Journal of Environmental Management 392 (2025) 126858

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

The potential of native microalgae consortia to remove pharmaceutical compounds present in treated wastewater

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ARTICLE INFO

Keywords:
Tertiary wastewater treatment
Native microalgae consortia
Pharmaceutical contaminants
Micropollutant removal

ABSTRACT

Wastewater treatment plants play a key role in the release of pharmaceuticals and other contaminants into the aquatic environment, causing negative effects on the ecosystems of receiving water bodies. This work aimed to assess the removal efficiency of real loads of these contaminants by wastewater-native microalgae consortia acting on treated wastewater previously subjected to secondary treatment. Wastewater sampling and removal efficiency experiments were conducted over 1 year at three different sampling points. Nineteen pharmaceutical compounds of different drug classes (stimulants, anticonvulsants, antidepressants, antibiotics, β-blockers, lipid regulators, and nonsteroidal anti-inflammatory drugs/analgesics), as well as one herbicide/algicide (Diuron) were detected and quantified. Native blooming microalgae consortia were grown in treated wastewaters, and their potential to remove these compounds was quantified. The removal efficiency of these compounds by native microalgae consortia was variable, ranging from almost no removal for Clofibric acid or Ketoprofen to near complete removal for Fluoxetine, Venlafaxine, Atenolol or Diuron. These variations were influenced not only by the molecular nature of the compounds but also by the microbial composition variability of the microalgae consortia, especially among the prokaryotes present. Overall, microalgae consortia successfully removed between 40 % and 83 % of the total detected compounds, preventing a significant part of these from entering the aquatic environment, contributing to enhance treated wastewater quality. Significant biomass growth was observed, reaching dry-weight concentrations up to 2.6 g.L^{-1}, indicative of good capacity of the grown consortia to deal with the toxicity effects of the pollutants. In addition to what is now reported, microalgae treatment also removes other pollutants, such as nutrients, metals or microplastic particles, constituting a versatile tertiary treatment for polishing treated wastewaters. These findings demonstrate the potential of native microalgae consortia-based systems to improve wastewater treatment processes, mitigating the environmental impact of pharmaceutical compounds while producing potentially useful biomass.

1. Introduction

The increasing use of pharmaceutical compounds (PhCs) in modern society poses a growing threat to both environmental and human health (Pérez-Lucas et al., 2023). A significant portion of these compounds is excreted unchanged or as biologically active metabolites, reaching aquatic ecosystems via domestic and industrial wastewater or surface runoff, contributing to the pool of potential environmental contaminants of emerging concern (CECs) (Paíga et al., 2019). Wastewater treatment plants (WWTPs) are major entry points of PhCs into the environment, as conventional treatment systems are not specifically designed to remove such contaminants, often functioning as permeable barriers (Gonzalez-Rey et al., 2015; Salgado et al., 2010; Sutherland et al., 2018). Additional relevant sources include hospital effluents and discharges from pharmaceutical manufacturing facilities (Moghaddam et al., 2023). As a result, PhCs have been detected in surface waters and in the tissues of aquatic organisms (Gonzalez-Rey et al., 2015; Samal et al., 2022; Szopińska et al., 2022).

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https://doi.org/10.1016/j.jenvman.2025.126858
Received 1 April 2025; Received in revised form 11 July 2025; Accepted 2 August 2025

Recognition of the risks associated with PhCs has led to new regulatory frameworks within European water policy, including Directive 2013/39/EU, the Urban Wastewater Treatment Directive (91/271/EEC), and the Watch List established by Implementing Decision (EU) 2022/1307, which includes ten PhCs, with six new additions, such as antibiotics (e.g., sulfamethoxazole and trimethoprim) and psychiatric drugs (e.g., venlafaxine). Over the past decades, the persistence of PhCs in treated effluents has been widely reported (Gaffney et al., 2017; Sutherland et al., 2018), with notable prevalence of non-steroidal anti-inflammatory drugs (NSAIDs), analgesics, antibiotics, antidiabetics, hormones, radiologic agents, β-blockers, and lipid regulators. Their occurrence varies according to demographic factors, population habits, and treatment technologies (Gaffney et al., 2017).

In Portugal, available data on PhCs in treated effluents are still limited, with most research focusing on influents or sludge (Silva et al., 2021b; Pereira et al., 2023). The compounds detected (Table 1) align with global trends, with antibiotics, NSAIDs, lipid regulators, and psychiatric drugs being most frequently reported. Paíga et al. (2016) found 19 out of 33 targeted PhCs in the Lis River (Leiria region), with increasing concentrations downstream of WWTPs. In southern Portugal, Rocha et al. (2013) reported the presence of pharmaceutical endocrine disruptors in the Formosa Lagoon, a protected natural area that receives treated effluents from one of the WWTPs studied. In the same region, Gonzalez-Rey et al. (2015) detected 13 pharmaceuticals in the Arade Estuary, with notable concentrations of caffeine (804 ng L^{-1}) and theophylline (186 ng L^{-1}).

The presence of emerging contaminants (CECs), such as PhCs, may compromise the safe reuse of wastewater, a crucial practice in water-scarce regions like the Mediterranean, where nearly 50 % of the population experiences water shortages during the dry season (Pereira et al., 2017). In southern Portugal, reservoir levels reached only 6 % of their useable capacity during the summer of 2024 (Águas do Algarve, 2024). In response, the Portuguese Environment Agency has launched a strategic plan to promote water efficiency, encouraging sustainable wastewater treatment methods that enable reuse, particularly in water-intensive sectors such as agriculture and tourism (Pereira et al., 2017). Both WWTPs analysed in this study operate under such conditions, being located near golf courses or orchards.

The need for improved wastewater treatment and reuse is also emphasized by international frameworks such as the EU Water Framework Directive and the United Nations Sustainable Development Goals (SDGs), particularly SDG 6, which aims to ensure availability and sustainable management of water and sanitation for all (Pereira et al., 2017). This need for sustainable water management is a global concern, impacting countries in Southern Europe, the Middle East, Asia, and Africa, due to climate change, population growth, agricultural challenges, and impaired water quality. Consequently, wastewater reuse has become a global priority, with increasing investments in innovative treatment solutions (UNESCO, 2024)

Microalgae-based systems are emerging as promising solutions for removing CECs, including PhCs, from treated effluents. These photosynthetic microorganisms are capable of metabolizing various organic compounds while producing biomass in nutrient-limited media (Maryjoseph and Ketheesan, 2020). This biomass can be further valorized within biorefinery contexts, through fractionation into high-value products (Aditya et al., 2022; Mojiri et al., 2022). Compared to other tertiary treatments, microalgae-based approaches offer advantages such as low operational costs, high efficiency, and potential for biomass recovery.

Initial studies focused primarily on the removal of nutrients such as phosphorus and nitrogen (Sutherland and Ralph, 2019). More recently, research has expanded to investigate the removal of emerging contaminants, with promising results under laboratory conditions (Gondi et al., 2022; Maryjoseph and Ketheesan, 2020).

Most studies have been conducted at lab scale with microalgae monocultures under controlled conditions (Maryjoseph and Ketheesan, 2020; Zhou et al., 2022). Furthermore, maintaining axenic conditions in real-world applications is costly and often impractical (Gururani et al., 2022). Although engineered microalgae-bacteria systems have been explored, fewer studies focus on native consortia from wastewater plants tested with real pharmaceutical loads. Recent research highlights their potential for removing contaminants (Sutherland et al., 2018), demonstrating advantages over monocultures, such as increased resilience and multiple removal mechanisms (Chen et al., 2019a; Chan et al., 2022;

Table 1
Studies targeting pharmaceutical compounds in wastewater effluents conducted in Portuguese WWTPs in the last fifteen years.

Number of WWTPs, Type and capacity (Pop. Eq.)	Location	Number of targeted molecules	Most frequent drug classes	Detection range (ng L^{-1})	Reference
15, secondary and tertiary 8700–256,000	North	11	Antibiotics	n.d – 9800	Pereira et al. (2015)
	Centre		Lipid regulator	n.d – 2400	
	South		Anti-inflammatory	n.d – 670	
5, secondary, 3164–78,601	South (Alentejo)	26	Analgesic	54–7800	Silva et al. (2021a)
			Stimulant	84–4800	
			NSAIDs	280–3300	
1, secondary, 213,500	Center (Lisboa)	32	Antidiabetics	50–58,000	Gaffney et al. (2017)
			Psychostimulants	n.d – 2900	
			NSAIDs	n.d - 3300	
1, Secondary, 248,685	Center (Leiria)	83	NSAIDs	n.d – 1934	Paíga et al. (2019)
			Antibiotics	n.d – 448	
			Psychiatric drugs	57–52,059	
1,secondary, 213,000	Center (Coimbra)	65	X-raycontrast agent	33,885–85,000	Santos et al. (2013)
			Antibiotics	<MDL – 1679	
			Psychiatric drugs	n.d - 496	
5, Secondary, 773–11,195	Center	65	NSAIDs	518 - 43,653	Salgado et al. (2010)
			B-blockers	624–19,888	
			Lipid regulator	198–7286	
2, Secondary, 49,351–248,685	Center (Leiria)	32	Analgesics	313–4909	Paíga et al. (2016)
			NSAIDs	<MDL – 3304	
			Psychiatric drugs	n.d – 374	
15, Secondary and tertiary, 8700–256,000	Northern	11	NSAIDs	n.d. – 32,000	Pereira et al., 2016
	Center		Antibiotic	n.d – 10,200	
	South		Lipid regulator	n.d –20,400	

*n.d. Not detected.

Aditya et al., 2022; Gururani et al., 2022; Sutherland and Ralph, 2019).

A further limitation is that most studies use spiked wastewater samples, artificially enriched with known contaminant concentrations, which may not reflect real-world conditions involving multiple compounds at trace levels. Even fewer studies have combined real effluent matrices and naturally occurring microbial consortia in the same experimental design.

This study aimed to evaluate the removal efficiency of naturally evolving microalgal consortia in the treatment of real WWTP effluents. Samples were collected from two wastewater treatment plants located in southern Portugal over one year, across different seasons. Twenty pharmaceutical compounds and one herbicide/algaecide were monitored. Treated effluent was used as the cultivation medium, enabling simultaneous assessment of biomass production and contaminant removal. The selected compounds were previously identified in regional studies (Pereira et al., 2016; Gonzalez-Rey et al., 2015) and are among the most consumed pharmaceuticals in Portugal (Infarmed, 2022).

Since pollutant loads and microbial compositions vary across WWTPs and regions, this study provides detailed insights into two representative southern Portuguese facilities, contributing valuable data for future comparative and applied research. To the best of our knowledge, this is among the first studies in which native microalgal consortia were allowed to evolve naturally throughout the experiments, without external manipulation of species composition. The integrated assessment of contaminant removal and biomass production underscores the feasibility of circular, sustainable, and environmentally sound wastewater treatment solutions.

2. Materials and methods

The samples of treated wastewater from two WWTPs were collected seasonally over one year: winter, spring, summer and autumn. The effluent samples were used for chemical analysis and microalgae growth. The loads of targeted pharmaceutical compounds were determined in each season, and their removal efficiency by native microalgae consortia was evaluated.

2.1. Wastewater collection

Samples were collected from two distinct WWTPs (WWTP-A and WWTP-B) effluents, as described in Afonso et al. (2024). Briefly, WWTP-A was originally designed to handle wastewater up to 31,500 m^3 day^{-1}, a population equivalent of 138,000, and is currently operating at an average flow rate of 10,000 m^3 day^{-1}. The treatment system comprises two parallel lines, with the first employing a trickling filter system during peak periods, while the second utilizes an activated sludge system. Following treatment, the effluent undergoes UV disinfection before entering an artificial maturation lagoon, where it has a retention time of about 48 h before being released to an adjacent water line. WWTP-B has a maximum treatment capacity of 26,700 m^3 day^{-1} (44,500 population equivalent) but is currently operating at an average flow rate of 4000 m^3 day^{-1}. This facility employs a biological treatment process using activated sludge under extended aeration conditions, housed in two oxidation ditches with surface aerators. As a post-secondary treatment, the wastewater undergoes UV disinfection before being discharged into the receiving waterbody, the Ria Formosa coastal lagoon.

Composite 24-h flow-weighted wastewater samples were collected seasonally (winter, spring, summer, and autumn) after the disinfection step (WWTP-A.1 and WWTP-B), and grab wastewater samples were collected after the maturation lagoon in WWTP-A (WWTP-A.2). All the water samples were collected in glass jars previously washed with sulfuric acid at 20 % (v/v). Upon arrival at the laboratory, the wastewater was characterized regarding its organic matter content, by measuring chemical oxygen demand (COD), following the American Public Health Association (APHA) method 5220D (APHA, 2017). Total phosphorus and total nitrogen (TP and TN) were also measured using APHA standard methods 4500-P and 4500-N respectively (APHA, 2017). Part of the collected wastewater was aliquoted and immediately stored at -20 ± 1 °C for the analysis of pharmaceuticals (PhCs) and other contaminants. The remaining wastewater was immediately used for characterization and microalgae growth experiments.

2.2. Reagents

HPLC-grade acetonitrile, methanol and water were supplied by Romil Ltd. (Barcelona, Spain). Analytical grade hydrochloric acid and formic acid were obtained from Panreac (Barcelona, Spain). Ammonium formate, acetaminophen, ampicillin, amoxicillin, atenolol, carbamazepine, ciprofloxacin, 10,11-epoxycarbamazepine, clofibric acid, diclofenac, 1-hydroxyibuprofen, 2-hydroxyibuprofen, ketoprofen, naproxen and salicylic acid, fluoxetine, venlafaxine (>97 %) were purchased from Sigma-Aldrich (Steinheim, Germany). Ibuprofen, diuron, sulfamethoxazole and trimethoprim (>98.5 %) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Caffeine was obtained from Merck (Darmstadt, Germany). 3 mL solid phase extraction (SPE) cartridges, packed with 60 mg of Oasis HLB were purchased from Waters (Milford, MA, USA). For each pharmaceutical compound, a stock solution of 1000 μg mL^{-1} was prepared in methanol, and stored at 4 °C. Working solutions were prepared by diluting the stock standard solution in methanol: water (50:50, v/v).

2.3. Experimental design

The mitigation experiments were performed as described in Afonso et al. (2024). Briefly, the growth experiments were conducted in 5 L glass jars with 2 L of working volume, where the treated wastewater (nutrient deficient and with no supplementation), was inoculated with the respective native microalgae consortia, previously obtained. The cultures were maintained over 30 days, in a fed-batch mode, replacing 30 % of the culture with fresh wastewater after 15 days of growth. Microalgae growth parameters were regularly monitored, such as pH (pH Meter Basic20, Crison), conductivity (MicroCM2202, Crison), dissolved oxygen (O_2) and dry weight (d.w.). For d.w. determination, 10 mL of culture were filtered through pre-weighed 1.2 μm fibreglass filters (Whatman 934-AH), dried for 1 h at 103 °C (Venti-line, VWR), and then weighed. The dry weight was calculated by subtracting the weight of the filter without biomass from the weight of the filter with biomass. After microalgae growth, the biomass was harvested by centrifuging the cultures at 14000 rpm, at 4 °C, in an ultracentrifuge (Gyrozene 2236R High-speed ultra-centrifuge, Korea), for 20 min. The solid fractions were kept for further analysis, and the liquid fractions were preserved at -20 ± 1 °C to quantify the targeted PhCs.

2.4. Detection and quantification of pharmaceutical compounds

Twenty pharmaceutical compounds and metabolites and one herbicide/algicide were selected for targeted analysis by liquid chromatography - coupled to triple quadrupole mass spectrometry (LC-MS/MS). The selected pharmaceutical compounds belong to six drug classes: stimulants (caffeine); antidepressants/anticonvulsants (carbamazepine and its metabolite: 10,11-epoxycarbamazepine; venlafaxine and fluoxetine); antibiotics (ampicillin, amoxicillin, ciprofloxacin, sulfamethoxazole and trimethoprim); lipid regulators (clofibric acid); β-blockers (atenolol) and NSAIDs/analgesics (diclofenac, Ibuprofen and its metabolites: 1-hydroxyibuprofen and 2-hydroxyibuprofen; ketoprofen, naproxen, acetaminophen and salicylic acid). The herbicide diuron was also targeted and quantified. This compound enters wastewater streams through agricultural runoff, as it is widely used in farming to control weeds. Additionally, diuron can reach wastewater from urban areas where it is applied for weed control on pavements and in gardens.

The compounds were extracted using solid phase extraction (SPE). The eluates were evaporated to dryness, then redissolved in methanol:

water (50:50, v/v), filtered through a 0.22 μm nylon filter (Whatman), and analysed by liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS). A detailed description of the extraction conditions and analytical method, including sensitivity, accuracy, and recovery rates, can be found in the supplementary material.

2.5. *Determination of microbial composition of the consortia*

The taxonomic classification of the microorganisms present on each consortia was based on 18S rRNA sequencing for eukaryotic organisms and 16S rRNA sequencing for prokaryotic organisms.

2.5.1. *DNA extraction*

Microalgal biomass DNA was extracted using the NZYSoil gDNA isolation kit (NZYTech, Portugal). The concentration and quality of the extracted DNA were assessed with a spectrophotometer (NanoDrop3300, Thermo Fisher Scientific, USA).

2.5.2. *Library preparation and sequencing workflow*

The library preparation and sequencing for full-length 16S and 18S rRNA gene amplicons were performed at the Integrated Microbiome Resource (IMR, https://imr.bio), as described at https://imr.bio/protocols.html. A detailed description is provided in the supplementary material.

2.5.3. *Bioinformatic analysis*

Taxonomic profiling of eukaryotic classes was conducted using a standardized bioinformatics pipeline from the Microbiome Helper repository (Comeau et al., 2017). The analysis followed the PacBio CCS Amplicon SOP v1 (QIIME 2) and Amplicon SOP v2 (QIIME 2 version 2020.8), available at the Microbiome Helper GitHub repository. Key steps included the following.

- Data Preparation: Sequence orientation was resolved to ensure uniformity across all reads. Primer sequences were trimmed using Cutadapt and reads not matching the correct primer sequences or outside the expected size range (1300–1800 nt) were filtered.
- Import and Quality Control: Trimmed FASTQ files were imported into QIIME 2 as artefacts. Quality metrics were summarized to ensure sufficient sequencing depth, and denoising was performed using the DADA2 plugin. Low-quality reads (expected errors >2) were removed, and sequences were trimmed based on quality score distributions. Amplicon sequence variants (ASVs) were inferred, and chimeric sequences were excluded.
- Taxonomic Classification: ASVs were taxonomically classified using a Naive Bayes classifier trained on the SILVA full-length 18S database. Chloroplast, mitochondrial, and unclassified sequences (at the phylum level) were removed.
- Data Filtration and Normalization: Highly rare ASVs (frequency <0.05 % of the mean sample depth) were excluded to reduce noise in the dataset.

Data Visualization: Relative abundance data at the class level were extracted and visualized in stacked bar plots to illustrate taxonomic diversity across samples.

2.6. *Statistical analysis*

Comprehensive statistical analysis was conducted using GraphPad Prism (version 10.1.2, GraphPad Software, Inc., San Diego, CA, USA). For graphical data, two-way ANOVA multiple-comparison tests were conducted to evaluate significant differences between data set groups. One-way ANOVA was performed for graphics with one data set. A correlation matrix was established using Spearman's rank correlation coefficient (Spearman r). This matrix incorporated the average values of the removal efficiency of each compound, the initial values of COD, TN and TP in the wastewaters, and the average values of pH, conductivity and dissolved oxygen (%) during the removal experiments. The significance level for all the analyses was set at $p < 0.05$.

3. Results and discussion

3.1. *Pharmaceutical loads*

In the present work, twenty pharmaceutical compounds and metabolites were targeted. Figs. 1 and 2 depict the loads of these compounds, over one year, on 12 samples analysed (four seasons in three different sampling stations). The antibiotics amoxicillin and ciprofloxacin were not detected, and ampicillin was only detected in one sample (WWTP-B in autumn) at a concentration of 102 ± 6.40 ng L^{-1}, therefore these are not represented in the graphical results. Ibuprofen was the compound found at the highest concentration (35.5 ± 3.6 μg L^{-1} in WWTP-B during autumn). The other compounds found at higher concentrations were diclofenac and venlafaxine. The other antibiotics, the antidepressant fluoxetine and the lipid regulator clofibric acid were found at lower concentrations, ranging between not detected (or below the minimum quantification limit) and 60.6 ± 2.5 ng L^{-1}. Overall, the compounds detected at higher concentrations belong to the stimulant, NSAIDs/analgesic and anticonvulsant/antidepressant drug classes, as illustrated in Fig. 1. This tendency is also observed when considering the annual average of the PhCs in the three effluents, shown in Table 2, where caffeine is the most abundant compound, followed by ibuprofen > diclofenac > venlafaxine > atenolol. The prevalence of ibuprofen and diclofenac in the samples correlates with the sales trends reported by the Portuguese National Health Authority (Infarmed, 2022), regarding medicine and healthcare products. According to the reports, anti-inflammatory and analgesic drugs were the most sold drug classes. Among these, ibuprofen and diclofenac were the second and third most frequently sold over-the-counter medications, accounting for 8 % and 6 % of the total sales volume, respectively (Infarmed, 2022). The most sold compound, acetaminophen, was found at lower loads in the wastewater effluents (12.25–16.76 ng L^{-1}), because it is efficiently removed by conventional WWTP treatments. Wu et al. (2023) reviewed the removal of acetaminophen in WWTPs worldwide and found an average removal of 94.7 %. Moreover, the remaining drug classes, antibiotics, β-blockers and lipid regulators, are listed as Portugal's top ten most prescribed drug classes (Infarmed, 2022).

Overall, these results are consistent with the data reported worldwide, where these classes are the most detected, both in treated wastewater and the receptor environment (Dey et al., 2019; Samal et al., 2022; Santos et al., 2013). In Portugal, available data regarding PhCs in WWTP effluents is summarized in Table 1 and shows a similar distribution of the predominant drug classes. Paíga et al. (2016) reported the prevalence of the same classes of PhCs in the treated effluents of two WWTPs with secondary treatment systems, and in the surface waters of the river where these treated wastewaters were discharged. Salgado et al. (2010) also detected high concentrations of NSAIDs, in the effluents of five WWTPs located in Portugal, where ibuprofen reached concentrations up to 50 μg L^{-1}. The occurrence of the compounds from the other three classes (antibiotics, β-blocker and lipid regulator), although at lower concentrations, was consistent, with a detection frequency close to 100 %. Contrarily, Salgado et al. (2010), found considerably higher concentrations of lipid regulators (ranging between 198 and 7286 ng L^{-1}) in five secondary WWTPs. The same trend was observed by Paíga et al. (2016), who found a prevalence of lipid regulators in the WWTPs located in the same region. However, different molecules were targeted by Paíga et al. (2016), which might explain these discrepancies. These classes of pharmaceuticals are commonly related to chronic diseases, often requiring long-term treatments, leading to a continuous discharge into the wastewater systems. These classes are also frequently detected in WWTPs worldwide, though their concentrations vary greatly, depending on the location, demographic characteristics, prescription

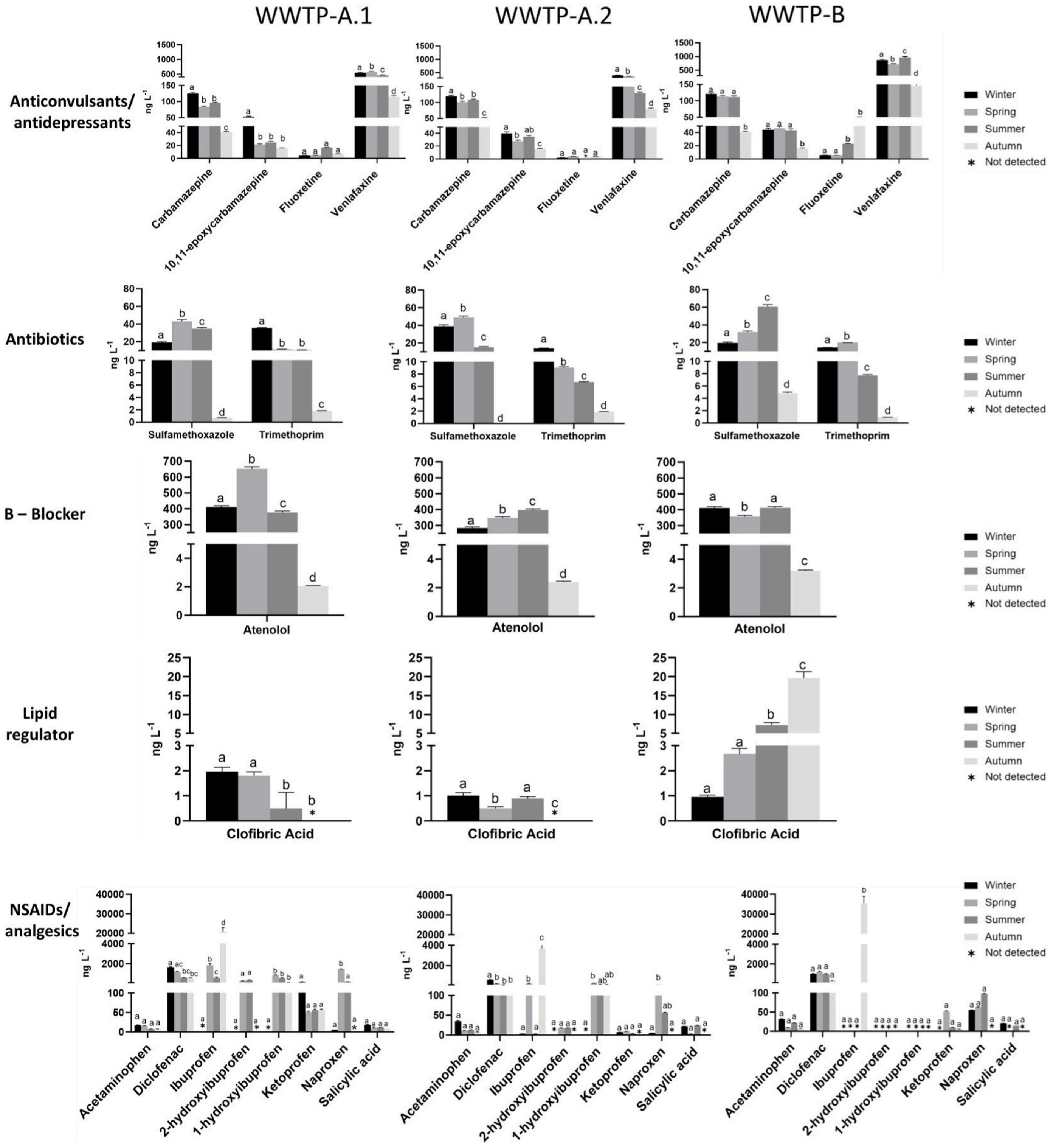

Fig. 1. Loads of the five classes of pharmaceutical compounds, in the winter, spring, summer and autumn, for each sampling site: WWTP-A.1, WWTP-A.2 and WWTP-B. Different Lower cases denote significant differences between seasons (groups), ($p < 0.05$).

habits, climatic conditions, and the type and capacity of WWTPs (Paíga et al., 2019; Santos et al., 2009; Salgado et al., 2010). The herbicide Diuron was detected in all samples analysed, at concentrations ranging between 4.02 ± 0.09 in WWTP-B in autumn, and 49.8 ± 1.10 μg L^{-1} in WWTP-A.1 in Winter, as depicted in Fig. 2. There is a trend for higher concentrations in winter and spring samples, coinciding with the wet season when weed control is most practised in the region where this study was conducted.

Analysing the loads of the samples within WWTP-A (WWTP-A.1 and WWTP-A.2 - Figs. 1 and 2 and Table 2), there is a tendency for a decreasing load after the maturation lagoon (WWTP-A.2). The concentrations of NSAIDs, anticonvulsants/antidepressants and lipid regulators, are generally higher before than after maturation. The final effluent released into the aquatic environment is discharged from the maturation lagoon. Significant differences were observed for ibuprofen (5.8 ± 8.7 μg L^{-1} in WWTP-A.1 and 0.98 ± 1.6 μg L^{-1} in WWTP-A.2) ($p < 0.05$).

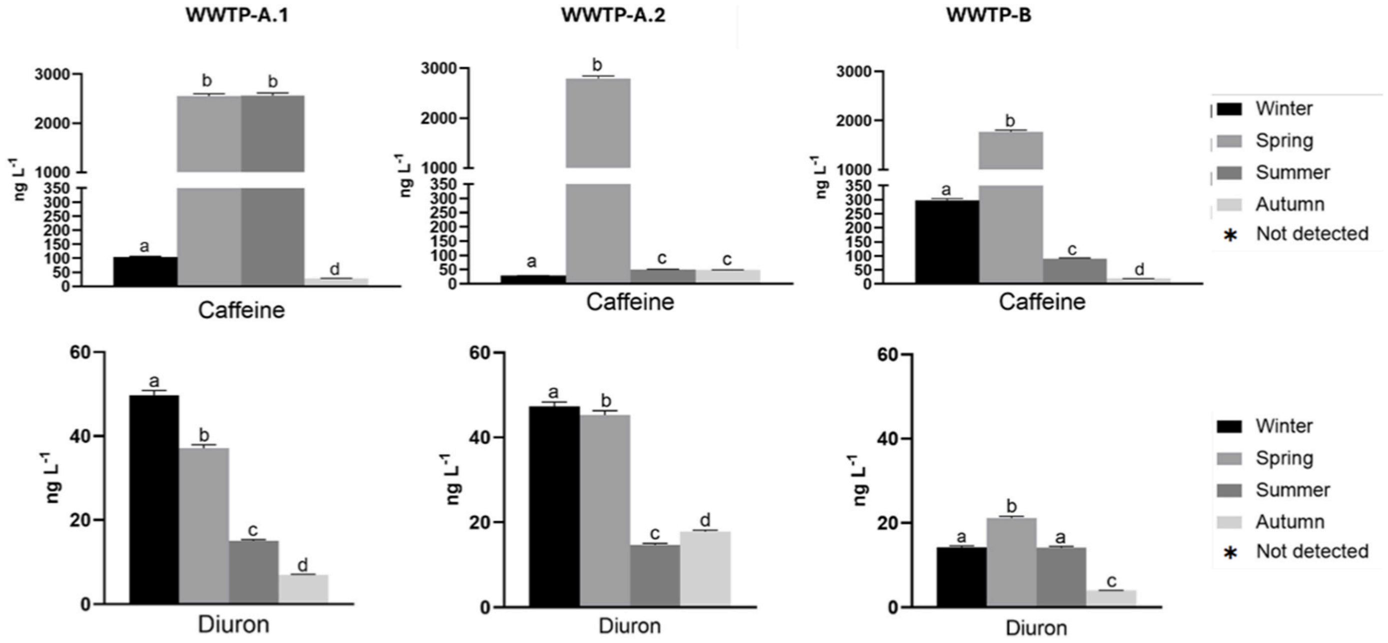

Fig. 2. Loads of the stimulant caffeine and the herbicide/algicide diuron, during winter, spring, summer and autumn, in the three wastewater effluents: WWTP-A.1, WWTP-A.2 and WWTP-B. Different lower cases denote significant differences between seasons (groups) ($p < 0.05$).

Table 2
Average loads, ng L^{-1} of each compound in the wastewater effluents: WWTP-A.1; WWTP-A.2; WWTP-B. of the four sampling campaigns (mean ± s.d). Different lower cases denote significant differences between columns of the same row.

	PhCs	WWTP-A.1	WWTP-A.2	WWTP-B
Anticonvulsants/Antidepressant	Fluoxetine	9 ± 5^{a}	3 ± 1^{a}	21 ± 18^{a}
	Venlafaxine	420 ± 181^{a}	238 ± 136^{a}	677 ± 319^{a}
	Carbamazepine	86 ± 30^{a}	94.83 ± 25.94^{a}	97 ± 32^{a}
	10,11-epoxycarbamazepine	29 ± 14^{a}	30 ± 9^{a}	38 ± 13^{a}
Antibiotics	Sulfamethoxazole	25 ± 16^{a}	26.18 ± 18.61^{a}	29 ± 21^{a}
	Trimethoprim	15 ± 13^{a}	8 ± 5^{a}	11 ± 7^{a}
B-blocker	Atenolol	360 ± 232^{a}	257 ± 152^{a}	295 ± 170^{a}
Lipid regulator	Clofibric acid	1 ± 1^{a}	0.7 ± 0.5^{a}	8 ± 7^{a}
Stimulant	Caffeine	1310 ± 1244^{a}	729 ± 1189^{a}	544 ± 715^{a}
NSAIDs/Analgesics	Naproxen	412 ± 604^{a}	57.00 ± 66.19^{a}	54 ± 35^{a}
	Ketoprofen	87 ± 57^{a}	5 ± 4^{a}	18 ± 20^{a}
	Ibuprofen	5832 ± 8727^{a}	983 ± 1560 ^{b}	8868 ± 15354^{a}
	2-hydroxyibuprofen	167 ± 160^{a}	14 ± 8^{a}	n.d.
	1-hydroxyibuprofen	407 ± 294^{a}	163 ± 56.72^{a}	<130
	Acetaminophen	12 ± 5^{a}	17 ± 11^{a}	17.66 ± 9.64^{a}
	Diclofenac	998 ± 439^{a}	267.75 ± 198^{a}	865 ± 326^{a}
	Salicylic acid	13 ± 4^{a}	13 ± 10^{a}	10 ± 8^{a}

This indicates that there is removal of compounds during the retention time in the lagoon, most likely promoted by exposure to UV radiation from sunlight, which causes photodegradation of organic molecules (Wang et al., 2020). In addition, microorganisms in the lagoon can incorporate these substances by assimilation or adsorption and further settling (Wang et al., 2020). Direct adsorption to sediments may also occur.

3.2. Removal of pharmaceutical compounds by microalgae consortia

The use of microalgae to remove pollutants from wastewater has been widely explored. However, most of the data available to date relies on laboratory experiments where few molecules were selected, and controlled microalgae cultures were used. Furthermore, most of the data reported is based on experiments where wastewater samples are spiked with the molecules of interest at higher concentrations than those found in real environmental samples. The present study investigates if wastewater-native microalgae consortia are efficient in removing the loads of the eighteen PhCs and one herbicide detected simultaneously in the different wastewater effluents. The wastewater samples used in this study were collected after secondary aerobic heterotrophic treatment, and the presence of the contaminants detected points out to the difficulty of these systems to remove the contaminants detected. Any further removal observed in this study is due to photosynthetic autotrophic activity or heterotrophic-autotrophic symbiosis. Even though the experiments were carried out at a laboratory scale, under controlled temperature and illumination conditions, further insights can be obtained from the present study. This study utilized real treated wastewater samples, characterized by their complex matrix and diverse array of trace compounds, along with spontaneously developed microbial consortia, known for their inherent variability and metabolic flexibility. This approach enhances the understanding of microalgal consortia in tertiary wastewater treatment.

Fig. 3 represents the composition of the consortia used at the beginning of the first mitigation experiments, each of which naturally developed in the wastewater collected from the respective sampling

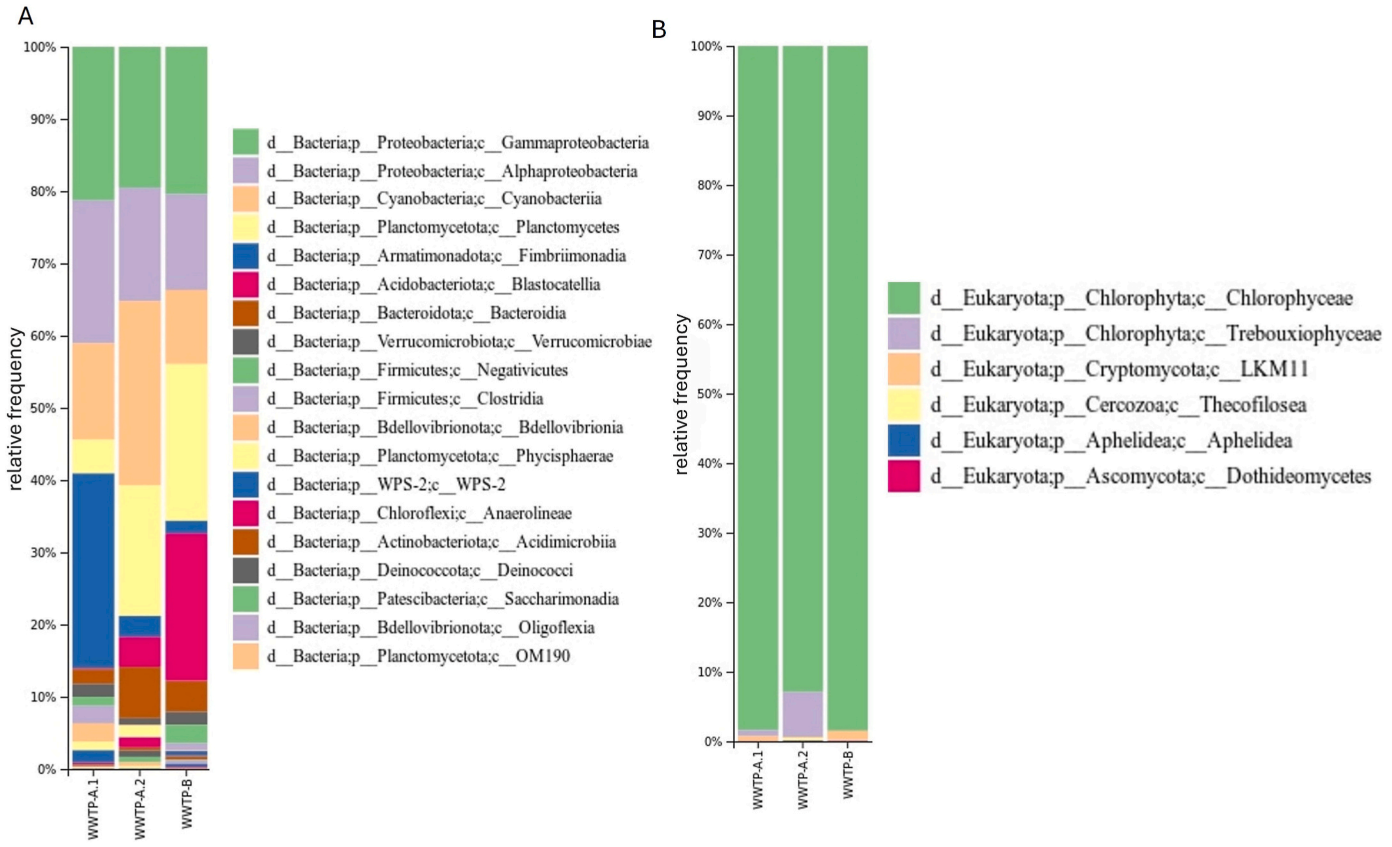

Fig. 3. Relative abundance of taxonomic classes in the three inocula used at the start of the mitigation experiments (winter) to inoculate the wastewater effluents: WWTP-A.1, WWTP-A.2, and WWTP-B. Taxonomic classification is based on 18S rRNA sequencing for eukaryotic classes (A) and 16S for prokaryotic classes. Each class is represented by a distinct colour as indicated in the legend.

points. Concerning the eukaryotic organisms (Fig. 3A), the predominant taxonomic class across all three consortia was Chlorophyceae, accounting for over 90 % of the detected organisms. In the WWTP-A.2 consortium, Trebouxiophyceae also exhibited a notable presence (~8 %), while the remaining classes collectively represented less than 2 %. The prevalence of photosynthetic green algae is expected given the culture conditions: irradiation, a moderate presence of nutrients and scarcity of biodegradable organic matter. These results align with those of Ibrahim et al. (2025) regarding the prevalence of Chlorophyceae (>90 %) as the dominant eukaryotic microalgae in wastewater-derived consortia, with Trebouxiophyceae practically representing the remaining microalgae, demonstrating their adaptability to wastewater environments. These authors reported a higher taxonomic diversity in summer conditions than in winter, but this is only visible with the breakdown of the genera present.

As to the abundance of prokaryotic organisms (Fig. 3B), the variability is much higher. Photosynthetic cyanobacteria were present ranging from 8 % (on WWTP-B consortium) to 25 % (on WWTP-A.2). Within each consortium there are at least four different taxonomic classes with an abundance higher than 5 %, but these and their abundance, are different on the three consortia. The composition of the consortia is expected to change during the mitigation experiments, as the microorganisms are grown in effluents with varying microbial and chemical compositions and exposed to varying temperatures and photoperiods. Thus, the abundance of prokaryotic organisms is highly dependent on the composition of the treated wastewater where the consortia were grown. This variability is expected to influence the performance of each consortium in the removal of some of the pollutants monitored. Nevertheless, the detected presence of Gammaproteobacteria, Alphaproteobacteria, Cyanobacteria, and Planctomycetes in the three consortia agrees with previous studies highlighting the role of these microbial groups in wastewater environments. Gammaproteobacteria have been associated with the removal of micropollutants in wastewater treatment systems (Unuofin et al., 2019; Cydzik-Kwiatkowska and Zielińska, 2016), suggesting their potential contribution to the bioremediation processes observed in the present study. Similarly, Alphaproteobacteria have been identified as temperature-sensitive in activated sludge systems, which may indicate that seasonal variations could influence their abundance in the experimental conditions (Muszyński et al., 2015). Moreover, Planctomycetes, frequently reported in activated sludge from multiple WWTPs (Chen et al., 2019b; Cydzik-Kwiatkowska and Zielińska, 2016), are known for their role in nitrogen cycling and organic matter degradation. The microbial composition of the three consortia shares similarities with those found in industrial WWTPs, where Proteobacteria dominate and are often responsible for the degradation of specific contaminants (Unuofin et al., 2019). These findings suggest that despite the differences in wastewater sources and treatment conditions, certain microbial groups consistently play key roles in wastewater bioremediation. Given the dynamic nature of the experimental conditions, further studies should be conducted to assess how the microbial composition evolves over time and how it impacts the degradation of specific pollutants.

It is important to highlight that the native consortia studied here are complex microbial communities where bacteria coexist with microalgae, creating symbiotic relationships that can significantly influence pharmaceutical degradation. Numerous studies have demonstrated that bacteria associated with microalgae enhance the biodegradation of contaminants via complementary metabolic pathways, co-metabolism, or facilitating bioavailability of compounds (e.g., Aditya et al., 2022; Phyu et al., 2024). The variability observed in removal efficiencies across consortia and seasons may partially reflect changes in the bacterial community composition and its interactions with microalgae.

Although this study focused primarily on the role of the microalgae consortia, future investigations targeting the specific contributions and mechanisms of bacterial partners within these communities could provide valuable insights for improving treatment performance.

Fig. 4 shows the average removal percentage of each PhC, during the four seasons (winter, spring, summer and autumn), while Fig. 5 depicts the same results for Caffeine and Diuron. Concerning the behaviour of the different pollutants monitored, three distinct categories can be identified.

1. Consistent removal above 60 % by all consortia used in all water samples.

   This is the case for Fluoxetine, Venlafaxine, Atenolol and Diuron.

2. Most frequent removal below 30 %, with few exceptions.

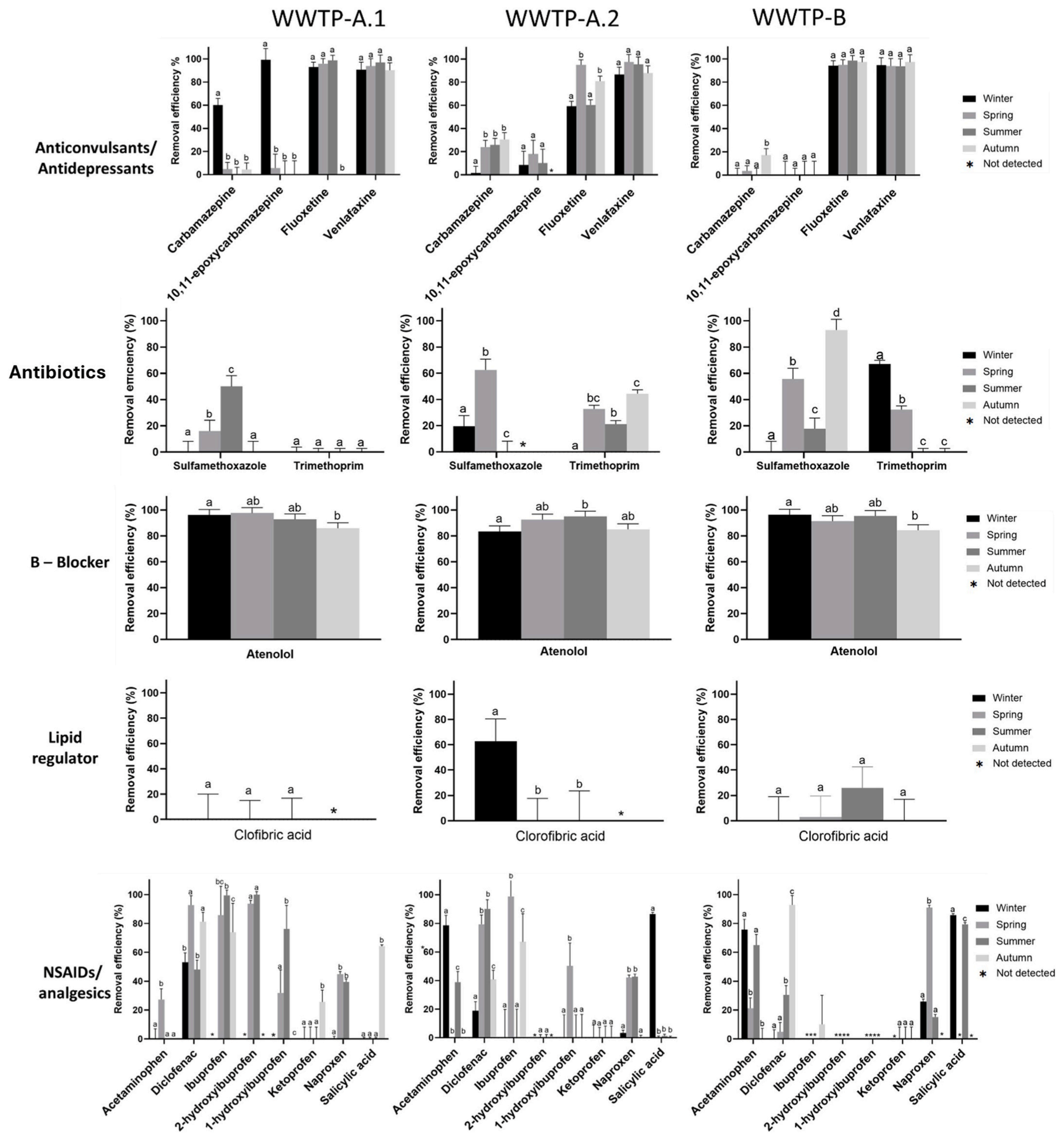

Fig. 4. Removal efficiencies of the drug classes: anticonvulsant/antidepressants, antibiotics, β-blocker, lipid regulator and NSAIDs; in the winter, spring, summer and autumn, for each sampling site: WWTP-A.1, WWTP-A.2 and WWTP-B. Different lower cases denote significant differences between seasons (groups) ($p < 0.05$).

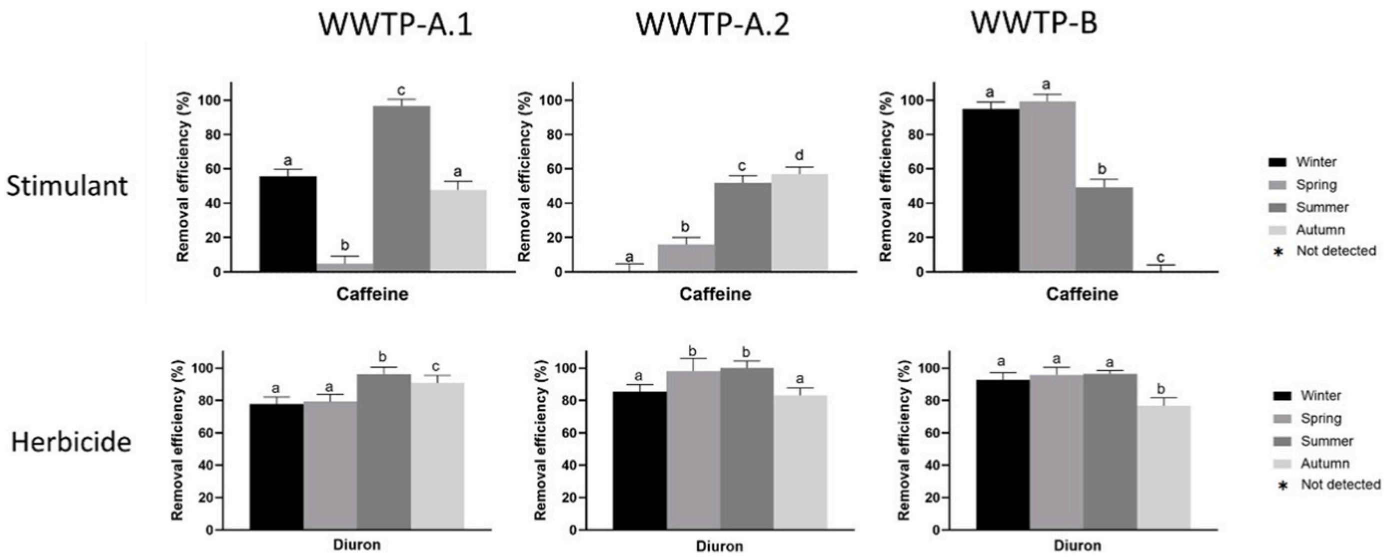

Fig. 5. Removal efficiencies of the stimulant caffeine and the herbicide/algicide diuron, in the winter, spring, summer and autumn, for each sampling site: WWTP-A.1; WWTP-A.2; WWTP-B. Different lower cases denote significant differences between seasons (groups) ($p < 0.05$).

This is the case for Carbamazepine and its metabolite, clofibric acid and ketoprofen.

3. Removal is highly dependent on the consortia used and/or water samples.

Observed for the antibiotics, NSAIDs/Analgesics (except for ketoprofen) and Caffeine.

3.2.1. *Category 1*

This includes the pollutants that were successfully removed by the native photosynthetic consortia. The chemical nature of these compounds contributes to their successful removal in all the samples and growth conditions tested with all consortia.

Venlafaxine, which is a tertiary amine compound, has a positive charge below pH 8.1, which enhances the affinity for the negatively charged cell walls, potentiating the adsorption of this molecule to the biomass (Akao et al., 2022). Fluoxetine is also basic, with a secondary amine group, and its removal is even more efficient. In this study, all the cultures for the mitigation experiments were initiated with pH levels between 7 and 8 (data not shown), and the average pH during microalgae growth varied between 8 and 9 (Table S5, supplementary material).

The β-blocker atenolol was consistently removed by all the consortia ranging from 83.4 % to 97.5 %. This aligns with the findings of Gentili and Fick (2017) and Hom-Diaz et al. (2017), who reported similar efficiencies using wild microalgae consortia cultivated in wastewater at a pilot scale under sunlight exposure.

Concerning the herbicide/algicide diuron (Fig. 5), this compound was consistently removed, in all experiments, with removal efficiencies ranging between 77.7 % and 100 %. On the contrary, García-Galán et al. (2020) reported a low removal efficiency for diuron on a closed PBR system with a mixed microalgae culture.

These contradictory data on the removal efficiencies may be attributed to differences in the microbial communities present in the treatment systems, the specific operational conditions of the reactors, and the initial concentration of diuron. Variations in light exposure, nutrient availability, and the cumulative contaminants present may also influence the degradation pathways and efficiency of diuron removal.

3.2.2. *Category 2*

The behavior of this category is also explained by the chemical nature of the pollutants, but in this case, these are recalcitrant and the photosynthetic consortia grown have no capacity to remove them.

Only the winter consortium on WWTP-A.1 was able to significantly remove carbamazepine (60.2 ± 5.8 %) and its metabolite 10,11-epoxy-carbamazepine (99.3 ± 9.7 %), with very low removal in all other experiments. Akao et al. (2022) obtained similar results using a microalgae-bacteria biofilm that removed 82–94 % of venlafaxine, but only 18–51 % of carbamazepine. Other authors have reported low removal efficiencies of carbamazepine by microalgae, bacteria and microalgae-bacteria consortia (García-Galán et al., 2020; Matamoros et al., 2016; Wilt et al., 2016). The hydrophilicity (Log P = 2.1) and recalcitrant characteristics of carbamazepine lead to low adsorption of this molecule to the cell walls; additionally, it possesses an electron-withdrawing functional group that makes it more resistant to biodegradation (Hai et al., 2018; Tadkaew et al., 2011; Wijekoon et al., 2013).

The lipid regulator (clofibric acid) showed minimal removal efficiency following microalgae growth. Only the winter consortium of the WWTP-A.2 effluent was able to remove 63 ± 17 % of this compound, and almost no removal by the other consortia was observed. Clofibric acid, along with carbamazepine, are recalcitrant compounds, that resist the different treatments of the WWTPs, and for this reason, they are frequently present in the aquatic environment (Tixier et al., 2003; Winkler et al., 2001). Its recalcitrant characteristics are attributed to the molecular structure, with two methyl groups on the carbon adjacent to the ether bond, that create steric hindrance, impairing not only the adsorption to the cell walls, but also the cleavage of the ether bond, which is the main mechanism known to occur during the biodegradation of its isomer (with only one methyl group near the ether bond) (Evangelista et al., 2010). In line with this data, Hemidouche et al. (2018) achieved a maximum removal of 35 % using a phenol-resistant strain of *Pseudomonas aeruginosa.* An exception to the low removal efficiencies was reported by Ungureanu et al. (2020), who achieved 60 % removal by optimising the culture conditions of the fungus *Trametes pubescens*.

In contrast with other NSAIDs, the removal efficiency of ketoprofen, was minimal, achieving only 26 % in WWTP-A.1 during autumn, and no removal was observed in the other seasons. These results were similar to those obtained by Hom-Diaz et al. (2017), who reported removal efficiencies of 36 % after microalgae treatment of toilet wastewater in a 1200 L multitubular PBR. Similarly, low degradation was reported on the removal rates of ketoprofen using consortia of *Chlorella* sp. – bacteria under continuous light (Ismail et al., 2016). The reduced biodegradability of ketoprofen under light exposure is attributed to the formation of by-products during photodegradation, which persist and hinder consortia biodegradation activity (Ismail et al., 2016). Additionally, the concentration of ketoprofen in the wastewater was relatively low (n.d. – 185 ng L^{-1}), which might contribute to less efficiency even in cases where partial removal occurred.

3.2.3. Category 3

For the compounds fitting into this category, over 30 % removal is frequent, but not the rule, as in most cases the same chemical compound can be almost totally removed or show almost no removal depending on the water sample and/or the microbial consortium growth.

The antibiotics monitored fit into this category (Fig. 4). The observed removal of sulfamethoxazole and trimethoprim was inconsistent, varying between no removal to 93 %. In WWTP-A.1, the summer consortium achieved the maximum removal (50.0 ± 8.2 %) for sulfamethoxazole, and no removal was observed for trimethoprim in all four consortia. In WWTP-A.2 the maximum removal was 62.6 ± 7.9 % in the spring consortium for sulfamethoxazole, while trimethoprim had a maximum removal of 44.5 ± 2.8 % in the autumn and no removal in Winter. In WWTP-B the removal efficiency was generally higher, reaching 93.0 ± 8.0 % for sulfamethoxazole by the autumn consortium, and 67.1 ± 2.8 % for trimethoprim in Winter. As stated above, the observed removal variability can be explained by the differences in the microalgae consortia composition through the four growth cycles, as different microorganisms may predominate in each growth cycle. As stated previously, the consortia evolve naturally through each cycle, with no attempt to influence the microbial composition of culture. Different microorganisms may use different mechanisms to remove these molecules, explaining the variability in the removal efficiencies between the consortia (Lu et al., 2023; Norvill et al., 2017; Tian et al., 2019). Lu et al. (2023) conducted a meta-analysis of the data reported in the literature on the removal of antibiotics by microalgae, concluding that there was a high heterogeneity among microalgae consortia. Furthermore, Kiki et al. (2020) found that the predominant mechanism for antibiotics removal was biodegradation, with the limited impact of biosorption, bioenrichment or abiotic factors.

As observed in Fig. 4, most NSAIDs/analgesics also fit into this category (except for ketoprofen, fitting in category 2 as previously discussed). Acetaminophen was removed with above 60 % efficiency whenever present at concentrations above 20 ng L^{-1} (winter consortium at WWTP-A.2 and winter and summer consortia at WWTP-B). Of twelve essays carried out, Diclofenac had over 60 % removal in five and below 30 % removal in only three of them. Ibuprofen and its metabolites were more than 60 % removed in nine out of fifteen cases where they were detected in the initial samples at significant concentrations. Very limited Ibuprofen removal was observed in the sample with the highest initial concentration (35.5 μg L^{-1} in WWTP-B is autumn), showing the incapacity of the consortium involved to deal with such an overload of this PhC. Naproxen showed nearly total removal in only one essay (WWTP-B in spring) but was also frequently more than 30 % removed. This variability corroborates the data reported by different authors, where different culture systems yield different removal efficiencies (Ismail et al., 2017; Escapa et al., 2015).

The removal of the stimulant, caffeine (Fig. 5), was inconsistent through all the consortia, with very low removal in WWTP-A.1 by the spring consortium, in WWTP-A.2 in winter, and in WWTP-B in the autumn consortium. In the other consortia, the removal efficiency varied between 16 and 100 %. Caffeine has a very low Log K_{OW} (−0.07) and consequently, low adsorption on the biomass or organic matter. Thus, the main mechanism responsible for eliminating caffeine in biological systems is attributed to biodegradation (Rempel et al., 2023).

For these pollutants in category 3 there is potential for their efficient removal using photosynthetic consortia, but in many cases this does not happen. This is probably related to the composition of the microbial consortium used, which is highly variable, particularly concerning the prokaryotic organisms present, as seen in Fig. 3B. Low removal is observed whenever microorganisms with the competence to remove the targeted chemicals have not been established in sufficient numbers. For these pollutants the microbial community seems to have a more substantial impact than the inherent properties of the molecules.

Table 3 displays the most significant positive correlations identified through the Spearman correlation matrix (Fig. S1on supplementary material), examining the influence of factors such as COD, TN, TP, and biomass production on removal efficiencies. A remarkable result is that there are almost no strong correlations involving pollutants outside category 3. Biomass production, measured by dry weight (d.w.), was strongly influenced by temperature (0.72), chemical oxygen demand (0.62) and total phosphorus (0.60). However, the only significant correlation between d.w. and PhC removal was observed for diclofenac (0.64), partially explaining the variable removal performance for this compound. Regarding initial nutrient concentrations, significant positive correlations were observed between TN concentration and the removal efficiency of acetaminophen (0.51), and TP concentrations and removal of naproxen (0.51). pH is one of the variables that shows influence on the removal efficiency of various PhCs, showing significant correlations with 1-hydroxyibuprofen (0.55), naproxen (0.50) and Diclofenac (0.64), which are all carboxylates. pH influences the electric charge of these molecules, consequently influencing biodegradability and/or adsorption. The removal of venlafaxine can be influenced by pH, as this molecule shows higher adsorption to the cell walls when it is positively charged, which is the case at pH 8.1. Furthermore, temperature also plays a vital role in microalgae growth, and physiological conditions. This was also supported by the strong correlation between dry weight and temperature (0.72). Fluoxetine was the only PhC which removal was slightly influenced by dissolved oxygen (0.58). Positive correlations were also observed between the removal of pairs of PhCs, most frequently those with similar structure, such as ibuprofen and its metabolites, carbamazepine and its metabolite or fluoxetine and venlafaxine. The similarities of their molecular structure lead to similar removal patterns.

These results show that in real-world scenarios, with no effort to influence the culture composition, temperature and light conditions, a system for wastewater treatment based on naturally evolving microalgae consortia will be unpredictable, and variable removal efficiencies

Table 3
Pairs of variables with the highest positive correlations (>0.5) obtained with the Spearman correlation matrix (Fig. S1).

Pair of variables	Correlation value
Ibuprofen – 1- hydroxyibuprofen	0.81
Ibuprofen – 2-hydroxyibuprofen	0.63
1-hydroxyibuprofen – 2-hydroxyibuprofen	0.78
1-hydroxyibuprofen – pH	0.55
Fluoxetine – Venlafaxine	0.58
Fluoxetine – sulfamethoxazole	0.60
Fluoxetine – 1-hydroxyibuprofen	0.52
Fluoxetine – 2-hydroxyibuprofen	0.53
Fluoxetine – O_2 (%)	0.58
Venlafaxine – Sulfamethoxazole	0.54
Carbamazepine – 10,11-epoxycarbamazepine	0.56
Carbamazepine – Diclofenac	0.55
Clofibric acid – Acetaminophen	0.61
Naproxen – pH	0.50
Naproxen – TP	0.51
Acetaminophen – Salicylic acid	0.76
Acetaminophen – TN	0.51
Diclofenac – pH	0.69
Diclofenac – d.w.	0.64
d.w. – Temperature	0.72
d.w. – COD	0.62
d.w. – TP	0.60

* TP- Total phosphorous; TN – Total Nitrogen.

must be expected. Further control must be exerted to ensure a more consistent removal, probably by carrying out acclimatization of the consortia to the presence of the targeted chemicals or inoculation using consortia that have proven their efficiency. Nonetheless, in this study, the overall aggregated removal of PhCs by the consortia in each experiment ranged from 40 to 83 %, improving water quality regarding PhCs contamination.

Even though the observed removal of PhCs was in many cases inconsistent and incomplete, there are other significant contributions of microalgae-based treatment for treated wastewater polishing. The most obvious is the removal of residual nutrients (N and P), preventing eutrophication on the receiving water bodies., Additionally, microplastics were also removed from these wastewater effluents (31 %–82 %), as detailed by Afonso et al. (2024). The proficient mitigation of various contaminants, including pharmaceuticals and microplastics, by native microalgae consortia underscores their potential to enhance wastewater treatment systems.

Recent studies have emphasized the critical role of microbial consortia – particularly the synergistic interactions between microalgae and bacteria – in improving wastewater treatment efficiency. Microalgae contribute directly by uptaking nutrients and certain micropollutants through photosynthesis and bioaccumulation, while also creating oxygenated conditions that support aerobic bacterial activity. In turn, bacteria can degrade more complex organic contaminants such as pharmaceuticals, and release CO_2 and metabolites that promote microalgal growth. This mutualistic relationship enhances the resilience, stability, and contaminant removal capacity of these systems across changing environmental conditions. By relying on native microalgae consortia, which include diverse bacterial communities, our study reflects this naturally occurring synergy and its role in the observed pharmaceutical mitigation (Su et al., 2022; Iqhrammullah et al., 2024)

While this study focused on evaluating the pharmaceutical removal performance of native consortia under real wastewater conditions, future research could incorporate controlled laboratory experiments to isolate specific microbial contributions and better elucidate the mechanistic pathways involved in pharmaceutical degradation. Overall, these findings highlight the capacity of native microalgae consortia, cultivated in wastewater effluents, to substantially reduce contamination levels, representing a promising solution for the development of sustainable wastewater treatment systems that can enable treated wastewater reuse. Additionally, the biomass produced, up to 2.6 kg/m^3 of treated water (Afonso et al., 2024), is a potential feedstock for biofuels and high-value product generation in a biorefinery concept, creating a circular economy system. The results of the study of the extraction of valuable bioactive compounds and the production of biofuels from this feedstock will be presented in a separate report currently under preparation.

To our knowledge, this is the first report where the same microalgae consortia have evolved naturally through four consecutive growth cycles over one year, with culture conditions adapted to each season, and where their performance in reducing pharmaceutical compounds in real treated effluents was assessed.

4. Conclusion

This work shows the potential of native microalgae consortia to remove real loads of pharmaceutical compounds and one herbicide from treated wastewater effluents. It is worth noting that all the cultures evolved naturally over the four growth cycles with no attempt to influence the microbial community. The wastewater effluents were nutrient deficient, and no supplementation was provided. Out of the twenty-one compounds targeted, nineteen were detected. The stimulant caffeine and the NSAID ibuprofen were the compounds that were present at higher concentrations (2.5 $\mu g\ L^{-1}$and 35.5 $\mu g\ L^{-1}$, respectively). Native microalgae consortia removed between 40 % and 83 % of the targeted molecules, with substantial variability among the different compounds and microalgae cultures. Of the 19 compounds targeted, all consortia consistently removed four and showed potential to remove eleven others. The removal was mainly influenced by the nature of the compound and by the microbial community of the consortia. Other variables, such as pH, and microalgal growth, also play a role in the removal efficiency, with positive correlations with the removal of specific pharmaceutical compounds. In conclusion, this work introduces a novel approach proposing the use of naturally evolving microalgae consortia in wastewater treatment, providing a comprehensive assessment of their effect on the removal of targeted contaminants in wastewater effluents. The biomass produced can be further used as a feedstock for high value biochemiclas and bioenergy generation, promoting the sustainability of both wastewater treatment and bioproduct production via a circular economy approach.

CRediT authorship contribution statement

Valdemira Afonso: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Brígida Rodrigues:** Validation, Resources. **Rodrigo Borges:** Validation, Resources. **Raúl Barros:** Writing – review & editing, Supervision, Resources, Conceptualization. **Maria João Bebianno:** Writing – review & editing, Supervision, Resources, Conceptualization. **Sara Raposo:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

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.

Acknowledgments

Authors acknowledge the support of FCT - Fundação para a Ciência e Tecnologia, Portugal, for the PhD grant UI/BD/150774/2020, and for the projects LA/P/0069/2020 awarded to the Associate Laboratory ARNET (https://doi.org/10.54499/LA/P/0069/2020), UIDB/00350/2020 (https://doi.org/10.54499/UIDB/00350/2020) and UIDP/00350/2020 (https://doi.org/10.54499/UIDP/00350/2020) awarded to CIMA of the University of the Algarve.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.126858.

Data availability

Data will be made available on request.

References

American Public Health Association (APHA), 2017. Standard Methods for the Examination of Water and Wastewater, 23rd ed. American Public Health Association, Washington, DC.

Aditya, L., Mahlia, T.M.I., Nguyen, L.N., Vu, H.P., Nghiem, L.D., 2022. Microalgae-bacteria consortium for wastewater treatment and biomass production. Sci. Total Environ. 838, 155871. https://doi.org/10.1016/j.scitotenv.2022.155871.

Afonso, V., Borges, R., Rodrigues, B., Barros, R., Bebianno, M.J., Raposo, S., 2024. Are native microalgae consortia able to remove microplastics from wastewater effluents? Environ. Pollut. 349, 123931. https://doi.org/10.1016/j.envpol.2024.123931.

Akao, P.K., Kaplan, A., Avisar, D., Dhir, A., Avni, A., Mamane, H., 2022. Removal of carbamazepine, venlafaxine and iohexol from wastewater effluent using coupled microalgal–bacterial biofilm. Chemosphere 308, 136399. https://doi.org/10.1016/j.chemosphere.2022.136399.

Chan, S.S., Khoo, K.S., Chew, K.W., Ling, T.C., Show, P.L., 2022. Recent advances biodegradation and biosorption of organic compounds from wastewater: microalgae-bacteria consortium -. A review. Bioresource Technology 344, 126159. https://doi.org/10.1016/j.biortech.2021.126159.

Chen, X., Hu, Z., Qi, Y., Song, C., Chen, G., 2019a. The interactions of algae-activated sludge symbiotic system and its effects on wastewater treatment and lipid accumulation. Bioresour. Technol. 292, 122017. https://doi.org/10.1016/j.biortech.2019.122017.

Chen, X., Lang, X.L., Xu, A.-L., Song, Z.-W., Yang, J., Guo, M.-Y., 2019b. Seasonal variability in the microbial community and pathogens in wastewater final effluents. Water 11 (12), 2586. https://doi.org/10.3390/w11122586.

Comeau, A.M., Douglas, G.M., Langille, M.G.I., 2017. Microbiome helper: a custom and streamlined workflow for microbiome research. mSystems 2. https://doi.org/10.1128/mSystems.00127-16 e00127–16.

Cydzik-Kwiatkowska, A., Zielińska, M., 2016. Bacterial communities in full-scale wastewater treatment systems. World J. Microbiol. Biotechnol. 32, 66. https://doi.org/10.1007/s11274-016-2012-9.

Dey, S., Bano, F., Malik, A., 2019. Pharmaceuticals and personal care product (PPCP) contamination-a global discharge inventory. Pharmaceuticals and Personal Care Products: Waste Management and Treatment Technology, pp. 1–26. https://doi.org/10.1016/B978-0-12-816189-0.00001-9.

Escapa, C., Coimbra, R.N., Paniagua, S., García, A.I., Otero, M., 2015. Nutrients and pharmaceuticals removal from wastewater by culture and harvesting of Chlorella sorokiniana. Bioresour. Technol. 185, 276–284. https://doi.org/10.1016/j.biortech.2015.03.004.

Evangelista, S., Cooper, D.G., Yargeau, V., 2010. The effect of structure and a secondary carbon source on the microbial degradation of chlorophenoxy acids. Chemosphere 79, 1084–1088. https://doi.org/10.1016/j.chemosphere.2010.03.018.

Gaffney, J.V., Cardoso, V.V., Cardoso, E., Teixeira, A.P., Martins, J., Benoliel, M.J., Almeida, C.M.M., 2017. Occurrence and behaviour of pharmaceutical compounds in a Portuguese wastewater treatment plant: removal efficiency through conventional treatment processes. Environ. Sci. Pollut. Control Ser. 24 (17), 14717–14734. https://doi.org/10.1007/s11356-017-9012-7.

García-Galán, M.J., Monllor-Alcaraz, L.S., Postigo, C., Uggetti, E., López de Alda, M., Díez-Montero, R., García, J., 2020. Microalgae-based bioremediation of water contaminated by pesticides in peri-urban agricultural areas. Environ. Pollut. 265. https://doi.org/10.1016/j.envpol.2020.114579.

Gentili, F.G., Fick, J., 2017. Algal cultivation in urban wastewater: an efficient way to reduce pharmaceutical pollutants. J. Appl. Phycol. 29, 255–262. https://doi.org/10.1007/s10811-016-0950-0.

Gondi, R., Kavitha, S., Yukesh Kannah, R., Parthiba Karthikeyan, O., Kumar, G., Kumar Tyagi, V., Rajesh Banu, J., 2022. Algal-based system for removal of emerging pollutants from wastewater: a review. Bioresour. Technol. 344. https://doi.org/10.1016/j.biortech.2021.126245.

Gonzalez-Rey, M., Tapie, N., Le Menach, K., Dévier, M.H., Budzinski, H., Bebianno, M.J., 2015. Occurrence of pharmaceutical compounds and pesticides in aquatic systems. Mar. Pollut. Bull. 96 (1–2), 384–400. https://doi.org/10.1016/j.marpolbul.2015.04.029.

Gururani, P., Bhatnagar, P., Kumar, V., Vlaskin, M.S., Grigorenko, A.V., 2022. Algal consortiums: a novel and integrated approach for wastewater treatment. Water 14, 22. https://doi.org/10.3390/w14223784.

Hai, F.I., Yang, S., Asif, M.B., Sencadas, V., Shawkat, S., Sanderson-Smith, M., Gorman, J., Xu, Z.Q., Yamamoto, K., 2018. Carbamazepine as a possible anthropogenic marker in water: occurrences, toxicological effects, regulations and removal by wastewater treatment technologies. Water 10 (2), 107. https://doi.org/10.3390/w10020107.

Hemidouche, S., Favier, L., Amrane, A., Dabert, P., Le Roux, S., Sadaoui, Z., 2018. Successful biodegradation of a refractory pharmaceutical compound by an Indigenous phenol-tolerant Pseudomonas aeruginosa strain. Water Air Soil Pollution 229, 103. https://doi.org/10.1007/s11270-018-3684-6.

Hom-Diaz, A., Jaén-Gil, A., Bello-Laserna, I., Rodríguez-Mozaz, S., Vicent, T., Barceló, D., Blánquez, P., 2017. Performance of a microalgal photobioreactor treating toilet wastewater: pharmaceutically active compound removal and biomass harvesting. Sci. Total Environ. 592, 1–11. https://doi.org/10.1016/j.scitotenv.2017.02.224.

Ibrahim, F.G.G., López-Serna, R., Muñoz Torre, R., Cabrerizo Pastor, A., de Godos Crespo, I., 2025. Evaluating emerging pollutant removal in a scale-down high rate algae pond. J. Environ. Chem. Eng. 13 (3), 116010. https://doi.org/10.1016/j.jece.2025.116010.

Iqhrammullah, M., Chiari, W., Hudaa, S., Irhamni, I., Fahrurrozi, Akbar, S.A., 2024. Microalgal–bacterial interactions: research trend and updated review. Heliyon 10 (15), e35324. https://doi.org/10.1016/j.heliyon.2024.e35324.

Ismail, M.M., Essam, T.M., Ragab, Y.M., Mourad, F.E., 2016. Biodegradation of ketoprofen using a microalgal–bacterial consortium. Biotechnol. Lett. 38 (9), 1493–1502. https://doi.org/10.1007/s10529-016-2145-9.

Ismail, M.M., Essam, T.M., Ragab, Y.M., El-Sayed, A.E. khair B., Mourad, F.E., 2017. Remediation of a mixture of analgesics in a stirred-tank photobioreactor using microalgal-bacterial consortium coupled with attempt to valorise the harvested biomass. Bioresour. Technol. 232, 364–371. https://doi.org/10.1016/j.biortech.2017.02.062.

Kiki, C., Rashid, A., Wang, Y., Li, Y., Zeng, Q., Yu, C.P., Sun, Q., 2020. Dissipation of antibiotics by microalgae: kinetics, identification of transformation products and pathways. J. Hazard Mater. 387. https://doi.org/10.1016/j.jhazmat.2019.121985.

Lu, W., Xu, C., Liu, F., Su, M., Cheng, S., Zhang, Y., 2023. Antibiotic removal efficiency by microalgae: a systematic analysis combined with meta-analysis. Process Saf. Environ. Prot. 174, 912–920. https://doi.org/10.1016/j.psep.2023.05.001.

Maryjoseph, S., Ketheesan, B., 2020. Microalgae based wastewater treatment for the removal of emerging contaminants: a review of challenges and opportunities. Case Stud. Chem. Environ. Eng. 2. https://doi.org/10.1016/j.cscee.2020.100046.

Matamoros, V., Uggetti, E., García, J., Bayona, J.M., 2016. Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: a laboratory scale study. J. Hazard Mater. 301, 197–205. https://doi.org/10.1016/j.jhazmat.2015.08.050.

Moghaddam, A., Khayatan, D., Esmaeili Fard Barzegar, P., Yazdanian, M., Tahmasebi, E., Alam, M., Abbasi, K., Esmaeili Gouvarchin Ghaleh, H., Tebyaniyan, H., 2023. Biodegradation of pharmaceutical compounds in industrial wastewater using biological treatment: a comprehensive overview. Int. J. Environ. Sci. Technol. 20, 5659–5696. https://doi.org/10.1007/s13762-023-04880-2.

Mojiri, A., Zhou, J.L., Ratnaweera, H., Rezania, S., Nazari V, M., 2022. Pharmaceuticals and personal care products in aquatic environments and their removal by algae-based systems. Chemosphere 288, 132580. https://doi.org/10.1016/j.chemosphere.2021.132580.

Muszyński, A., Tabernacka, A., Miłobędzka, A., 2015. Long-term dynamics of the microbial community in a full-scale wastewater treatment plant. Int. Biodeterior. Biodegrad. 100, 44–51. https://doi.org/10.1016/j.ibiod.2015.02.008.

Norvill, Z.N., Toledo-Cervantes, A., Blanco, S., Shilton, A., Guieysse, B., Muñoz, R., 2017. Photodegradation and sorption govern tetracycline removal during wastewater treatment in algal ponds. Bioresour. Technol. 232, 35–43. https://doi.org/10.1016/j.biortech.2017.02.011.

Paíga, P., Correia, M., Fernandes, M.J., Silva, A., Carvalho, M., Vieira, J., Jorge, S., Silva, J.G., Freire, C., Delerue-Matos, C., 2019. Assessment of 83 pharmaceuticals in WWTP influent and effluent samples by UHPLC-MS/MS: hourly variation. Sci. Total Environ. 648, 582–600. https://doi.org/10.1016/j.scitotenv.2018.08.129.

Paíga, P., Santos, L.H.M.L.M., Ramos, S., Jorge, S., Silva, J.G., Delerue-Matos, C., 2016. Presence of pharmaceuticals in the Lis river (Portugal): sources, fate and seasonal variation. Sci. Total Environ. 573, 164–177. https://doi.org/10.1016/j.scitotenv.2016.08.089.

Pereira, A., de Morais, E.G., Silva, L., Pena, A., Freitas, A., Teixeira, M.R., Varela, J., Barreira, L., 2023. Pharmaceuticals removal from wastewater with microalgae: a pilot study. Applied Sciences 13 (11), 6414. https://doi.org/10.3390/app13116414.

Pereira, A.M.P.T., Silva, L.J.G., Laranjeiro, C.S.M., Meisel, L.M., Lino, C.M., Pena, A., 2017. Human pharmaceuticals in Portuguese rivers: the impact of water scarcity in the environmental risk. Sci. Total Environ. 609, 1182–1191. https://doi.org/10.1016/j.scitotenv.2017.07.200.

Pereira, A.M.P.T., Silva, L.J.G., Lino, C.M., Meisel, L.M., Pena, A., 2016. Assessing environmental risk of pharmaceuticals in Portugal: an approach for the selection of the Portuguese monitoring stations in line with directive 2013/39/EU. Chemosphere 144, 2507–2515. https://doi.org/10.1016/j.chemosphere.2015.10.100.

Pereira, A.M.P.T., Silva, L.J.G., Meisel, L.M., Lino, C.M., Pena, A., 2015. Environmental impact of pharmaceuticals from Portuguese wastewaters: geographical and seasonal occurrence, removal and risk assessment. Environ. Res. 136, 108–119. https://doi.org/10.1016/j.envres.2014.09.041.

Pérez-Lucas, G., Aatik, A. El, Aliste, M., Navarro, G., Fenoll, J., Navarro, S., 2023. Removal of contaminants of emerging concern from a wastewater effluent by solar-driven heterogeneous photocatalysis: a case study of pharmaceuticals. Water Air Soil Pollut. 234, 55. https://doi.org/10.1007/s11270-023-06075-4.

Phyu, K.K., Zhi, S., Liang, J., Chang, C.-C., Liu, J., Cao, Y., Wang, H., Zhang, K., 2024. Microalgal-bacterial consortia for the treatment of livestock wastewater: removal of pollutants, interaction mechanisms, influencing factors, and prospects for application. Environ. Pollut. 349, 123864. https://doi.org/10.1016/j.envpol.2024.123864.

Rempel, A., Gutkoski, J.P., Biolchi, G.N., Biduski, B., Hoff, R.B., Perin, M., Treichel, H., Colla, L.M., 2023. Microalgae growth using treated domestic effluent added to emerging pollutants: removal mechanism and generation of by products. Journal of Water Process Engineering 55, 104175. https://doi.org/10.1016/j.jwpe.2023.104175.

Rocha, M.J., Cruzeiro, C., Reis, M., Rocha, E., Pardal, M., 2013. Determination of seventeen endocrine disruptor compounds and their spatial and seasonal distribution in Ria Formosa Lagoon (Portugal). Environ. Monit. Assess. 185 (10), 8215–8226. https://doi.org/10.1007/s10661-013-3168-5.

Salgado, R., Noronha, J.P., Oehmen, A., Carvalho, G., Reis, M.A.M., 2010. Analysis of 65 pharmaceuticals and personal care products in 5 wastewater treatment plants in Portugal using a simplified analytical methodology. Water Sci. Technol. 62 (12), 2862–2871. https://doi.org/10.2166/wst.2010.985.

Samal, K., Mahapatra, S., Hibzur Ali, M., 2022. Pharmaceutical wastewater as emerging contaminants (EC): treatment technologies, impact on environment and human health. Energy Nexus 6, 100076. https://doi.org/10.1016/j.nexus.2022.100076.

Santos, J.L., Aparicio, I., Callejón, M., Alonso, E., 2009. Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain). J. Hazard Mater. 164 (2–3), 1509–1516. https://doi.org/10.1016/j.jhazmat.2008.09.073.

Santos, L.H.M.L.M., Gros, M., Rodriguez-Mozaz, S., Delerue-Matos, C., Pena, A., Barceló, D., Montenegro, M.C.B.S.M., 2013. Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: identification of ecologically relevant pharmaceuticals. Sci. Total Environ. 461–462, 302–316. https://doi.org/10.1016/j.scitotenv.2013.04.077.

Silva, C., Almeida, C.M.M., Rodrigues, J.A., Silva, S., Coelho, M. do R., Martins, A., Lourinho, R., Cardoso, E., Cardoso, V.V., Benoliel, M.J., Rosa, M.J., 2021a. Occurrence and seasonality of pharmaceutical compounds in urban wastewaters in two Portuguese regions. Urban Water J. 18 (6), 465–478. https://doi.org/10.1080/1573062X.2021.1893365.

Silva, S., Cardoso, V.V., Duarte, L., Carneiro, R.N., Almeida, C.M.M., 2021b. Characterization of five Portuguese wastewater treatment plants: removal efficiency of pharmaceutical active compounds through conventional treatment processes and environmental risk. Applied Sciences 11 (16), 7388. https://doi.org/10.3390/app11167388.

Su, Y., Zhu, X., Zou, R., Zhang, Y., 2022. The interactions between microalgae and wastewater indigenous bacteria for treatment and valorization of brewery wastewater. Resour. Conserv. Recycl. 182, 106341. https://doi.org/10.1016/j.resconrec.2022.106341.

Sutherland, D.L., Heubeck, S., Park, J., Turnbull, M.H., Craggs, R.J., 2018. Seasonal performance of a full-scale wastewater treatment enhanced pond system. Water Res. 136, 150–159. https://doi.org/10.1016/j.watres.2018.02.046.

Sutherland, D.L., Ralph, P.J., 2019. Microalgal bioremediation of emerging contaminants - opportunities and challenges. Water Res. 164, 114921. https://doi.org/10.1016/j.watres.2019.114921.

Szopińska, M., Potapowicz, J., Jankowska, K., Luczkiewicz, A., Svahn, O., Björklund, E., Nannou, C., Lambropoulou, D., Polkowska, Ż., 2022. Pharmaceuticals and other contaminants of emerging concern in admiralty Bay as a result of untreated wastewater discharge: status and possible environmental consequences. Sci. Total Environ. 835, 155400. https://doi.org/10.1016/j.scitotenv.2022.155400.

Tadkaew, N., Hai, F.I., McDonald, J.A., Khan, S.J., Nghiem, L.D., 2011. Removal of trace organics by MBR treatment: the role of molecular properties. Water Res. 45 (8), 2439–2451. https://doi.org/10.1016/j.watres.2011.01.023.

Tian, Y.J., Wei, L.X., Yin, Z., Feng, L., Zhang, L.R., Liu, Y.Z., Zhang, L.Q., 2019. Photosensitization mechanism of algogenic extracellular organic matters (EOMs) in the phototransformation of chlortetracycline: role of chemical constituents and structure. Water Res. 164, 114940. https://doi.org/10.1016/j.watres.2019.114940.

Tixier, C., Singer, H.P., Oellers, S., Müller, S.R., 2003. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. Environ. Sci. Technol. 37 (6), 1061–1068. https://doi.org/10.1021/es025834r.

Ungureanu, C.V., Favier, L., Bahrim, G.E., 2020. Improving biodegradation of clofibric acid by Trametes pubescens through the design of experimental tools. Microorganisms 8 (8), 1243. https://doi.org/10.3390/microorganisms8081243.

Unuofin, J.O., Okoh, A.I., Nwodo, U.U., 2019. Recovery of laccase-producing gammaproteobacteria from wastewater. Biotechnology Reports 21, e00320. https://doi.org/10.1016/j.btre.2019.e00320.

Wang, Y., Fan, L., Crosbie, N., Roddick, F.A., 2020. Photodegradation of emerging contaminants in a sunlit wastewater lagoon, seasonal measurements, environmental impacts and modelling. Environmental Science: Water Research & Technology 6 (12), 3380–3390. https://doi.org/10.1039/D0EW00527D.

Wijekoon, K.C., Hai, F.I., Kang, J., Price, W.E., Guo, W., Ngo, H.H., Nghiem, L.D., 2013. The fate of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters and pesticides during MBR treatment. Bioresour. Technol. 144, 247–254. https://doi.org/10.1016/j.biortech.2013.06.097.

Wilt, D.A., Butkovskyi, A., Tuantet, K., Leal, L.H., Fernandes, T.V., Langenhoff, A., Zeeman, G., 2016. Micropollutant removal in an algal treatment system fed with source separated wastewater streams. J. Hazard Mater. 304, 84–92. https://doi.org/10.1016/j.jhazmat.2015.10.033.

Winkler, M., Lawrence, J.R., Neu, T.R., 2001. Selective degradation of Ibuprofen and clofibric acid in tow model river biofilm systems. Water Res. 35 (13), 3197–3205. https://doi.org/10.1016/S0043-1354(01)00026-4.

Wu, J.L., Liu, Z.H., Ma, Q.G., Dai, L., Dang, Z., 2023. Occurrence, removal and risk evaluation of ibuprofen and acetaminophen in municipal wastewater treatment plants: a critical review. Sci. Total Environ. 891 (Jun), 164600. https://doi.org/10.1016/j.scitotenv.2023.164600.

Zhou, J.L., Yang, L., Huang, K.X., Chen, D.Z., Gao, F., 2022. Mechanisms and application of microalgae on removing emerging contaminants from wastewater: a review. Bioresour. Technol. 364, 128049. https://doi.org/10.1016/j.biortech.2022.128049.

Web references

Águas do Algarve, 2024. https://www.aguasdoalgarve.pt/content/disponibilidades-hidricas visited at March 25th, 2024.

EU, 2019. https://commission.europa.eu/news/evaluation-eu-legislation-urban-waste-water-treatment-finds-it-fit-purpose-its-effectiveness-could-2019-12-17_en visited at March 22nd, 2024.

Infarmed. https://www.infarmed.pt/web/infarmed, 2022 visited at March 22nd, 2024.

Integrated Microbiome Resource, 2024. Protocols. n.d. https://imr.bio/protocols.html. visited at December 7th.

UNESCO, 2024. The United Nations World Water Development Report 2024: Water for Prosperity and Peace. United Nations Educational, Scientific and Cultural Organization at. https://www.unwater.org/publications/un-world-water-development-report-2024.