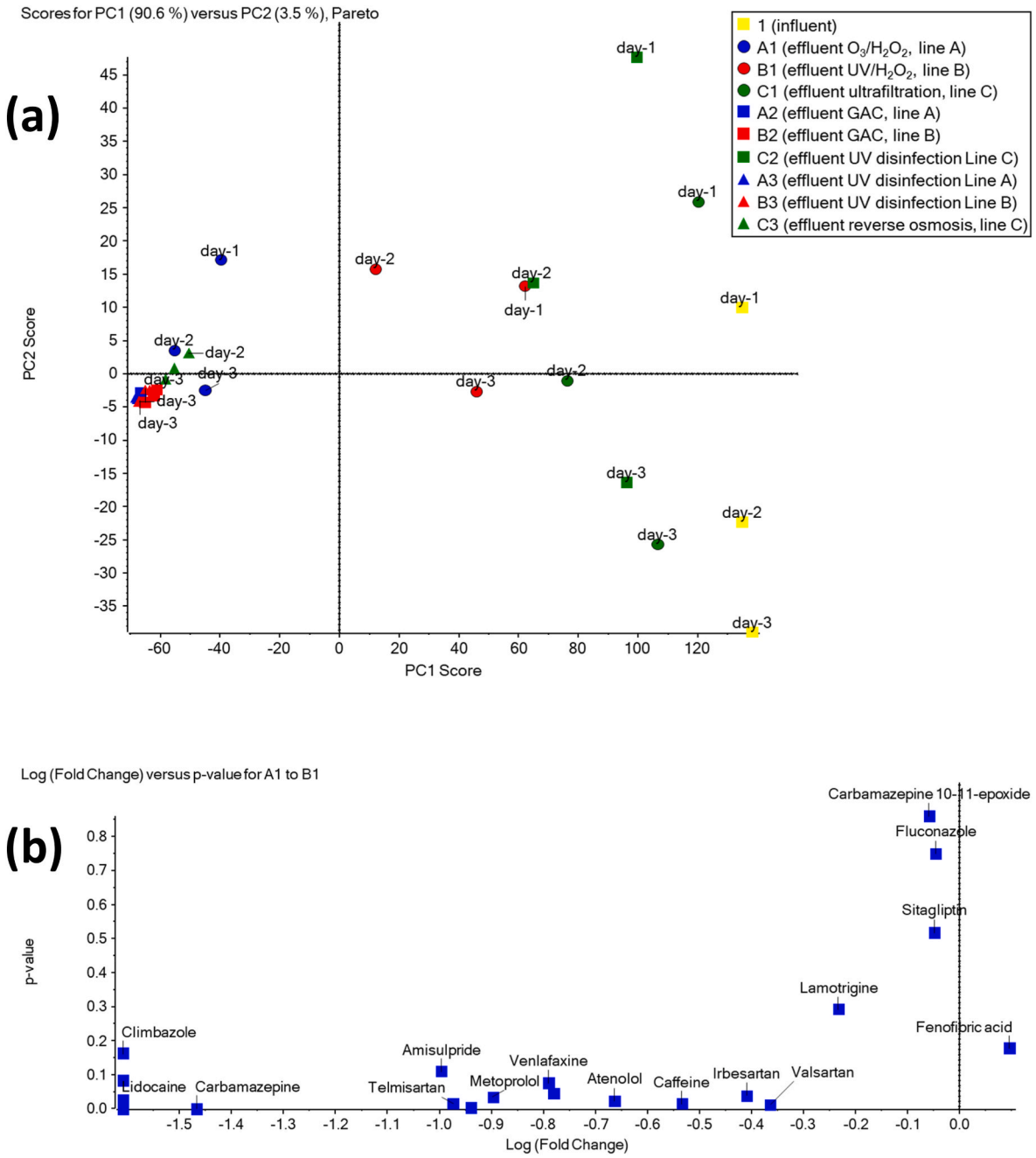


Fig. 4. (a): PCA score plot resulting from processing of the 29 monitored CECs in all sampling points of the second experimental phase. In the score plot selected replicate samples, taken at different sampling days, are shown. Sampling points are those displayed in Fig. 1. (b): *t*-test obtained by comparing samples A1 (effluent of ozone/H_2O_2) and B1 (effluent of UV/H_2O_2) showing the CECs mostly present in each effluent.

At the terminal points of each treatment line (A3, B3, C3), the median load of coliforms, *E. coli*, Enterococci and *C. perfringens* was lower than 1 cfu/100 mL. Only *P. aeruginosa* was found in high load in all terminal points of the three treatment lines, namely 195 cfu/250 mL (range 70–2000 cfu/250 mL) at point A3, 111 cfu/250 mL, range (32–500 cfu/250 mL) at point B3, and 80 cfu/250 mL (range <1–3000 cfu/250 mL) at point C3.

In the first experimental phase, all three treatment lines showed good performance in the full removal of mandatory bacterial parameters (*E. coli*, coliforms and Enterococci) according to limit value (<1 cfu/100 mL) established by the national and new european law. Among the accessory parameters, only *Clostridium perfringens* was found to be compliant to the national and new european law (limit value = <1 cfu/100 mL), while *P. aeruginosa* resulted resistant to the all treatments to which the wastewater was subjected revealing values above those allowed (limit value = <1 cfu/250 mL).

P. aeruginosa is a Gram-negative bacterium belonging to a large group of free-living bacteria that are ubiquitous in the environment. It is often found in natural waters such as lakes and rivers, but also in wastewater and is the most prevalent antibiotic resistant, opportunistic pathogen of humans (Stover et al., 2000). Its presence in water, especially if intended for human consumption and if the microorganism is present in high concentrations, can represent a risk in healthcare settings or where vulnerable individuals live. In immunocompromised patients can cause endocarditis, osteomyelitis, pneumonia, urinary tract infections, gastrointestinal infections, meningitis and is a leading cause of septicemia (Crone et al., 2020). It is able to persist in drinking water for extended periods and it is often difficult to eliminate for its ability to

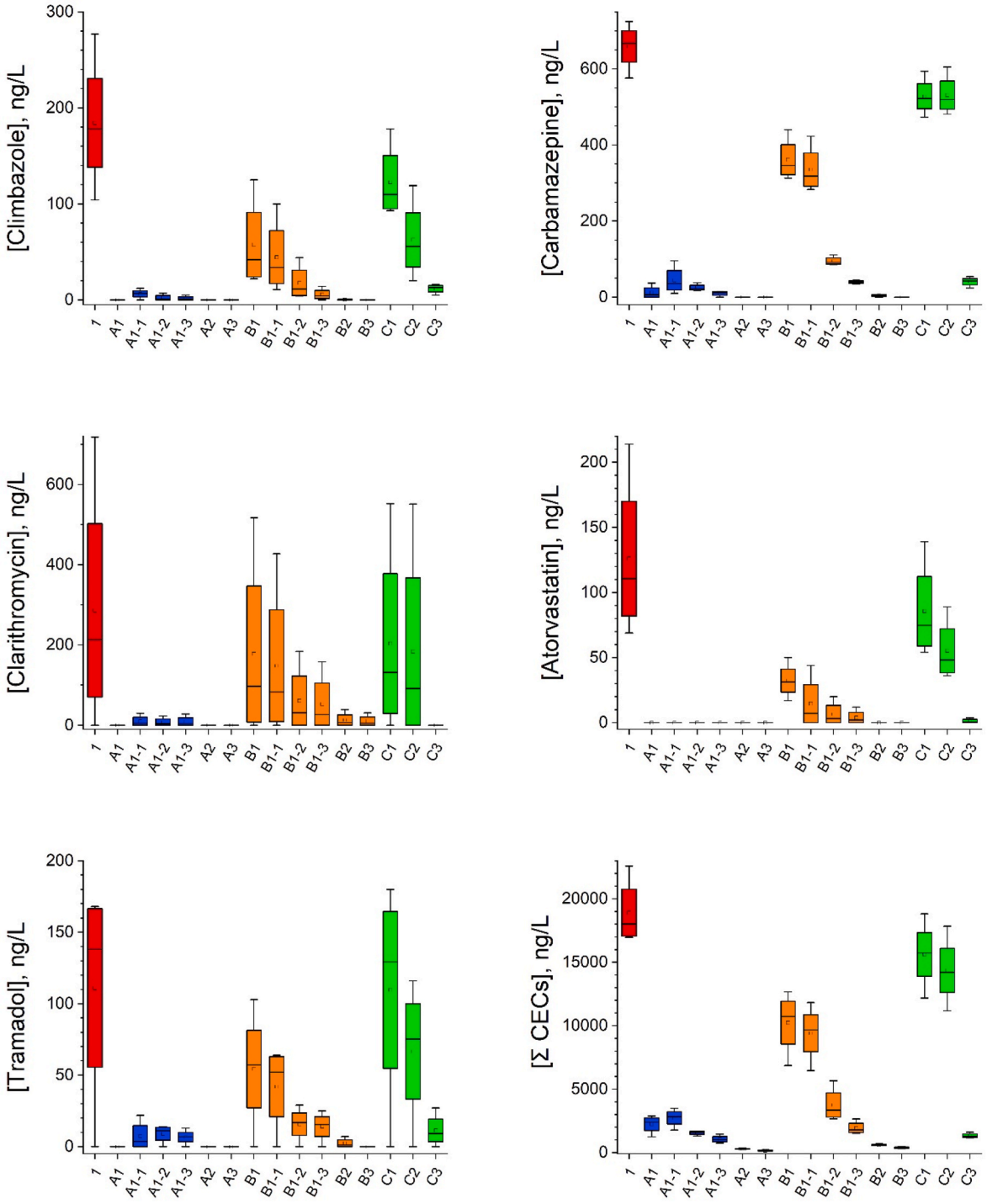


Fig. 5. Box-whisker plots of removal of selected CECs obtained by the three parallel treatment lines, namely ozone/H_2O_2 (line A), UV/H_2O_2 (line B) and the conventional treatment (line C) during the second experimental phase. Sampling points are as follows: 1: influent; A1: effluent O_3/H_2O_2, line A, A2: effluent GAC, line A; A3: effluent UV disinfection, Line A; B1: effluent UV/H_2O_2, line B; B2: effluent GAC, line B; B3: effluent UV disinfection, Line B; C1: effluent ultrafiltration, line C; C2: effluent UV disinfection, Line C; C3: effluent reverse osmosis, line C.

colonize biofilms in plumbing systems (e.g. taps, shower heads, etc.) (Crone et al., 2020). Our results showed that *P. aeruginosa* appeared with highest concentration in all three treatment lines after step of UV disinfection (A3 = 195 cfu/250 mL, range 70–2000 cfu/250 mL; B3 = 111 cfu/250 mL, range 32–500 cfu/250 mL; C2 = 1750 cfu/250 mL, range 50–2000 cfu/250 mL).

UV irradiation, which is considered a promising physical disinfectant in the water treatment without generating toxic by-products, can penetrate bacterial cell walls and is directly absorbed by nucleic acids (Zhang et al., 2019). Previous experimental studies have shown that duration, dose, temperature of irradiation exposure and suspended particles were a crucial determinant of the performance of the UV-C device (Zhang et al., 2019). Some authors reported that exposure to low doses of UV increases biofilm formation by *Pseudomonas aeruginosa* (Said et al., 2011).

Lines A and B showed an increase in the concentration of coliforms in

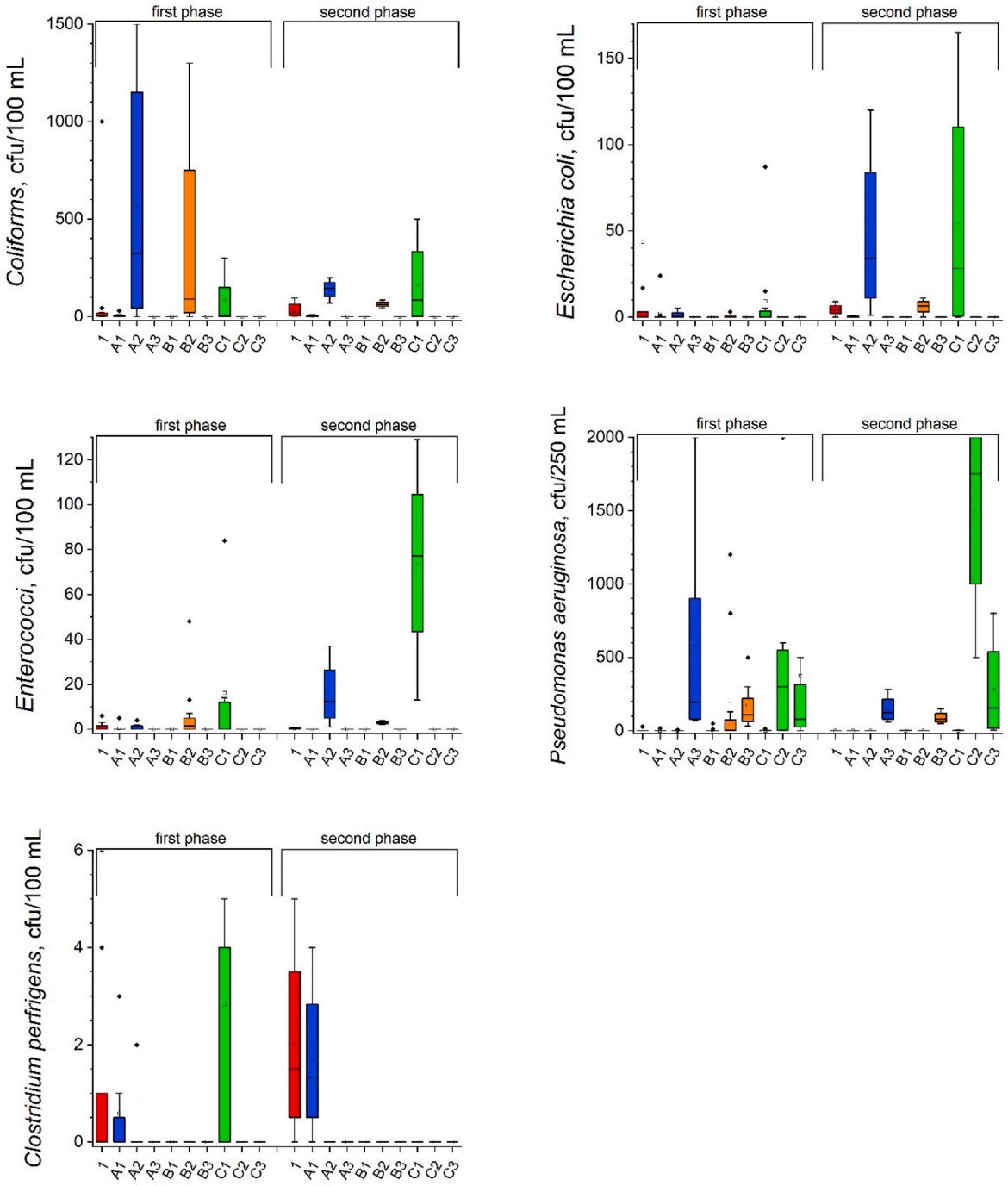


Fig. 6. Box-whisker plots of removal of the microbial parameters obtained by the three parallel treatment lines, namely ozone/H_2O_2 (line A), UV/H_2O_2 (line B) and the conventional treatment (line C) during the first and the second experimental phases. Sampling points are as follows: 1: influent; A1: effluent O_3/H_2O_2, line A, A2: effluent GAC, line A; A3: effluent UV disinfection, Line A; B1: effluent UV/H_2O_2, line B; B2: effluent GAC, line B; B3: effluent UV disinfection, Line B; C1: effluent ultrafiltration, line C; C2: effluent UV disinfection Line C; C3: effluent reverse osmosis, line C.

sampling points coming from the GACs (median value = 325 cfu/100 mL, range <1–1500 cfu/100 mL in A2; 90 cfu/100 mL, range <1–1300 cfu/100 mL in B2). Line B, instead, showed an increase of Enterococci and *P. aeruginosa* also. This phenomenon was probably due to the carbon particles in corrispondence of GAC that may be densely colonized by bacteria causing problems when particles penetrate treatment barriers and enter finished drinking water. In general, the extent of colonization by pathogens may be limited by the indigenous microrganisms present on GAC particles. However, competition for nutrients, space, production of inhibitory secondary metabolites were probably the basis for the increasing in pathogen levels reported here (Camper et al., 1985). Line C showed an increase in Coliforms, Enterococci and Clostridia after ultrafiltration treatment. Some authors reported that mainly for drinking water treatment, no membrane filtration system can be considered as an absolute barrier for all microorganisms (Bodzek et al., 2019; Bray et al., 2021). The imperfections of membranes and membrane modules due to pressure deformation enable secondary bacterial growth in water after passing through the membrane (Bodzek et al., 2019). Therefore, to ensure full epidemiological safety, the ultrafiltration process should be combined with other disinfection technologies for example, UV or

reverse osmosis (Campos et al., 2016; Hembach et al., 2019; Scholes et al., 2021). Specifically, our results proved the capacity of reverse osmosis in removing waterborne pathogens as potable water reuse systems already used in other countries, namely Southern California and Singapore (Gerrity et al., 2013).

Second experimental phase. Overall, the point 1 showed a higher level of microbial contamination than in the previous phase. In particular, the results reported the presence of coliforms (median load = 18.8 cfu/100 mL, range 2–95 cfu/100 mL), *E. coli* (median load = 4.2 cfu/100, range <1–9 cfu/100 mL), and *C. perfringens* (median load = 1.5 cfu/100 mL, range <1–5 cfu/100 mL). *Salmonella* spp and *S. aureus* were never present. Finally, Enterococci and *P. aeruginosa* had a median load <1 cfu/100 and < 1 cfu/250 mL, respectively. Some outliers of microbial contamination in the first phase were probably due to a higher temperature linked to the summer-autumn season compared to the winter one of the second experimental phase. Also in this phase, lines A and B, showed an increase in the concentration of Coliforms (median value = 145 cfu/100 mL, range 70–200 cfu/100 mL and 61.7 cfu/100 mL, range 45–85 cfu/100 mL in A2 and B2 respectively), *E. coli* (median value = 34.2 cfu/100 mL, range 1–120 cfu/100 mL and 6.5 cfu/100 mL, range <1–11 cfu/100 mL in A2 and B2 respectively) and Enterococci (median value = 15.7 cfu/100 mL, range 1–37 cfu/100 mL and 3 cfu/100 mL, range 2–4 cfu/100 mL in A2 and B2, respectively) in correspondence with the GACs. In general, particular attention should be paied to GAC filters when they are first replaced, since it is during this time that pathogens can easily colonize and persist in the carbon beds (Camper et al., 1985). The integration of pretreatment with GAC filter is important even for filters having a well-developed biofilm to reduce the levels of pathogenic bacteria (Piras et al., 2022).

Regarding the microbial removal of the final steps of the three treatment lines (A3, B3, C3), the results obtained in the second phase confirm those obtained in the previous phase of the study. At the terminal points of each treatment line, the microbial contamination of coliforms, *E. coli*, Enterococci and *C. perfringens* was completely removed. Also in this phase all samples were positive only for *P. aeruginosa* resistant to all three treatment lines with highest median concentration after step of UV disinfection (A3 = 123.7 cfu/250 mL, range 60–82 cfu/100 mL; B3 = 80 cfu/250 mL, range 50–150 cfu/100 mL; C2 = 1750 cfu/250 mL, range 50–2000 cfu/100 mL. In reality, among the three lines, the line B shows more comforting results regarding *P. aeruginosa* contamination, probably due to the lower flow rate (0.6 m^3/h vs 1.2 m^3/h) and by major quantity of UV dose (366 mJ/cm^2 vs 275 mJ/cm^2) performed in the second phase. For the same reasons illustrated in the phase 1, line C showed an increase in Coliforms, and Enterococci after ultrafiltration treatment. Regarding *E. coli* STEC/VTEC detection, all the water samples resulted negative in two phases of the study.

As for the viruses detection, water samples of all the three treatment lines were found to be compliant according to Italian regulation providing only Enterovirus detection (Legislative Decree, 2001). The search for HAV, HEV, Adenovirus, Norovirus and Rotavirus, always gave negative results in all phases of the study. Only PMMoV was found both in the first and in the second phase. In particular, in the first phase, it was found in 66.7 % of water samples coming from point 1. In the waters leaving each treatment line, PMMoV was detected in points A3 (16.7 %), B3 and C3 (both 33.3 %). While, in the second experimental phase, PMMoV was detected in 50 % of the samples coming from point 1, A3, B3, respectively. They were absent in all the wastewater samplings conducted at terminal point (C3) of line C.

PMMoV is a pathogenic virus for the pepper plant (*Capsicum* spp.) belonging to the Virgoviridae family, which has been proposed as a potential indicator of fecal contamination in aquatic environments and of the effectiveness of treatment systems (Gyawali et al., 2019).

Currently the European Directive 2020/2184 does not impose the viruses detection in water intended for human consumption but establishes the search for somatic coliphages in untreated water in order to control the effectiveness of treatment processes against microbiological risks such as pathogenic viruses.

It is recognized that the detection of all viral pathogens in water matrices is impractical, due to the complexity, high cost, and time and labor requirements in analytical procedures (Farkas et al., 2020).

Recent studies suggest identifying other indicators of water contamination, for example PMMoV, to reduce human health risks (Bonanno Ferraro et al., 2021). To date, although in Italy their presence is still poorly documented, PMMoV presence is reported worldwide (Kitajima et al., 2018) both in drinking water and in wastewater, surface, irrigation and groundwater (Malla et al., 2019). Further studies are required in order to assess the suitability of these viruses as indicators of fecal contamination.

Results of the SOS Chromotest applied to the samples (preconcentrated 10, 100, and 1000 times) of the first and second experimental phases are reported in Table 4 and expressed as induction factors. No genotoxic activity was detected in any sample analyzed at the three preconcentration factors, since in all cases the IF values found were below the 1.5 threshold.

3.3. CECs removal effectiveness and operative costs analysis

According to the employed operative conditions (Table 2) the influent total CECs daily load for the first experimental phase was 0.41 ± 0.09 g/d, for lines A and B, and 0.24 ± 0.06 g/d for line C. Thus, it is evident the greater effectiveness of the ozone/H_2O_2 step with respect to the UV/H_2O_2 one in removing CECs. Specifically, total CECs daily load released by treated effluents was 0.136 ± 0.067 and 0.334 ± 0.117 g/d for ozone/H_2O_2 and UV/H_2O_2, respectively, corresponding to the

Table 4
SOS chromotest data of the first and second phases of the study expressed as IF. Each sample was analyzed applying the following preconcentration factors: 1:10, 1:100; 1:1000. Data are expressed as mean ± SD.

Sample	Preconcentration factor	First experimental phase	Second experimental phase
		Induction factor (IF)	Induction factor (IF)
1	10X	1.10 ± 0.05	1.01 ± 0.06
	100X	1.19 ± 0.06	1.03 ± 0.04
	1000X	1.23 ± 0.07	1.09 ± 0.05
A1	10X	1.09 ± 0.05	0.95 ± 0.04
	100X	1.14 ± 0.07	1.11 ± 0.06
	1000X	1.15 ± 0.06	1.19 ± 0.08
C1	10X	1.10 ± 0.06	1.12 ± 0.05
	100X	1.14 ± 0.05	1.14 ± 0.06
	1000X	1.24 ± 0.05	1.17 ± 0.06
A2	10X	1.12 ± 0.05	1.00 ± 0.06
	100X	1.26 ± 0.07	1.03 ± 0.07
	1000X	1.27 ± 0.08	1.17 ± 0.07
A3	10X	0.96 ± 0.05	1.01 ± 0.06
	100X	1.03 ± 0.06	1.09 ± 0.06
	1000X	1.07 ± 0.05	1.20 ± 0.05
B1	10X	1.08 ± 0.05	0.98 ± 0.07
	100X	1.13 ± 0.05	0.99 ± 0.06
	1000X	1.14 ± 0.07	1.02 ± 0.08
B2	10X	1.03 ± 0.07	1.01 ± 0.07
	100X	1.15 ± 0.06	1.03 ± 0.07
	1000X	1.31 ± 0.06	1.07 ± 0.08
B3	10X	1.01 ± 0.06	0.95 ± 0.06
	100X	1.05 ± 0.07	0.96 ± 0.06
	1000X	1.14 ± 0.08	1.05 ± 0.06
C1	10X	1.10 ± 0.07	1.12 ± 0.07
	100X	1.14 ± 0.08	1.14 ± 0.06
	1000X	1.24 ± 0.07	1.17 ± 0.08
C2	10X	1.15 ± 0.08	1.04 ± 0.06
	100X	1.21 ± 0.08	1.09 ± 0.06
	1000X	1.30 ± 0.07	1.11 ± 0.05
C3	10X	1.05 ± 0.05	0.91 ± 0.06
	100X	1.19 ± 0.08	1.05 ± 0.06
	1000X	1.20 ± 0.08	1.10 ± 0.06

removal percentages of 66.8 ± 18 and 18.4 ± 12 %. The following step of GAC adsorption allowed the removal efficiency to increase up to 92.0 ± 8.0 and 91.6 ± 8.2 %, respectively. The conventional treatment (Line C), employed as a baseline reference treatment, showed that the ultrafiltration step only allowed lowering the total CECs daily load to 0.185 ± 0.042 g/d corresponding to a removal percentage of 22.5 ± 1.0 %. With the following step by reverse osmosis the CECs daily load decreased to 0.002 ± 0.001 g/d (99.0 ± 1.0 % removal efficiency) thus reaching treatment equivalency of zero-liquid-discharge lines consisting of AOPs followed by GAC.

During the second experimental phase, in which the UV dose was increased from 275 to 366 mJ/cm^2, the effectiveness of the UV/H_2O_2 step in removing CECs significantly increased. During this testing period influent total CECs daily load was 0.27 ± 0.05 and 0.32 ± 0.05 g/d, for lines A-B and line C, respectively, due to flow rate reduction. The total CECs daily load in the effluent was 0.032 ± 0.013 and 0.147 ± 0.044 g/d for the ozone/H_2O_2 and UV/H_2O_2, respectively, corresponding to removal percentages of 88.2 ± 6 and 45.8 ± 12 %. Once again, the following step by GAC adsorption allowed the removal efficiency to raise up to 98.5 ± 1.5 and 96.8 ± 1.2 %, respectively. The conventional treatment (Line C) showed similar performance as during the first experimental phase being the removal efficiencies of ultrafiltration and reverse osmosis steps 17.4 ± 7.4 and 93.0 ± 1.5 %, respectively.

Overall, results showed that the higher UV dose allowed to improve the CECs removal efficiency of the UV/H_2O_2 step, but still insufficiently to meet the performance of ozone/H_2O_2. The almost complete CECs removal achieved by the reverse osmosis step was also obtained when GAC adsorption was coupled with AOPs in the lines A and B. It is worth noting that the employment of reverse osmosis has as the drawback of management and disposal of the retentate that contains the concentrated CECs.

At an average energy cost of 0.25 €/kWh, the overall energy cost for removing from a tertiary effluent, on average, 50 % of the CECs entering GAC adsorption is 0.21 kWh/m^3 x 0.25 €/kWh = 0.053 €/m^3 and 1.178 kWh/m^3 x 0.25 €/kWh = 0.295 €/m^3 for ozone/H_2O_2 and UV/H_2O_2, respectively. To these costs, the operational expenses associated with H_2O_2 should be added to both AOPs. Specifically, since both lines were operated with a H_2O_2 concentration of 11 mg/L, the incremental cost for H_2O_2 dosage is 0.130 €/m^3 (Table 5). It should be also mentioned that the cost of UV lamp replacement is minimal relative to the other operational costs, therefore it has been neglected in this cost analysis. In summary, the electrical costs required to achieve 50 % average CECs removal in a tertiary effluent projected to a full-scale plant of 250 m^3/h (or 25,000 inhabitants) is 0.183 and 0.425 €/m^3 for ozone/H_2O_2 and UV/H_2O_2, respectively.

4. Conclusion

Based on experimental results of the present investigation, it is possible to draw the following conclusions:

- AOPs coupled with GAC adsorption and UV disinfection are effective zero-liquid-discharge process for implementing IPR strategies of secondary and tertiary wastewater effluents.
- Ozone/H_2O_2 is more effective than UV/H_2O_2 for removing CECs. Reverse osmosis, after ultrafiltration and UV disinfection, also yields similar results for CEC removal but poses a retentate management problem.
- As for the comparison between the two AOPs, under the investigated conditions it was found that a 50% CECs removal objective could be cost-effectively achieved in a full-scale plant of 250 m^3/h with 0.183 and 0.425 €/m^3 for ozone/H_2O_2 and UV/H_2O_2, respectively.

All three treatment strategies employed in this pilot study showed good performance in removing regulated and emerging water quality parameters, including viruses, exception made for *P. aeruginosa*. The presence of high chlorides concentration in the influent wastewater limited the water reuse options to IPR. That said, IPR practices shall be encouraged as they would bring additional benefits to the local context (Apulian coastal aquifer of Fasano, IT) including creating a freshwater barrier, via aquifer recharge, to contain seawater intrusion.

Table 5
Overall energy cost calculated for both the ozone/H_2O_2 and UV/H_2O_2 steps for removing 50% of the CECs entering the GAC. The average energy cost of 0.25 €/kWh was employed.

	Operating cost of the AOP step (€/m^3)	Cost of H_2O_2 (€/m^3)	Cost of the whole treatment
ozone/H_2O_2	0.053	0.13	0.183
UV/H_2O_2	0.295	0.13	0.425

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117661.

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