Harnessing water fleas for water reclamation

Abdullahi, Muhammad; Stead, Iestyn; Bennett, Sophie; Orozco, Rafael; Abdallah, Mohamed Abou-elwafa; Jabbari, Sara; Macaskie, Lynne E.; Tzella, Alexandra; Krause, Stefan; Al-Duri, Bushra; Lee, Robert G.; Herbert, Ben; Thompson, Peter; Schalkwyk, Megan; Getahun, Samuel; Dearn, Karl D.; Orsini, Luisa

DOI:
10.1016/j.scitotenv.2023.167224

Citation for published version (Harvard):
Abdullahi, M, Stead, I, Bennett, S, Orozco, R, Abdallah, MA, Jabbari, S, Macaskie, LE, Tzella, A, Krause, S, Al-Duri, B, Lee, RG, Herbert, B, Thompson, P, Schalkwyk, M, Getahun, S, Dearn, KD & Orsini, L 2023, 'Harnessing water fleas for water reclamation: A nature-based tertiary wastewater treatment technology', *Science of the Total Environment*, vol. 905, 167224. https://doi.org/10.1016/j.scitotenv.2023.167224

Science of the Total Environment 905 (2023) 167224

Contents lists available at ScienceDirect

Science of the Total Environment

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

Harnessing water fleas for water reclamation: A nature-based tertiary wastewater treatment technology

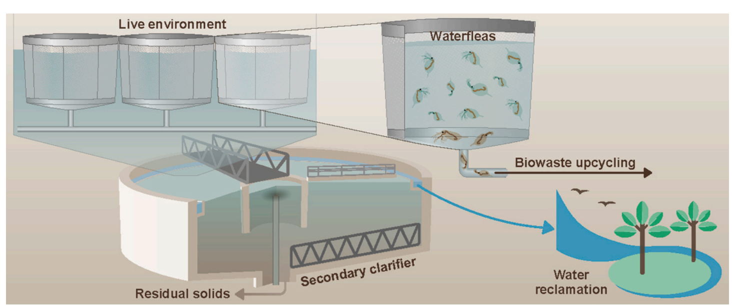
Muhammad Abdullahi [a,1], Iestyn Stead [a,b,c,1], Sophie Bennett [d], Rafael Orozco [e], Mohamed Abou-Elwafa Abdallah [f], Sara Jabbari [d], Lynne E. Macaskie [e], Alexandra Tzella [d], Stefan Krause [f,g], Bushra Al-Duri [h], Robert G. Lee [c,i], Ben Herbert [j], Peter Thompson [k], Megan Schalkwyk [k], Samuel Getahun [k], Karl D. Dearn [b,c,2], Luisa Orsini [a,c,l,*,2]

[a] Environmental Genomics Group, School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK
[b] Department of Mechanical Engineering, University of Birmingham, B15 2TT, UK
[c] Daphne Water Solution Limited, B168JB Birmingham, UK
[d] School of Mathematics, University of Birmingham, B15 2TT, UK
[e] School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK
[f] School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham B15 2TT, UK
[g] LEHNA - Laboratoire d'ecologie des hydrosystemes naturels et anthropises, University of Lyon, Darwin C & Forel, 3-6 Rue Raphaël Dubois, 69622 Villeurbanne, France
[h] School of Chemical Engineering, College of Engineering and Physical Sciences, University of Birmingham, Birmingham B15 2TT, UK
[i] Birmingham Law School, University of Birmingham, Birmingham B15 2TT, UK
[j] Stopford Ltd – Technology and Innovation Service Group, Mere Hall Farm Business Centre, Bucklow Hill Lane, Mere, Knutsford, Cheshire WA16 6LE, UK
[k] Umgeni Water Amanzi, South Africa
[l] The Alan Turing Institute, British Library, 96 Euston Road, London NW1 2DB, UK

HIGHLIGHTS

- A scalable, low-cost, low-carbon, and retrofittable tertiary water treatment biotechnology for the removal of persistent chemical pollutants
- At laboratory scale, removal efficiency is 90% for diclofenac, 60% for arsenic, 59% for atrazine and 50% for PFOS.
- Sustained removal efficiency of diclofenac was validated over four weeks at prototype scale.
- The *Daphnia*-based technology has technical, commercial and sustainability advantages over established and emerging treatments.

GRAPHICAL ABSTRACT



Modular interconnected devices are introduced in secondary clarifiers (live environment) to sustain a population of *Daphnia* (waterfleas) that removes chemical pollutants, generating clean effluent. Dead *Daphnia* is recycled after further treatment.

* Corresponding author at: Environmental Genomics Group, School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK.
E-mail addresses: mxa1132@student.bham.ac.uk (M. Abdullahi), i.m.n.stead@bham.ac.uk (I. Stead), r.l.orozco@bham.ac.uk (R. Orozco), m.abdallah@bham.ac.uk (M.A.-E. Abdallah), s.jabbari@bham.ac.uk (S. Jabbari), l.e.macaskie@bham.ac.uk (L.E. Macaskie), a.tzella@bham.ac.uk (A. Tzella), s.krause@bham.ac.uk (S. Krause), b.al-duri@bham.ac.uk (B. Al-Duri), r.g.lee@bham.ac.uk (R.G. Lee), ben.herbert@stopford.co.uk (B. Herbert), peter.thompson@umgeni.co.za (P. Thompson), megan.schalkwyk@umgeni.co.za (M. Schalkwyk), k.d.dearn@bham.ac.uk (K.D. Dearn), l.orsini@bham.ac.uk (L. Orsini).
[1] These authors contributed equally.
[2] Shared senior authorship.

https://doi.org/10.1016/j.scitotenv.2023.167224
Received 15 May 2023; Received in revised form 5 September 2023; Accepted 18 September 2023
Available online 20 September 2023

A R T I C L E I N F O

Editor: Damia Barcelo

Keywords:
Water reclamation
Biotechnology
Tertiary wastewater treatment
Pharmaceuticals
Pesticides
PFOS

A B S T R A C T

Urbanisation, population growth, and climate change have put unprecedented pressure on water resources, leading to a global water crisis and the need for water reuse. However, water reuse is unsafe unless persistent chemical pollutants are removed from reclaimed water. State-of-the-art technologies for the reduction of persistent chemical pollutants in wastewater typically impose high operational and energy costs and potentially generate toxic by-products (e.g., bromate from ozonation). Nature-base solutions are preferred to these technologies for their lower environmental impact. However, so far, bio-based tertiary wastewater treatments have been inefficient for industrial-scale applications. Moreover, they often demand significant financial investment and large infrastructure, undermining sustainability objectives. Here, we present a scalable, low-cost, low-carbon, and retrofittable nature-inspired solution to remove persistent chemical pollutants (pharmaceutical, pesticides and industrial chemicals). We showed *Daphnia*'s removal efficiency of individual chemicals and chemicals from wastewater at laboratory scale ranging between 50 % for PFOS and 90 % for diclofenac. We validated the removal efficiency of diclofenac at prototype scale, showing sustained performance over four weeks in outdoor seminatural conditions. A techno-commercial analysis on the *Daphnia*-based technology suggested several technical, commercial and sustainability advantages over established and emerging treatments at comparable removal efficiency, benchmarked on available data on individual chemicals. Further testing of the technology is underway in open flow environments holding real wastewater. The technology has the potential to improve the quality of wastewater effluent, meeting requirements to produce water appropriate for reuse in irrigation, industrial application, and household use. By preventing persistent chemicals from entering waterways, this technology has the potential to maximise the shift to clean growth, enabling water reuse, reducing resource depletion and preventing environmental pollution.

1. Introduction

Urbanisation, population growth, unsustainable food production and climate change have put unprecedented pressure on water resources, leading to a global water crisis (Joseph et al., 2020; Mancosu et al., 2015; Mishra et al., 2021). Sustainable use of water resources including the reclamation of previously used water are needed for sustaining people's societal, economic and environmental future. However, persistent chemical pollutants in treated wastewater (pharmaceuticals, pesticides, and industrial chemicals), originating from domestic and industrial processes, escape conventional wastewater treatment and prevent its safe reuse (K'Oreje et al., 2016; Rimayi et al., 2018). Inefficient wastewater treatment contributes significantly to the high number of chemicals found in the environment (Naidu et al., 2021; Wang et al., 2014). Wastewater effluent discharged into rivers is one of the main routes of distribution of chemical pollutants, as rivers are the main component of water reservoirs, irrigation and aquifer recharges. Through these uses of surface water, chemical pollutants make their way to humans through the food chain and water supply, adversely affecting the health of million people every year (Fuller et al., 2022). Contamination of water resources not only impacts human health, but also contributes to the loss of biodiversity and the deterioration of ecosystem services worldwide (Backhaus et al., 2012; Cardinale et al., 2012).

Wastewater treatment only removes a small proportion of chemical pollutants (Blum et al., 2017; Sutherland and Ralph, 2019) through adsorption onto activated sludge (Tran et al., 2018) or biotransformation, even if a mechanistic understanding of the latter is still superficial (van Bergen et al., 2021). Tertiary wastewater treatments designed to reduce micropollutants, such as ozonation and chlorination have high operational and energy costs, require large infrastructure, and can generate toxic by-products (e.g., bromate from ozonation; organochlorine compounds from chlorination) (Jahan et al., 2021; Li and Mitch, 2018). Moreover, the removal efficiency of these tertiary treatment technologies is affected by the hydrophobicity and ionization characteristics of chemical pollutants (Ma et al., 2018), meaning that the substrate determines the contaminants that can be removed. Often, multiple tertiary treatment technologies must be combined to remove a wide range of chemical pollutants (Skouteris et al., 2015). Biological tertiary wastewater treatments (e.g., phytoremediation and phycoremediation) have been advocated to manage wastewater sustainably, enabling water reuse and allowing the recovery of valuable resources such as nutrients and energy (Duque et al., 2021). However, these solutions are too slow for industrial-scale operations, requiring days rather than the needed hours to treat wastewater effluent, and require large infrastructure, undermining sustainability objectives (Wollmann et al., 2019).

Here, we pioneered and prototyped a scalable, low-cost, low-carbon, and retrofittable nature-inspired tertiary treatment technology for municipal wastewater. It uses *Daphnia* (waterfleas) to non-selectively remove chemical pollutants from secondary treated wastewater, improving wastewater effluent quality. *Daphnia* populations are retrofitted in containment devices within secondary clarifiers to polish effluent before final discharge. Once installed, the technology is largely self-sustaining, thanks to the ability of *Daphnia* to reproduce clonally. *Daphnia*'s exceptionally long dormancy (hundreds of years) enables the resurrection of dormant populations that have experienced different historical pollution pressures. Our pioneering research on *Daphnia* enabled us to leverage these properties and source strains with different tolerance to chemical pollutants to be employed in the technology development (Abdullahi et al., 2022a; Abdullahi et al., 2022b). In a previous laboratory study, we benchmarked the *Daphnia*'s removal of 16 pharmaceuticals from wastewater against other biological agents, i.e., algae and bacteria (Abdullahi et al., 2022a). Here, we show removal efficiency of an industrial chemical [e.g., perfluorooctanesulfonic acid (PFOS), a pesticide (atrazine), a heavy metal (arsenic) and a pharmaceutical (diclofenac)] by *Daphnia* strains, both as individual chemicals and as mixtures in secondary treated wastewater. These chemicals are on the priority list of regulatory agencies world-wide and have been shown to cause adverse health effects in humans and wildlife (Fuller et al., 2022). We show that chemicals taken up by *Daphnia* are not released back into the water, both in laboratory settings and in seminatural outdoor conditions. We show sustained performance of the technology at prototype scale, using diclofenac as proxy chemical, mimicking conditions of secondary clarifiers in wastewater plants and using volumes of water comparable to the wastewater generated by a single household. The technology has the potential for applications in municipal wastewater treatment. It can maximise the shift to clean growth, enabling water reuse, reducing resource depletion and environmental pollution.

2. Materials and methods

We present a nature-inspired technology that removes persistent chemical pollutants from secondary treated wastewater, providing a

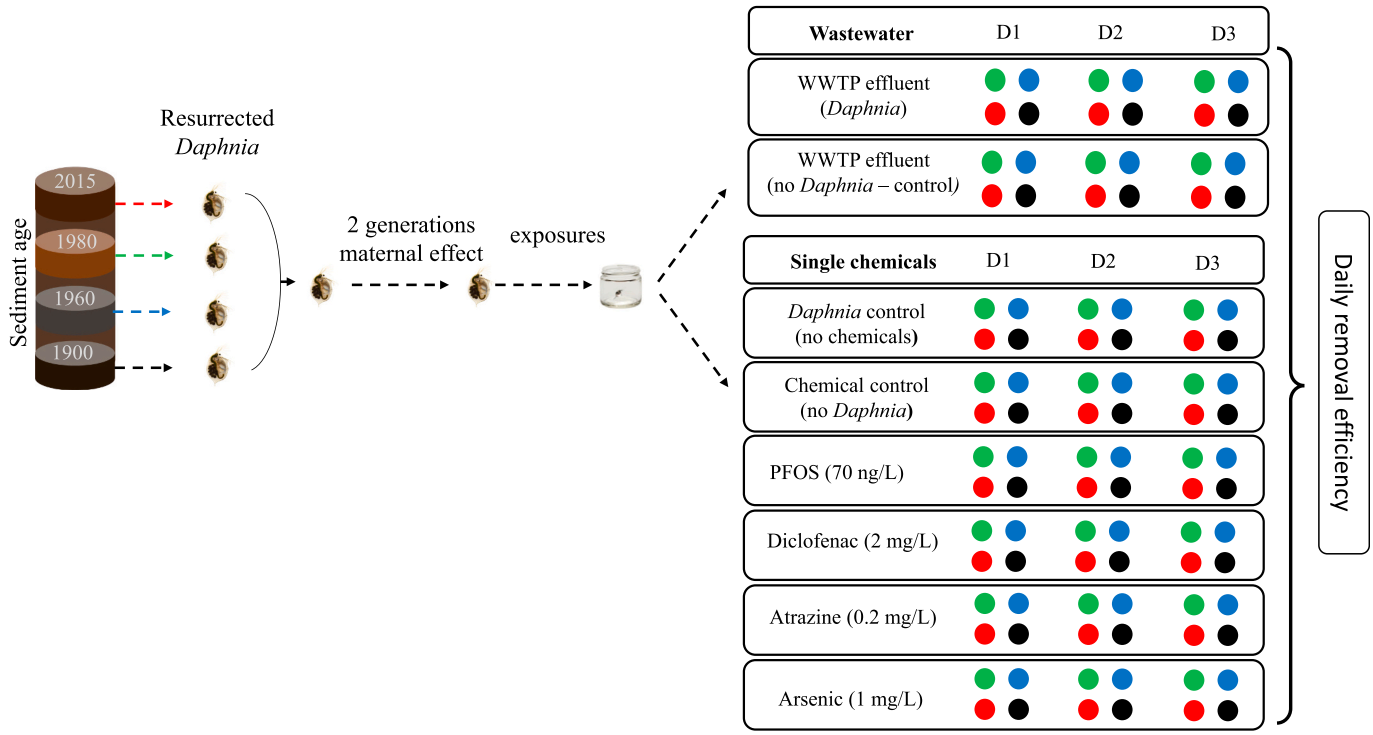


Fig. 1. Experimental design for laboratory exposures. Resurrected *Daphnia magna* genotypes from lake sediment deposits were maintained in controlled laboratory conditions (20 ± 2 °C, 16:8 h light:dark photoperiod and fed daily with 0.8 mg C/L of *C. vulgaris*) for two generations before exposure to single chemicals and wastewater secondary treated effluent. Two generations in these conditions were used to control for maternal effect. Single chemicals were quantified following exposure of four *D. magna* strains over 3 days to PFOS - 70 ng/L; diclofenac - 2 mg/L; atrazine - 0.2 mg/L and arsenic - 1 mg/L. The same four *D. magna* strains were also exposed to wastewater effluent. *Daphnia* and chemical controls were used as reference. The same four chemicals used in the individual chemical exposures were quantified in the effluent before and after exposure to *Daphnia*.

polishing step for wastewater before effluent discharge. We use methodologies from different disciplines, including biology to identify *Daphnia* strains tolerant to chemicals, environmental chemistry to quantify chemical removal efficiency and engineering to prototype the technology at small scale in semi-natural conditions. We prove removal efficiency of four chemical pollutants in the priority list of regulatory agencies worldwide at laboratory scale. We also compare removal efficiency of individual chemicals and mixture in wastewater. This exercise shows that the chemicals are not released back into the water. We use one of the chemicals used in the laboratory settings as a proxy to prove the performance of the technology at prototype scale in semi-natural outdoor conditions. Finally, we complete a techno-commercial analysis to identify comparative advantages of our nature-inspired technology over established tertiary treatments.

2.1. Exposures of Daphnia strains to chemical pollutants in controlled laboratory conditions

Daphnia magna strains used for the technology development were revived (resurrected) from multiple sedimentary archives with a well-documented history of anthropogenic change. To protect commercially sensitive information, the proprietary strains are only labelled with DM (*Daphnia magna*) and the approximate age of the strain. For this study, we used four *Daphnia* strains of different ages, spanning a century: DM1900; DM1960; DM1980; and DM2015.

Our first action was to assess chemical removal efficiency of different chemical pollutants by strains of *Daphnia magna* with different histories of exposure to chemicals. For this assessment, we exposed four strains, in triplicates over 3 days, to borehole water spiked with known concentrations of an industrial chemical (PFOS: 70 ng/L; CAS:2795-39-3), a biocide (atrazine:0.2 mg/L; CAS:1912-24-9), a pharmaceutical (diclofenac: 2 mg/L; CAS:15307-79-6) and a heavy metal (arsenic 1 mg/L; CAS: 7784-46-5), as well as to secondary treated wastewater. The chosen concentrations of individual chemicals were identified from literature research to be observed in surface and/or wastewater; the concentrations used in the exposures were matching some of the higher concentrations reported, as explained in the following. Nonsteroidal anti-inflammatory drugs such as diclofenac, acetaminophen and ibuprofen can be found at μg L^{-1} to g L^{-1} concentrations in seawater (Weigel et al., 2001) and surface waters (Fick et al., 2009), whereas their concentration is significantly lower in ground and drinking waters (ng/L; Godfrey et al., 2007). A concentration between 1 and 2 mg/L can be found in surface water downstream of drug production factories (Fick et al., 2009) and in wastewater (Wang and Wang, 2016; Wang et al., 2017).

Atrazine is one of the most widely used photosynthesis-inhibiting pre-emergent biocides worldwide (Prado et al., 2014). Typically, it occurs in drinking waters at concentrations of μg L^{-1} (Graziano et al., 2006). However, like other herbicides their concentrations in connection with floods caused by surface run-off (Petersen et al., 2012) can be several order of magnitude higher. This is especially true for region with intense land-use (Dalton et al., 2014). Metal contamination of groundwater is common in low and middle income countries (Winkel et al., 2011), even if average concentrations of up to 30 μg L^{-1} can be found in high income economies as well (Barrett et al., 2018). Occasionally in high income economies (e.g. North America, Smith et al., 2016), and frequently in low-and-middle-income countries (Chakraborti et al., 2018; Le Luu, 2019), arsenic concentrations in ground water can range between 500 μ/L and 1000 μ/L (Chakraborti et al., 2018). Perfluorinated compounds and other personal care products are ubiquitous in surface and wastewater, where they can occur in concentrations ranging between 8 and 390 ng/L (Subedi et al., 2015; Thompson et al., 2022). We opted for the concentration that was analytically detectable and on the higher end spectrum reported in literature.

The borehole water used for the individual chemical exposures was collected from a deep aquifer well. It is routinely used in our laboratory and its chemical properties are checked quarterly, showing a stable composition for the past 10 years.

Prior to the exposures, clonal replicates of the four *Daphnia* strains were maintained for at least two clonal generations in common garden conditions (20 ± 2 °C, 16:8 h light:dark photoperiod and fed daily with 0.8 mg C/L of *Chlorella vulgaris*) to control for maternal effect following (Abdullahi et al., 2022b; Cuenca-Cambronero et al., 2018). Following this acclimation phase, 24 h-old juveniles from the third or following broods of the second generation were exposed to known concentrations of individual chemicals and to wastewater (Fig. 1). The exposures were conducted over three days to assess whether the chemicals were excreted or retained by *Daphnia*. This was a critical step for the use of *Daphnia* as biological filter. The *Daphnia* density (10 *Daphnia* in 100 ml of borehole water) in each experimental exposure and biological replicate was chosen to ensure that the spiked borehole water or the wastewater were filtered completely every 24 h – this was based on previously tests showing the filtering capacity of *Daphnia magna* (Pau et al., 2013; Serra et al., 2019).

We did two types of exposures: 1) four individual chemicals, in which borehole water was spiked with known concentrations of chemicals and compared to a *Daphnia* control (borehole water, no chemicals, plus *Daphnia*). In these exposures, a control for chemical degradation consisted of borehole water spiked with individual chemicals without *Daphnia* at the same concentration used in the exposures. Limit of detection for these chemicals are reported in Appendix A and Table S1; and 2) treated wastewater in which the four chemicals were quantified before and after exposure to *Daphnia*. In these experiments, wastewater minus *Daphnia* was used as reference. Of the four chemicals tested in the individual chemical exposures, diclofenac and PFOS were found in the treated wastewater, whereas atrazine and arsenic were not detected. This is not surprising for UK-sourced wastewater, but it was not known at the beginning of the experiment. A total of 216 exposures were completed to quantify the removal efficiency of the four chemicals by the four genotypes of *Daphnia* (Fig. 1). The four chemicals used in individual exposures were quantified in wastewater before and after exposure to *Daphnia*. The secondary treated wastewater was sourced from the Finham wastewater treatment plant managed by Severn Trent Water, serving a population equivalent of 430,470 (Coventry, UK) and meeting accepted discharge standards for the UK and the EU [ammonia (1 mg/L); COD (120 mg/L); total nitrogen (1 mg/L); total phosphorus (1 mg/L); pH (5–9); suspended solids (25 mg/L)]. All exposures were conducted at 20 ± 2 °C, 16:8 h light:dark photoperiod. *Daphnia* in the individual chemical exposures were fed ad libitum with 0.8 mg Carbon/L of algal suspension (*Chlorella vulgaris*). *Daphnia* in wastewater exposures fed on organics already present in wastewater.

2.2. Quantifying chemicals in laboratory experiments

Following exposures, individual chemicals were quantified in the growth medium (borehole water spiked with chemicals and wastewater) using mass spectrometry following (Abdallah et al., 2019; Harrad et al., 2019) as explained in the Supplemental methods (Appendix A). The concentration of PFOS, atrazine, and diclofenac, both in the medium from individual chemical exposures and in wastewater were quantitated with ultraperformance liquid chromatography (UPLC) coupled with high-resolution mass spectrometry following (Abdallah et al., 2019; Abdullahi et al., 2022a). Arsenic concentration in both water and *Daphnia* tissue was quantified by using a Nexion 300× ICP-MS (Perkin Elmer, Seer Green, U.K.) fitting with a cyclonic spray chamber. Calibration curves spanning 1–20 ppb for borehole water and 1–10 ppb for *Daphnia* were constructed in DI water. For quantification of arsenic in the *Daphnia* tissue 50 ppb germanium was infused as an internal standard. Ideally, all chemicals would have been quantified in the *Daphnia* tissue to understand biotransformation mechanisms. However, protocols are lacking for chemical quantification in invertebrate tissue.

2.3. Removal efficiency at laboratory scale

The removal efficiency of the four chemicals was quantified both in borehole water and wastewater following *Daphnia* exposure as explained in Appendix A (Section 1.1) and calculated as [starting concentration − final concentration / starting concentration] × 100. On these quantitations, we applied an analysis of variance (ANOVA) with the Satterthwaite's method (lmerTest package; Kuznetsova et al., 2017) to assess if removal efficiency varied by genotype, day and their interaction term, nesting clonal replicates within genotype. Before applying the ANOVA analysis, the data were tested to meet the parametric assumption of the model by plotting the model residual vs fitted values (Q-Q plots) with the "car" package (Fox and Weisberg, 2018; Zuur et al., 2010). The removal efficiency was plotted using the ggplot2 package (Gentleman et al., 2009), with the standard error calculated using the 'summarySE' function in Rmisc package in R (Team, 2022).

2.4. Chemical pollutants removal at prototype scale

Having identified the strains with the highest removal efficiency in the laboratory exposures, we tested removal efficiency of a population of these strains in outdoor conditions for four weeks. We built a closed environment of 300 L (hereunder 'prototype'), holding borehole water spiked with diclofenac at the same concentration used in laboratory exposures (2 mg/L). Diclofenac was used as proxy chemical to demonstrate the scalability of the technology. It was chosen because it is among the most common chemicals found in effluent wastewater. The amount of spiked borehole water was equivalent to wastewater produced daily by a single household of 3.5 people. Diclofenac was spiked at the beginning of each week and removal efficiency measured daily, 5 times a week for 4 weeks, on 3 replicated samples of 100 ml, randomly collected from the top 1 m of water. Removal efficiency was quantitated with ultraperformance liquid chromatography coupled with high-resolution mass spectrometry as described above, using optimised methods (Abdallah et al., 2019).

Dissolved oxygen and water temperature were recorded daily in the prototype using a HANNA sensor (H19146). The prototype was protected from the rain with a tarpaulin and topped up every other week to compensate for evaporation. This strategy was applied to mimic a real-world open system (secondary clarifier) in which water levels are constant. The average temperature of the tank was regulated by a thermostat. However, fluctuations occurred due to the prototype being outdoors, reflecting realistic operational conditions. An algal suspension of *Chlorella vulgaris* was added daily to the prototype at a concentration comparable to the laboratory conditions described above. These conditions mimic a constant flow of organic matter entering the secondary clarifiers in a real-word wastewater treatment process. To mimic the flow of a secondary treatment tank in a wastewater treatment process, the prototype was aerated using a small submersible pump that recirculated the water at a flow rate of 1900 L/h through a diffused nozzle maintaining laminar flow conditions at a macroscopic scale. The retention time of the prototype was 4-6 h. These conditions were consistent with secondary clarifiers in wastewater treatment works. Diclofenac removal efficiency was calculated as in the laboratory experiments above. We tested whether removal efficiency significantly differed among weeks by using the lmer Test package (Kuznetsova et al., 2017), nesting days within weeks.

2.5. Engineering a self-sustaining technology prototype

For future installations in wastewater treatment works, we engineered and tested a system comprising of a live and a back-up environment. The live environment consisted of filtration vessels which are retrofitted within secondary clarifiers for effluent polishing, whether the

Table 1
ANOVA. An analysis of variance (ANOVA) is used to test whether the removal efficiency of individual chemicals spiked in borehole water (A) and of the same chemicals from wastewater (B) varied by genotype, day and their interaction term. Clonal replicates were nested with genotype. Atrazine and Arsenic were not found in the wastewater sampled from the Finham treatment plant (UK).

Effect	DF	A. Individual compounds								B. Wastewater			
		PFOS		Diclofenac		Atrazine		Arsenic		PFOS		Diclofenac	
		F	*Pr*	F	*Pr*	F	*Pr*	F	*Pr*	F	*Pr*	F	*Pr*
Genotype	3	3.81	**0.04**	3.87	**0.04**	2.10	0.16	0.81	0.51	3.13	0.07	327.08	**<0.001**
Day	2	0.10	0.91	1.65	0.23	3.49	0.07	0.48	0.63	5.13	**0.02**	84.60	**<0.001**
Genotype:day	6	2.60	0.08	1.00	0.47	1.31	0.33	1.58	0.23	7.28	**0.002**	10.05	**0.002**

Significant terms ($P < 0.05$) are in bold.

back-up environment consists of tandem bioreactors comprising a feedstock (algae *Chlorella vulgaris* CCAP 211/11b) growing chamber linked to algal medium (BBM; Appendix B; Table S2), and a *Daphnia* chamber (Appendix B; Fig. S1). This system produces *Daphnia* used to seed the secondary clarifiers and as a backup system in case of shock events (Appendix A, Section 1.2).

The vessels in the live environment are interconnected by a manifold system, and float in the top metre of the prototype. These vessels allow for the movement of water through porous meshes, sustaining *Daphnia* populations, and the collection of biowaste (dead *Daphnia* at the end of their life cycle). The latter happens via a system of interconnected valves that isolate the individual devices while the biowaste is funnelled into further treatment. Possible treatments of his biomass are discussed in Section 4.3.

Three vessels of 20 L capacity each were introduced in a 300 L outdoor prototype and seeded with *Daphnia* to assess removal efficiency at prototype scale. As *Daphnia* is self-sustaining via clonal reproduction, the *Daphnia* population density is expected to increase exponentially until it reaches carrying capacity (Bruijning et al., 2018). Following the *Daphnia* population introduction in the prototype, the number of *Daphnia* was allowed to reach the same density of the laboratory exposures described in Section 2.1 before removal efficiency of diclofenac was quantified. During the experiment, the *Daphnia* density was monitored in each vessel at the beginning and the end of each week over four weeks by collecting triplicate 100 ml-samples. *Daphnia* individuals were counted in the sampled volume and the density in the prototype estimated from these counts.

The controlled semi-automated back-up environment is maintained in controlled lighting, temperature and fluid transfer as described in Supporting Methods (Appendix A; Section 1.2). To understand the impact of shock events on the *Daphnia* population dynamics with consequences on removal efficiency in real world environments, we developed a model to capture the dynamics of both juvenile *Daphnia* ($J(t)$) and adult *Daphnia* ($A(t)$) that uses delay differential equations to capture the maturation time (τ) of juveniles. This model is described in the Supplemental methods (Appendix A, Section 1.3).

2.6. Techno-economic assessment of the Daphnia-based technology

The *Daphnia*-based technology performance was benchmarked against a range of existing tertiary wastewater treatment technologies, including established and emerging technologies through a desk techno-economic analysis. The competitor technologies assessed were ultraviolet irradiation, ozonation, chlorination, activated carbon, and multimedia filters. The operating and performance parameters used for this analysis, subject to availability, were: i) contaminant removal, ii) capital

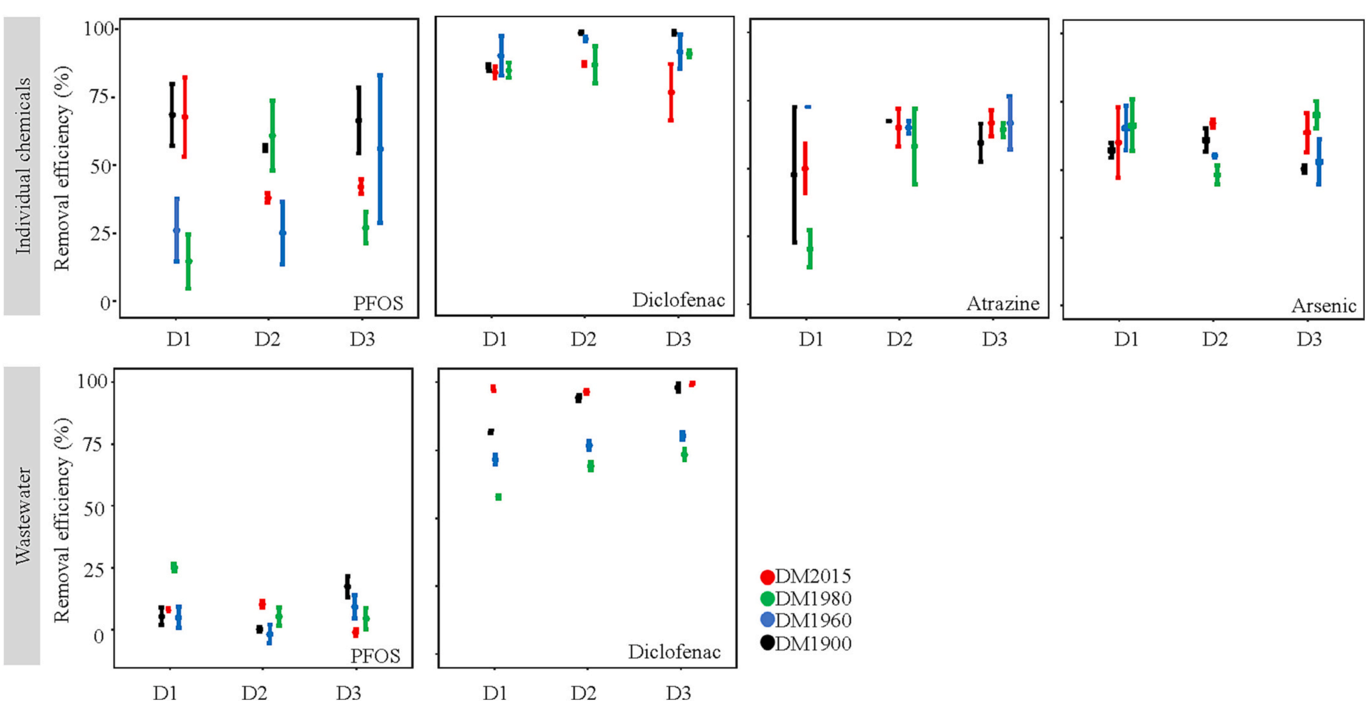


Fig. 2. Removal efficiency of chemical pollutants at laboratory scale. Removal efficiency (%) of 4 individual chemical compounds from borehole water and from wastewater: Atrazine and arsenic were not found in the wastewater sampled from the Finham treatment plant (UK). Error bars show variance among biological replicates in the experiments. The four *Daphnia* genotypes are colour coded: DM2015 (red); DM1980 (green); DM1960 (blue); and DM1900 (black).

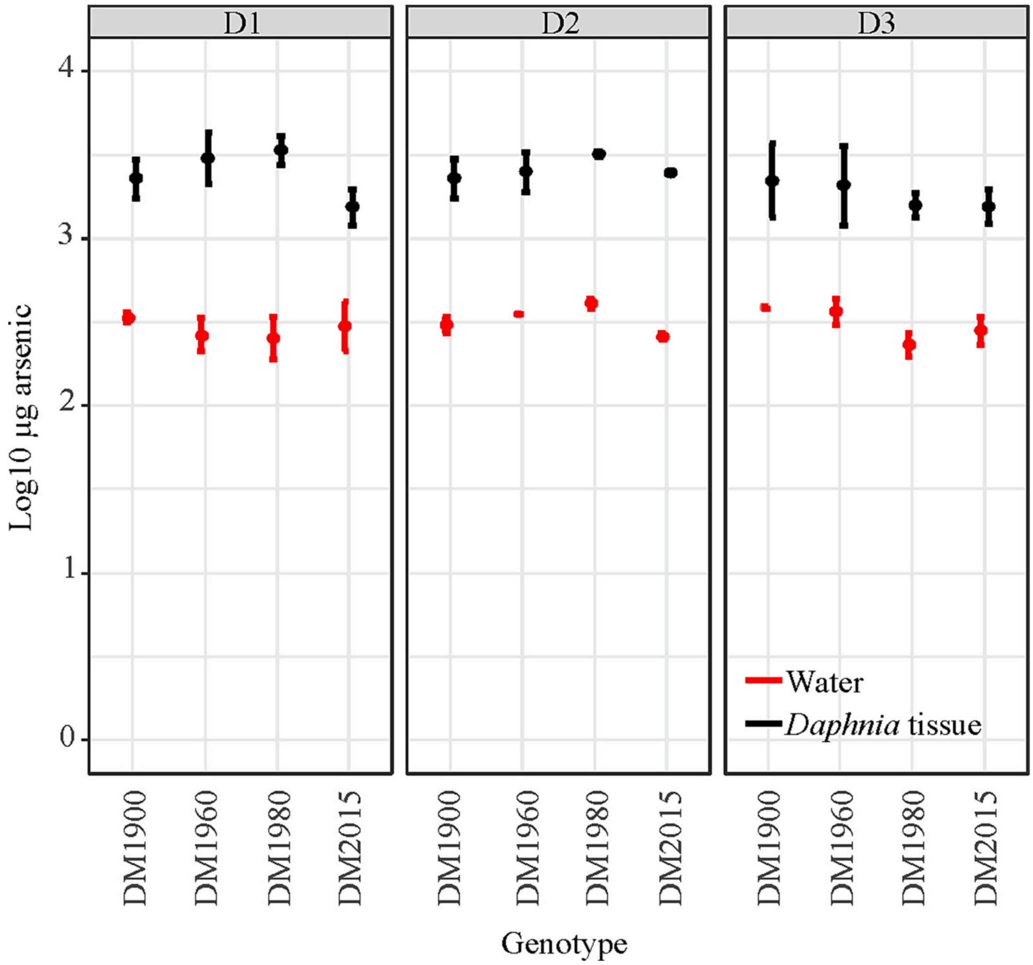


Fig. 3. Concentration of arsenic in the *Daphnia* tissue and removal from medium. Removal of arsenic from spiked borehole water and arsenic quantified in the tissue of four *Daphnia* strains across three days. The *Daphnia* strains are: DM1900, DM1960, DM1980 andDM2015. The two data series are not significantly different (chi-square Pearson correlation $P = 1$).

expenditure (CAPEX), iii) operational expenditure (OPEX), iv) by-products generation, v) energy used, and vi) carbon footprint. The same criteria were used to benchmark the technology against emerging biological tertiary wastewater treatment technologies including DRAM microbial technology, membrane biofilm reactors (MBfR), rotating biological contactors (RBC), phytoremediation, packed bed reactor (PBR), and photobioreactors.

3. Results

3.1. Exposures of Daphnia strains to chemical pollutants in controlled laboratory conditions

We quantified the recovery of each chemical from the medium after exposure to *Daphnia* as compared to a control (Appendix B - Table S3), and the removal efficiency of the four *Daphnia* strains in water spiked with known concentrations of four chemical pollutants belonging to four chemical classes (Appendix B – Table S4) The removal efficiency of PFOS and diclofenac in individual chemical exposures varied significantly by *Daphnia* genotype, whereas the four genotypes did not significantly differ in their removal efficiency of arsenic and atrazine (Table 1A; Fig. 2). The average removal efficiency across the three days of exposure was 90 % for Diclofenac, 50 % for PFOS, 59 % for atrazine and 60 % for arsenic (Fig. 2; Appendix B – Table S4). The historical *Daphnia* strain DM1900 showed the highest removal efficiency for diclofenac (95 %) and PFOS (65 %) (Fig. 2). The removal of the four individual chemicals did not significantly differ across the three days, suggesting that once taken up by *Daphnia*, the chemical pollutants were not excreted back into the water (Table 1A).

We quantified the removal efficiency of the four strains in wastewater. Whereas PFOS and diclofenac were recovered from the secondary treated wastewater, atrazine and arsenic were not detected (Appendix B – Table S5). This was not known at the beginning of the experiment. PFOS in the sampled wastewater was found at an average concentration of 35 ng/L, whereas diclofenac was found at an average concentration of 530 ng/L (Appendix B - Table S6), in line with previously reported concentrations in European effluent wastewaters (Ebele et al., 2017; Stulten et al., 2008). The removal efficiency of PFOS from wastewater did not significantly differ among genotypes but varied significantly by day. Some genotypes (e.g., DM1980) showed higher removal efficiency in day one and lower in days 2 and 3, whereas other genotypes (e.g., DM1900) showed increasing removal over the three days (Table 1B; genotype:day; Fig. 2). The four genotypes differed significantly in their removal efficiency of diclofenac from wastewater (Table 1B; genotype). The removal efficiency differed significantly across the three days of the experiment, with two out of four genotypes showing an increasing removal efficiency from day 1 to day 3 (Table 1B; genotype: day; Fig. 2; DM1900 and DM 2015). The average removal efficiency was lower for both chemicals when they occurred in mixtures (wastewater) than in individual chemical exposures, especially for PFOS (Fig. 2).

Our water analysis revealed that chemicals removed by *Daphnia* were not excreted back into the water. Using the heavy metal arsenic, we

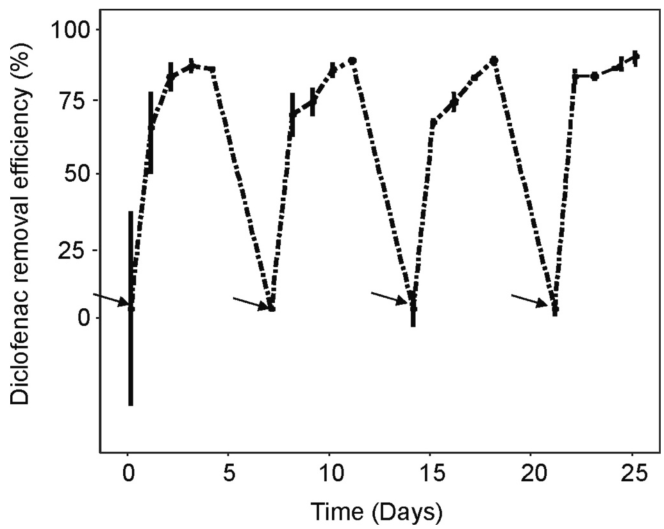


Fig. 4. Sustained performance at prototype scale. Removal efficiency of diclofenac spiked in a prototype-scale environment resembling volumes of wastewater produced daily by a single household of 3.5 people (arrows indicate spiking times). Removal efficiency was assessed with mass spectrometry analysis and calculated as follows: [starting concentration − final concentration / starting concentration] × 100.

quantified the amount of chemical present in the *Daphnia* tissue to assess whether the amount removed from the water corresponded to the one found in the *Daphnia* tissue, providing a first indication of the mechanisms of chemical removal by the biological agent. We worked under the hypothesis that if the amount of chemical removed from water corresponded to the amount found in the *Daphnia* tissue, the compound was bioaccumulated. To understand biotransformation mechanisms a mass balance analysis would be needed but not possible at this point due to lack of optimised protocols. The amount of arsenic removed from water was not significantly different from the concentration of arsenic recovered from the *Daphnia* tissue across the three days, suggesting that the arsenic removed from water was bioaccumulated in the *Daphnia* tissue, as expected for heavy metals (Fig. 3). The quantification of the other chemicals in the *Daphnia* tissue would have been useful but protocols are not yet established.

3.2. Prototype-scale demonstrator

We tested the removal efficiency of diclofenac by a population of *Daphnia* strains in the outdoor prototype over a period of 4 weeks (Appendix B – Table S7). A population of *Daphnia* comprising an equal proportion of DM1900 and DMV2015 was used. The highest removal efficiency in the prototype was 90 %, with an average across all weeks of 78 % (Fig. 4). The technology performance was constant across the four weeks regardless of the outdoor conditions (the removal efficiency did not significantly differ between weeks; ANOVA; $P = 1$) (Fig. 4). The sustained removal efficiency was accomplished by a thriving population of *Daphnia* for the duration of the trial, expressing an oscillation around an average density of 101 individuals/L (Appendix A, Fig. S2A).

The prototype was designed to mimic, as much as possible, operation conditions of secondary clarifiers in wastewater treatment works (Fig. 5A). To this end, a diffused nozzle maintained a laminar flow of water (Fig. 5B). The water temperature averaged 21 °C, with daily oscillations between 17 °C and 24 °C (Fig. S2B). Dissolved oxygen was on average 6 ppm, ranging between 4 and 9 ppm (Fig. S2B). Visual inspection of the water in the prototype showed no evidence of leakage of

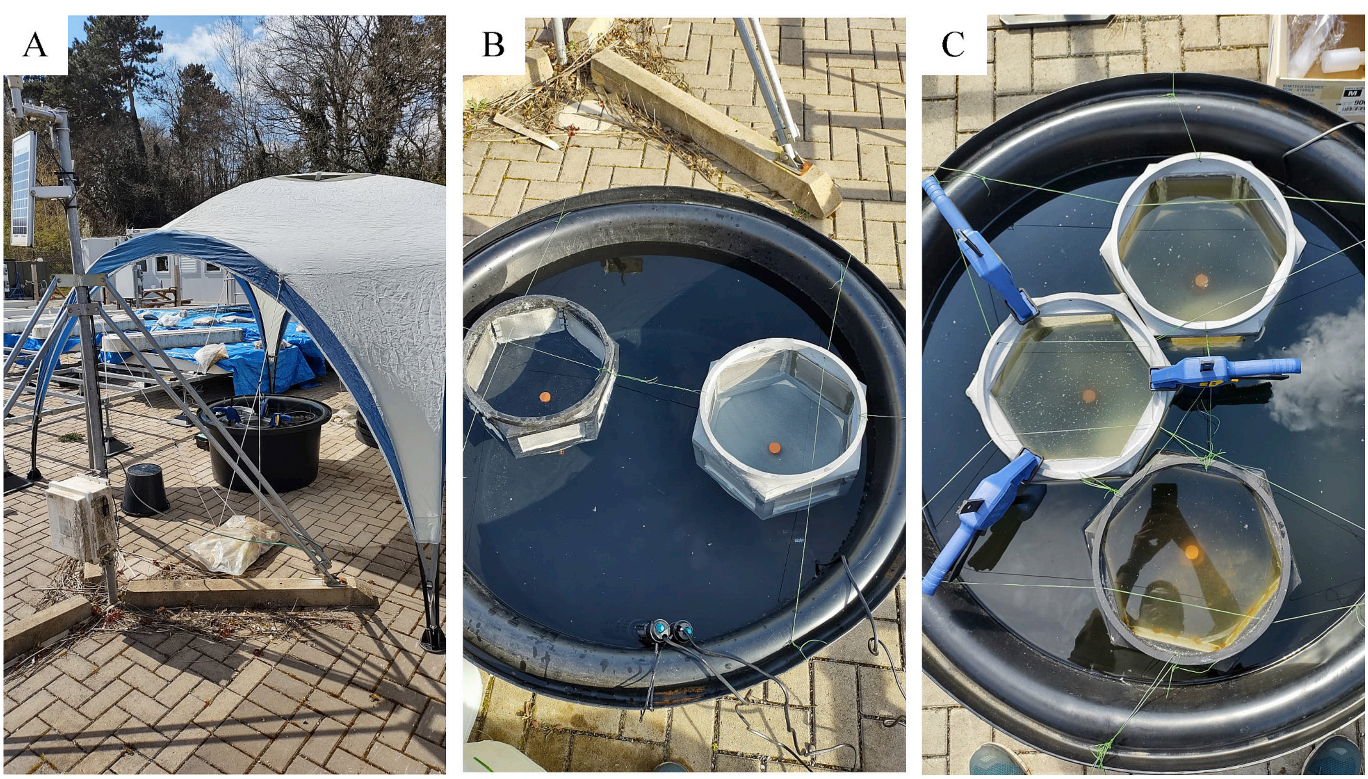


Fig. 5. *Daphnia*-based wastewater technology prototype. The *Daphnia*-based technology was tested at prototype scale in outdoor conditions (A). A tarpaulin protected the prototype from rainfall. Filtration devices were suspended in the top 1 m of the prototype tank to assess the technology performance over 4 weeks (B). At inoculation, the vessels are clear. Over time, the self-sustaining population of *Daphnia* grows to carrying capacity populating the vessels (C). The water flows freely through the walls of the vessels that are made of porous meshes. The solid base collects biowaste (dead *Daphnia* and shed carapaces) that is syphoned out to further treatment by using a system of interconnected valves (the red plug at the bottom of the vessel is the connection to the valve system).

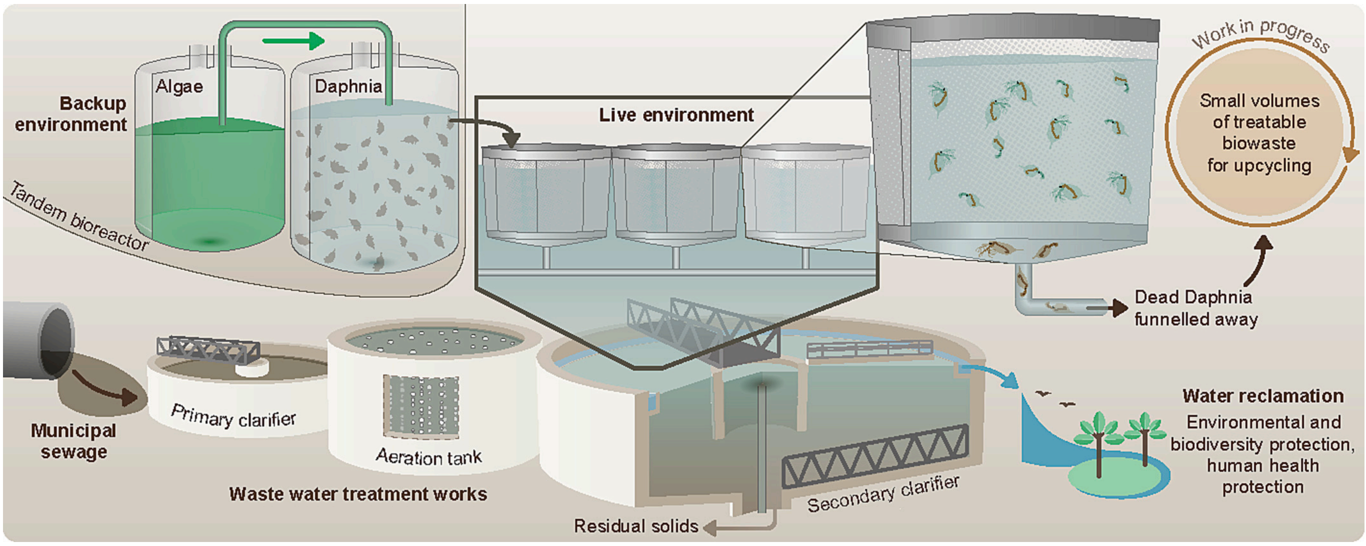


Fig. 6. Conceptual flow diagram of the water bioremediation process. The *Daphnia*-based water bioremediation technology consists of a live environment and a backup environment. Modular interconnected devices are introduced in secondary clarifiers (live environment) to sustain a population of *Daphnia* that removes chemical pollutants, generating clean effluent. At the end of the *Daphnia* life cycle and after having bioaccumulated/biotransformed persistent pollutants, dead *Daphnia* settle at the bottom of the containment devices and are syphoned into a waste treatment process of upcycling. The backup environment consists of a tandem *Daphnia*/algae bioreactor used to seed the initial population of *Daphnia* in the live environment and to top-up this population in case of shock events.

the filtration devices (Fig. 5C). At the end of the trial period a visual inspection of the filtration devices base confirmed the collection of debris (Fig. 5C), including *Daphnia* chitin exoskeleton that was shed at each moult cycle, confirming that the design allows for the collection of biowaste (dead *Daphnia* and other debris) without disturbing the live *Daphnia* population in the filtration vessels.

The live *Daphnia* population was contained in vessels consisting of a solid frame and a porous mesh that allows for the movement of water, while retaining the live *Daphnia* population and allowing for the collection of biowaste (dead *Daphnia*) at the base of the vessel (Fig. 6). The interconnected valves at the bottom of the vessels allow the isolation of the individual devices while the biowaste is funnelled into further treatment, as discussed in Section 4.3 below. A backup environment consists of tandem bioreactors for the on-site production of *Daphnia*, supported by the feedstock (algae) (Fig. 6). This backup environment has the critical function to generate *Daphnia* to seed the initial population in the live environment and to replenish this population in case of shock events. In the next result section, we present the first modelling results to determine the impact of shock events on the *Daphnia* population.

3.3. Modelling Daphnia population dynamics to prevent the impact of shock events

To ensure constant performance of the technology, and based on filtration efficiency in a laminar flow environment, we estimated that the *Daphnia* population density should be between 100 and 200 individuals/L. The health and stability of the *Daphnia* population can be affected by shock events (e.g., extreme temperature, pulses of chemical pollutants above accepted thresholds), affecting the removal efficiency of chemical pollutants. Predicting population crashes is critical to ensuring constant chemical removal. We derived a first set of simulations to understand how changes in population dynamics can cause loss of pollutant removal performance (Appendix A, Section 1.3). We, therefore, simulated the *Daphnia* population dynamics in a closed environment using a delay differential equation model, which accounts for juvenile and adult stages of the *Daphnia* population (Fig. S3). While future work will facilitate calibration of the model against data collected under a variety of environmental conditions, the model is already able to qualitatively distinguish between two scenarios; i) the first is where the balance of birth and death rates results in oscillatory dynamics, but ultimately the population is able to reach a stable positive equilibrium where chemical pollutants are removed efficiently (Fig. S3, solid line); ii) in the second scenario, an excess inflow of pollutants results in increased death rate and a population crash (Fig. S3; dashed line); in this scenario, the resident population would be unable to remove chemical pollutants. For operational practice, it will be essential to use calibrated models to identify dynamics leading to population crashes before they happen, guiding the top-up of the *Daphnia* populations from the side stream reactors.

3.4. Techno-economic assessment of the Daphnia-based technology

The techno-economic analysis served to benchmark the *Daphnia*-based technology against both established mechanical/chemical processes and emerging bio-based solutions. This exercise evidenced that our technology presents several technical, commercial and sustainability advantages over established and emerging treatments at comparable removal efficiency, benchmarked on available data on individual chemicals. The advantages of the *Daphnia*-based technology include the removal of a wide range of chemical pollutants; capital expenditure (CAPEX) and operational expenditure (OPEX) several orders of magnitude lower than conventionally adopted technologies, both mechanical and bio-based; low infrastructure requirements and non-toxic by-products generation. The techno-commercial analysis showed that the technology behaves equally or better than most technologies on the market for individual chemicals removal. For example, the *Daphnia*-based technology removes up to 99 % of diclofenac. This is comparable to UV treatment, ozonation (Plakas et al., 2016; Ziylan and Ince, 2011), reverse osmosis (Plakas et al., 2016), and chlorination (Hey et al., 2012) but it is more efficient that granular activated carbon (80 %) and multi-media filtration (3 %) (Snyder et al., 2007). Our technology removed up to 59 % of atrazine; reverse osmosis (95 %; Plakas et al., 2016) and granular activated carbon (74 %; Snyder et al., 2007) are more efficient than the *Daphnia*-based technology. However, multi-media filtration (17 %; Snyder et al., 2007), UV and ozonation (40 %; Plakas et al., 2016) are less efficient than our technology. Similarly, removal efficiency of diclofenac by phytoremediation (constructed wetlands; 50–80 %; Snyder et al., 2007) and photobioreactors (40–60 %; Salama et al., 2017) was lower than in the *Daphnia*-based technology. The techno-commercial analysis also revealed that the environmental impact of the technology in terms of odour, visual and noise impact was negligible and smaller than the one of both mechanical/chemical (Table S8) and bio-based solutions (Table S9). Data on energy consumption was not available for the *Daphnia*-based technology at the time of the techno-commercial analysis. However, as the energy input is negligible, it is anticipated that the carbon footprint will be low.

4. Discussion

4.1. Removal efficiency of micropollutants

Our previous research showed that *Daphnia* can remove 7 out of 16 pharmaceuticals more efficiently than algae and bacteria and the remaining 9 at a comparable rate in controlled laboratory conditions (Abdullahi et al., 2022a). Here, we demonstrated the removal efficiency by four carefully selected *Daphnia* strains of four chemical pollutants belonging to four distinct classes: pharmaceuticals, pesticides, heavy metals and industrial chemicals. We showed their removal efficiency both in single chemical exposures and in mixtures found in secondary treated wastewater. We found significant differences in removal efficiency among *Daphnia* strains. This difference can be explained by different histories of exposure, and hence tolerance, to chemical pollution. The strains used in our study were revived from sedimentary archives of lakes with known exposure history to chemical pollution. The removal efficiency of these strains aligns with their evolved tolerance to recurring chemical stress and higher tolerance of naïve strains to novel chemical stress, confirming previous findings (Abdullahi et al., 2022b). The selection of *Daphnia* strains based on their tolerance to pollution is unique to our innovation. Our understanding of the ecological and evolutionary properties of the biological agent allows the tailoring of *Daphnia* strains to different wastewater sources, providing unprecedented flexibility for the highest efficiency for specific wastewater sources.

In a first attempt to understand the fate of chemicals in the *Daphnia* tissue, we quantified the amount of arsenic in the *Daphnia* tissue and compared it to the amount removed from the growth medium (borehole water spiked with known concentrations of arsenic) after exposure. The concentration in the tissue was not significantly different from the concentration of arsenic removed from the exposure medium. Whereas a mass balance analysis is required to understand the mechanisms of biotransformation, our preliminary results suggest bioaccumulation of arsenic in line with previous studies in *Daphnia* (Tan et al., 2012). Generally, limited information is available on the mechanisms of removal of freshwater invertebrates because of technological limitations in detecting biotransformation products and parent compounds at low concentrations within the tissue of these organisms. Recent advances in liquid chromatography high-resolution mass spectrometry (e.g., Orbitrap) are promising for metabolite profiling due to their sensitivity and selectivity (Abdallah et al., 2019). Yet, lack of reference standards for metabolites in non-model species makes the quantification of parent compounds and metabolites in these species challenging. It is noteworthy that whereas we envision to tackle these challenges soon, understanding these mechanisms is not necessary to demonstrate the potential of the *Daphnia*-based technology. Critically, the system of valves at the bottom of the containment devices in the live environment, funnels away dead *Daphnia* preventing the release of chemicals back into the water.

An area often overlooked in studies assessing bio-based applications for water treatment is the effect of chemical mixtures on removal efficiency. Studying the response to individual chemicals as well as mixtures is vital because combined effects of chemicals may produce cumulative or synergistic effects that are more than or different from the sum of individual effects (Sprinkle and Payne-Sturges, 2021). In our study, we demonstrated comparable removal efficiency of diclofenac in single chemical and mixture (wastewater) exposures. Conversely, the removal of PFOS was lower in wastewater than in the individual chemical exposures. This could be explained by lower recovery rate of the reverse phase extraction cartridge used to capture mixtures, which may have affected the quantification of this compound in wastewater. It is also possible that the removal efficiency of PFOS in wastewater was dampened by synergistic effects with other chemicals (Ahrens and Bundschuh, 2014; Yang et al., 2019). Further studies are needed to pinpoint the reason for the observed discrepancy. The results of our study provide important insights for further development of the innovation.

4.2. Technology performance, risk and mitigation

The *Daphnia* technology presents performance, economic and engineering advantages. By non-selectively taking up different chemical pollutants, the technology overcomes the limitation of some state-of-the-art technologies affected by the hydrophobicity and ionization characteristics of chemicals (Ma et al., 2018). By providing a retrofittable solution it negates the need for major infrastructure modifications, which are required for other bio-based solutions (e.g. phycoremediation; Škufca et al., 2021). The technology is sympathetic to current secondary clarifier designs, which have been identified through co-development with the water industry. Whereas variations of the engineering design for secondary clarifiers with variation in tank profile, inlet and outlet geometry and scraping technologies (Trianni et al., 2021), may require adaptation of the containment devices in the live environment, the inherent agility and scalability of our technology, as well as the positioning of the containment devices in the top 1 m of the treatment tanks, enables their installation into most treatment plants without impacting the existing infrastructure. Modifications of the containment devices may be required for off-grid and small work installations. However, the modularity of the system permits these adjustments without major modifications of the wastewater plants and the technology. Because of these properties the *Daphnia*-based technology has potential for applications in low- and middle-income countries, meeting key sustainable development goals.

The outdoor prototype served to demonstrate that the removal efficiency of the proxy chemical (diclofenac) was comparable to removal efficiency measured in the laboratory and was sustained over time, regardless of the varying outdoor climatic conditions. The next step in the technology development is the further upscaling in a continuous open-flow environment holding real wastewater. This process will likely be stepwise, with validation in an intermediate scale prototype before installation is attempted within wastewater workflows. We expect success of upscaling based on prototype-scale demonstrators and co-development with end-users. Should the technology fail in upscaling, we will revert to smaller scale off-grid or smaller wastewater treatment applications, which are in demand for rural areas and smaller treatment plants. These smaller scale applications also meet the demand of low- and middle-income countries, as well as of higher income countries where <80 % of the population is connected to public urban wastewater treatment systems (e.g., Albania, Croatia, Slovenia and Poland) (Commission, 2014).

Extreme climates can potentially affect *Daphnia* performance impacting the removal efficiency of persistent chemicals. It is possible to overcome these limitations by either using strains naturally adapted to different climates - similar approaches have been used in phytoremediation (Ferro et al., 2018) - or by reverse engineering *Daphnia* strains. The latter approach is possible as shown by reverse genetics application in *Daphnia* (Fatimah et al., 2022; Nakanishi et al., 2014).

With respect to the potential release of the bioremediation agent into waterways, while the system is designed to prevent such release, *Daphnia* do not pose an environmental threat. Additionally, if released accidentally, *Daphnia*'s chance of survival in running water is very low because it is lake-dwelling species. In case of other shock events that can affect the chemical removal efficiency due to a crash of the resident

Daphnia population, the innovation uses sidestream reactors as a backup and top-up system.

4.3. Outstanding challenges and future technological developments

4.3.1. Waste management

We designed a manifold system regulated by a valve system that enables the dead *Daphnia* collection (biowaste) without disturbing the live population, as demonstrated in our prototype. This biowaste consists of an organic matrix (dead *Daphnia*) and persistent inorganics that are not biotransformed by *Daphnia* (e.g., heavy metals, as our results on arsenic suggest). Incineration of this biomass is feasible given the estimated modest average of 1 to 2 tonnes of biomass per clarifier/wastewater plant/year (Environment, 2019). For a fully circular system that enables the reuse of the biomass produced yearly as e.g. fertiliser, sustainably sourced 2D photocatalysts are an exciting new avenue for the reduction of persistent chemicals without the production of toxic by-products (Pérez-Álvarez et al., 2022). The development of a sustainable system to treat the refuse biomass is underway but will require significant efforts and optimisation. An inline mixer or other mechanical shearing of biomass may be needed prior to treatment with 2D photocatalysts.

4.3.2. Pushing sustainability and circularity

Pushing the concept of circularity that underpins our technology, we explored the market needs for other valuable by-products of the technology. One such product is chitin, which comprises the exoskeleton of *Daphnia*. Market research identified a potential market for chitin and its derivative chitosan into existing supply chains by serving applications in agriculture, textiles, food preservation, filtration, bioprinting, and as fuel cell catalysts (Environment, 2019). In response to these findings, a proof-of-concept was completed to test the separation between the organic matrix and the protein-chitin shell in *Daphnia* for onward valorisation. This preliminary work suggests that significant removal of the organic soft body is achieved at 240 °C in hot compressed water and preserves the chitin structure (Fig. S4). However, the impact of the treatment on the deacetylation, deproteinization and demineralization of the chitin fraction and how this may impact deacetylation to chitosan for onward valorisation are yet to be determined. At least the separation of the organic and chitin material in hot compressed water at high temperatures ensures that no residual pollutants are presence in the chitin matrix.

4.3.3. Plug-in and forget

The population dynamics modelled through the delay differential equations was able to distinguish population dynamics of a thriving population from the one of a population nearing a tipping point, eventually affecting the technology performance. Our next challenge is the encapsulation of this population model into a user-friendly interface to enable the application of the technology in a commercial environment by prompting a top-up of the population from the back-up environment before the *Daphnia* population reaches critical low density in the live environment, affecting chemical removal.

5. Conclusions

The *Daphnia*-based technology presented here provide a potentially ground-breaking process for the sustainable removal of persistent chemical pollutants, such as pharmaceutical, pesticides, industrial chemicals, and heavy metals, from wastewater. Preventing the discharge of these chemicals in the environment will avoid environmental deterioration and prevent impact on biodiversity. The low carbon footprint of the technology, combined with prevention of pollution of surface water provides a practical solution to meet increasingly stringent regulations (e.g., Urban Wastewater Directive on micropollutants removal; European Directive 2008/1/EC for pollution prevention and control; EU chemical strategy for sustainability 2020). By providing an add on polishing step to the traditional wastewater treatment, the technology contributes to delivering higher quality effluent, and prevents additional treatment to produce water appropriate for reuse in e.g., irrigation, industrial applications and use household use, such as toilet flushing.

CRediT authorship contribution statement

MA and IS – Investigation; formal analysis; writing; visualisation; SB – software; RO – Investigation; M A-E – resources, supervision; SJ, LEM AT - visualisation; software; supervision; SK, B A-D, RGL, BH, PT, MS and SG – Resources; KDD and LO – conceptualisation; methodology; writing; supervision; funding acquisition. All authors – writing.

Declaration of competing interest

LO and KDD declare potential financial conflict of interest as majority shareholders of Daphne Water Solutions Limited, a start-up which aims at commercialising the *Daphnia*-based technology. The data and engineering designs presented in this manuscript were realised through grants awarded to LO and KDD in their academic roles and are part of the technology proof of concept prior to commercialisation.

Data availability

The mass spectrometry data for laboratory exposures and prototype performance can be found at the dryad entry: https://datadryad.org/stash/share/IqavWPfhp8nPycJbV_Jw76y8JDJdtnX3AMDzYjdTvX0.

The codes used to generate the ANOVA analysis and the delay differential equations are available at the github entries (DWS/Anova.rmd at main · madbullahi/DWS (github.com) and DWS/MathscriptsDaphnia2022.zip at main · madbullahi/DWS (github.com)).

Acknowledgements

We thank Chantal Jackson for the artwork of Fig. 1. This work was funded by the NERC highlights grant deCODE (NE/N016777/1), the Alan Turing Institute (under EPSRC grant R-BIR-001). MA is supported by the Petroleum Technology Development Fund, Nigeria (PTDF/ED/OSS/POF/1369/18).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.167224.

References

Abdallah, M.A.-E., Nguyen, K.-H., Ebele, A.J., Atia, N.N., Ali, H.R.H., Harrad, S., 2019. A single run, rapid polarity switching method for determination of 30 pharmaceuticals and personal care products in waste water using Q-Exactive Orbitrap high resolution accurate mass spectrometry. J. Chromatogr. A 1588, 68–76.

Abdullahi, M., Li, X., Abdallah, M.A., Stubbings, W., Yan, N., Barnard, M., et al., 2022a. Daphnia as a sentinel species for environmental health protection: a perspective on biomonitoring and bioremediation of chemical pollution. Environ. Sci. Technol. 56, 14237–14248.

Abdullahi, M., Zhou, J., Dandhapani, V., Chaturvedi, A., Orsini, L., 2022b. Historical exposure to chemicals reduces tolerance to novel chemical stress in Daphnia (waterflea). Mol. Ecol. 31, 3098–3111.

Ahrens, L., Bundschuh, M., 2014. Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: a review. Environ. Toxicol. Chem. 33, 1921–1929.

Backhaus, T., Snape, J., Lazorchak, J., 2012. The impact of chemical pollution on biodiversity and ecosystem services: the need for an improved understanding. Integr. Environ. Assess. Manag. 8, 575–576.

Barrett, P.M., Hull, E.A., King, C.E., Burkart, K., Ott, K.A., Ryan, J.N., et al., 2018. Increased exposure of plankton to arsenic in contaminated weakly-stratified lakes. Sci. Total Environ. 625, 1606–1614.

Blum, K.M., Andersson, P.L., Renman, G., Ahrens, L., Gros, M., Wiberg, K., et al., 2017. Non-target screening and prioritization of potentially persistent, bioaccumulating

and toxic domestic wastewater contaminants and their removal in on-site and large-scale sewage treatment plants. Sci. Total Environ. 575, 265–275.
Bruijning, M., ten Berge, A.C.M., Jongejans, E., 2018. Population-level responses to temperature, density and clonal differences in Daphnia magna as revealed by integral projection modelling. Funct. Ecol. 32, 2407–2422.
Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., et al., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67.
Chakraborti, D., Singh, S.K., Rahman, M.M., Dutta, R.N., Mukherjee, S.C., Pati, S., et al., 2018. Groundwater arsenic contamination in the Ganga River Basin: a future health danger. Int. J. Environ. Res. Public Health 15.
Commission E, 2014. Communication on the Review of the List of Critical Raw Materials for the EU and the Implementation of the Raw Materials Initiative. European Commission, Brussels, p. 297.
Cuenca-Cambronero, M., Marshall, H., De Meester, L., Davidson, A., Beckerman, A.P., Orsini, L., 2018. Predictability of the impact of multiple stressors on the keystone species Daphnia. Sci. Rep. 8, 17572.
Dalton, R.L., Pick, F.R., Boutin, C., Saleem, A., 2014. Atrazine contamination at the watershed scale and environmental factors affecting sampling rates of the polar organic chemical integrative sampler (POCIS). Environ. Pollut. 189, 134–142.
Duque, A.F., Campo, R., Val Del Rio, A., Amorim, C.L., 2021. Wastewater valorization: practice around the world at pilot- and full-scale. Int. J. Environ. Res. Public Health 18.
Ebele, A.J., Abdallah, M.A.-E., Harrad, S., 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerg. Contam. 3, 1–16.
Environment SEa, 2019. Novel Tertiary Wastewater Treatment Technology and Process by Products (Birmingham).
Fatimah, R.M., Adhitama, N., Kato, Y., Watanabe, H., 2022. Development of transgenic Daphnia magna for visualizing homology-directed repair of DNA. Sci. Rep. 12, 2497.
Ferro, L., Gentili, F.G., Funk, C., 2018. Isolation and characterization of microalgal strains for biomass production and wastewater reclamation in northern Sweden. Algal Res. 32, 44–53.
Fick, J., Soderstrom, H., Lindberg, R.H., Phan, C., Tysklind, M., Larsson, D.G., 2009. Contamination of surface, ground, and drinking water from pharmaceutical production. Environ. Toxicol. Chem. 28, 2522–2527.
Fox, J., Weisberg, S., 2018. An R Companion to Applied Regression. SAGE Publications, Inc.
Fuller, R., Landrigan, P.J., Balakrishnan, K., Bathan, G., Bose-O'Reilly, S., Brauer, M., et al., 2022. Pollution and health: a progress update. Lancet E535–E547.
Gentleman, R., Hornik, K., Parmigiani, G., 2009. ggplot2. Springer, Heidelberg, London, New York.
Godfrey, E., Woessner, W.W., Benotti, M.J., 2007. Pharmaceuticals in onsite sewage effluent and ground water, western Montana. Groundw. Res. 45, 263–271.
Graziano, N., McGuire, M.J., Roberson, A., Adams, C., Hang, H., Blute, N., 2006. National atrazine occurrence monitoring program using the abraxis ELISA method. Environ. Sci. Technol. 40, 1163–1171.
Harrad, S., Wemken, N., Drage, D.S., Abdallah, M.A.-E., Coggins, A.-M., 2019. Perfluoroalkyl substances in drinking water, indoor air and dust from Ireland: implications for human exposure. Environ. Sci. Technol. 53, 13449–13457.
Hey, G., Ledin, A., Jansen, J., Andersen, H.R., 2012. Removal of pharmaceuticals in biologically treated wastewater by chlorine dioxide or peracetic acid. Environ. Technol. 33, 1041–1047.
Jahan, B.N., Li, L., Pagilla, K.R., 2021. Fate and reduction of bromate formed in advanced water treatment ozonation systems: a critical review. Chemosphere 266, 128964.
Joseph, N., Ryu, D., Malano, H.M., George, B., Sudheer, K.P., 2020. A review of the assessment of sustainable water use at continental-to-global scale. Sustain. Water Resour. Manag. 6, 18.
K'Oreje, K.O., Vergeynst, L., Ombaka, D., De Wispelaere, P., Okoth, M., Van Langenhove, H., et al., 2016. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. Chemosphere 149, 238–244.
Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmer test package: tests in linear mixed effects models. J. Stat. Softw. 82, 1–26.
Le Luu, T., 2019. Remarks on the current quality of groundwater in Vietnam. Environ. Sci. Pollut. Res. Int. 26, 1163–1169.
Li, X.F., Mitch, W.A., 2018. Drinking water disinfection byproducts (DBPs) and human health effects: multidisciplinary challenges and opportunities. Environ. Sci. Technol. 52, 1681–1689.
Ma, J., Dai, R., Chen, M., Khan, S.J., Wang, Z., 2018. Applications of membrane bioreactors for water reclamation: micropollutant removal, mechanisms and perspectives. Bioresour. Technol. 269, 532–543.
Mancosu, N., Snyder, R.L., Kyriakakis, G., Spano, D., 2015. Water scarcity and future challenges for food production. Water 7, 975–992.
Mishra, B.K., Kumar, P., Saraswat, C., Chakraborty, S., Gautam, A., 2021. Water security in a changing environment: concept, challenges and solutions. Water 13, 490.
Naidu, R., Biswas, B., Willett, I.R., Cribb, J., Kumar Singh, B., Paul Nathanail, C., et al., 2021. Chemical pollution: a growing peril and potential catastrophic risk to humanity. Environ. Int. 156, 106616.
Nakanishi, T., Kato, Y., Matsuura, T., Watanabe, H., 2014. CRISPR/Cas-mediated targeted mutagenesis in Daphnia magna. PLoS One 9, e98363.
Pau, C., Serra, Teresa, Colomer, J., Casamitjana, X., Sala, L., Kamp, R., 2013. Filtering capacity of Daphnia magnaon sludge particles untreated wastewater. Water Res. 47, 181–186.
Pérez-Álvarez, D.T., Brown, J., Elgohary, E.A., Mohamed, Y.M.A., El Nazer, H.A., Davies, P., et al., 2022. Challenges surrounding nanosheets and their application to solar-driven photocatalytic water treatment. Mater. Adv. 3, 4103–4131.
Petersen, J., Grant, R., Larsen, S.E., Blicher-Mathiesen, G., 2012. Sampling of herbicides in streams during flood events. J. Environ. Monit. 14, 3284–3294.
Plakas, K.V., Georgiadis, A.A., Karabelas, A.J., 2016. Sustainability assessment of tertiary wastewater treatment technologies: a multi-criteria analysis. Water Sci. Technol. 73, 1532–1540.
Prado, B., Duwig, C., Hidalgo, C., Müller, K., Mora, L., Raymundo, E., et al., 2014. Transport, sorption and degradation of atrazine in two clay soils from Mexico: andosol and vertisol. Geoderma 232, 628–639.
Rimayi, C., Odusanya, D., Weiss, J.M., de Boer, J., Chimuka, L., 2018. Contaminants of emerging concern in the Hartbeespoort Dam catchment and the uMngeni River estuary 2016 pollution incident, South Africa. Sci. Total Environ. 627, 1008–1017.
Salama, E.-S., Kurade, M.B., Abou-Shanab, R.A.I., El-Dalatony, M.M., Yang, I.-S., Min, B., et al., 2017. Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. Renew. Sust. Energ. Rev. 79, 1189–1211.
Serra, T., Muller, M.F., Colomer, J., 2019. Functional responses of Daphnia magna to zero-mean flow turbulence. Sci. Rep. 9, 3844.
Skouteris, G., Saroj, D., Melidis, P., Hai, F.I., Ouki, S., 2015. The effect of activated carbon addition on membrane bioreactor processes for wastewater treatment and reclamation - a critical review. Bioresour. Technol. 185, 399–410.
Škufca, D., Kovačič, A., Prosenc, F., Griessler Bulc, T., Heath, D., Heath, E., 2021. Phycoremediation of municipal wastewater: removal of nutrients and contaminants of emerging concern. Sci. Total Environ. 782, 146949.
Smith, A.E., Lincoln, R.A., Paulu, C., Simones, T.L., Caldwell, K.L., Jonesc, R.L., et al., 2016. Assessing arsenic exposure in households using bottled water or point-of-use treatment systems to mitigate well water contamination. Sci. Total Environ. 544, 701–710.
Snyder, S.A., Adham, S., Redding, A.M., Cannon, F.S., DeCarolis, J., Oppenheimer, J., et al., 2007. Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. Desalination 202, 156–181.
Sprinkle, R.H., Payne-Sturges, D.C., 2021. Mixture toxicity, cumulative risk, and environmental justice in United States federal policy, 1980–2016: why, with much known, was little done? Environ. Health 20, 104.
Stulten, D., Zuhlke, S., Lamshoft, M., Spiteller, M., 2008. Occurrence of diclofenac and selected metabolites in sewage effluents. Sci. Total Environ. 405, 310–316.
Subedi, B., Codru, N., Dziewulski, D.M., Wilson, L.R., Xue, J., Yun, S., et al., 2015. A pilot study on the assessment of trace organic contaminants including pharmaceuticals and personal care products from on-site wastewater treatment systems along Skaneateles Lake in New York State, USA. Water Res. 72, 28–39.
Sutherland, D.L., Ralph, P.J., 2019. Microalgal bioremediation of emerging contaminants - opportunities and challenges. Water Res. 164, 114921.
Tan, C., Fan, W.H., Wang, W.X., 2012. Role of titanium dioxide nanoparticles in the elevated uptake and retention of cadmium and zinc in Daphnia magna. Environ. Sci. Technol. 46, 469–476.
Team RC, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Thompson, K.A., Mortazavian, S., Gonzalez, D.J., Bott, C., Hooper, J., Schaefer, C.E., et al., 2022. Poly- and perfluoroalkyl substances in municipal wastewater treatment plants in the United States: seasonal patterns and meta-analysis of long-term trends and average concentrations. Enviorn. Sci. Technol. Water 2, 690–700.
Tran, N.H., Reinhard, M., Gin, K.Y., 2018. Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. Water Res. 133, 182–207.
Trianni, A., Negri, M., Cagno, E., 2021. What factors affect the selection of industrial wastewater treatment configuration? J. Environ. Manag. 285, 112099.
van Bergen, T.J.H.M., Rios-Miguel, A.B., Nolte, T.M., Ragas, A.M.J., van Zelm, R., Graumans, M., et al., 2021. Do initial concentration and activated sludge seasonality affect pharmaceutical biotransformation rate constants? Environ. Biotechnol. 105, 6515–6527.
Wang, J., Wang, S., 2016. Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: a review. J. Environ. Manag. 182, 620–640.
Wang, T., Zhao, L., Liu, M., Xie, F., Ma, X., Zhao, P., et al., 2014. Oral intake of hydrogen-rich water ameliorated chlorpyrifos-induced neurotoxicity in rats. Toxicol. Appl. Pharmacol. 280, 169–176.
Wang, Y., Liu, J., Kang, D., Wu, C., Wu, Y., 2017. Removal of pharmaceuticals and personal care products from wastewater using algae-based technologies: a review. Rev. Environ. Sci. Biotechnol. 16, 717–735.
Weigel, S., Bester, K., Huhnerfuss, H., 2001. New method for rapid solidphase extraction of large-volume water samples and its application to non-target screening of North Sea water for organic contaminants by gas chromatography-mass spectrometry. J. Chromatogr. A 912, 151–161.
Winkel, L.H., Pham, T.K., Vi, M.L., Stengel, C., Amini, M., Nguyen, T.H., et al., 2011. Arsenic pollution of groundwater in Vietnam exacerbated by deep aquifer exploitation for more than a century. Proc. Natl. Acad. Sci. U. S. A. 108, 1246–1251.
Wollmann, F., Dietze, S., Ackermann, J.U., Bley, T., Walther, T., Steingroewer, J., et al., 2019. Microalgae wastewater treatment: biological and technological approaches. Eng. Life Sci. 19, 860–871.
Yang, H.B., Zhao, Y.Z., Tang, Y., Gong, H.Q., Guo, F., Sun, W.H., et al., 2019. Antioxidant defence system is responsible for the toxicological interactions of mixtures: a case study on PFOS and PFOA in Daphnia magna. Sci. Total Environ. 667, 435–443.
Ziylan, A., Ince, N.H., 2011. The occurrence and fate of anti-inflammatory and analgesic pharmaceuticals in sewage and fresh water: treatability by conventional and non-conventional processes. J. Hazard. Mater. 187, 24–36.
Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14.