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Research article

Impact of parabens on drinking water bacteria and their biofilms: The role of exposure time and substrate materials

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ABSTRACT

Parabens have been detected in drinking water (DW) worldwide, however, their impact on DW microbial communities remains to be explored. Microorganisms can easily adapt to environmental changes. Therefore, their exposure to contaminants of emerging concern, particularly parabens, in DW distribution systems (DWDS) may affect the microbiological quality and safety of the DW reaching the consumers tap. This work provides a pioneer evaluation of the effects of methylparaben (MP), propylparaben (PP), butylparaben (BP), and their combination (MIX), in bacterial biofilms formed on different surfaces, representative of DWDS materials - high-density polyethylene (HDPE), polypropylene (PPL) and polyvinyl chloride (PVC). *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia*, isolated from DW, were used to form single and dual-species biofilms on the surface materials selected. The exposure to MP for 7 days caused the most significant effects on biofilms, by increasing their cellular culturability, density, and thickness up to 233%, 150%, and 224%, respectively, in comparison to non-exposed biofilms. Overall, more pronounced alterations were detected for single biofilms than for dual-species biofilms when HDPE and PPL, demonstrating that the surface material used affected the action of parabens on biofilms. Swimming motility and the production of virulence factors (protease and gelatinase) by *S. maltophilia* were increased up to 141%, 41%, and 73%, respectively, when exposed to MP for 7 days. The overall results highlight the potential of parabens to interfere with DW bacteria in planktonic state and biofilms, and compromise the DW microbiological quality and safety.

1. Introduction

Drinking water (DW) safety imposes high concerns for public health. Despite all the strategies used to guarantee chemically and microbiologically safe DW, biofilm development in drinking water distribution systems (DWDS) is unavoidable, hindering the efficacy of disinfection (Chan et al., 2019). This may lead to potential public health issues as bacteria in biofilms (microbial communities living adhered to a surface and embedded in a matrix of extracellular polymeric substances - EPS - produced by themselves) are protected against environmental threats, and their presence can affect DW quality (Chan et al., 2019). Aside from microbiological problems, DW is also affected by chemical pollution. The presence of emerging contaminants (ECs) in DW is a recognized concern, whose consequences for DW microbial quality and safety have been disregarded so far. Parabens, which are used as preservatives and antimicrobial agents in personal care products (PCPs), pharmaceuticals, and food have been detected in DW worldwide (Wei et al., 2021). Their popular use arises from their low toxicity, broad spectrum against pathogens, biodegradability, and low cost (Wei et al., 2021).

Parabens reach the aquatic environment through domestic wastewater, effluents from wastewater treatment plants (WWTPs), or by deposition of particles from the atmosphere (Wei et al., 2021). The concentrations of parabens detected in WWTPs influents are the mirror of society. Thereby, regions with greater population density, industrial activity, and low restrictive regulation for their use have the highest levels of contamination by parabens in WWTPs and surface waters (Wei et al., 2021). Among the different methods used to mitigate environmental pollutants (Rasheed et al., 2021), the removal of parabens in WWTPs and drinking water treatment plants (DWTPs) is not completely effective (Wei et al., 2021). Therefore, parabens are still found in surface

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waters (the most susceptible to parabens contaminations) and DW, at environmental concentrations ranging from ng/L–μg/L (Wei et al., 2021). The maximum concentration of parabens detected in WWTPs influents in the last two years (2020/2022) was in Tunisia (560000 ng/L) for methylparaben (MP) (Haddaoui and Mateo-Sagasta, 2021) and the lowest concentration was detected in Spain (783 ng/L), for the same compound. This huge difference may reflect the more restrictive regulation for parabens use in Europe than in other regions (Sadutto et al., 2021). This also highlights the wide and greater use of MP in comparison to other parabens (Wei et al., 2021). Although the knowledge about the presence of parabens in DW is limited, most of the studies reported maximum concentrations of MP, ethylparaben (EP), propylparaben (PP) and butylparaben (BP) in the range of 50–250 ng/L (Bolujoko et al., 2022; Gomes et al., 2020a; Le et al., 2022; Wei et al., 2021).

Parabens have also been found in life underwater (up to 3600 ng/g in fish samples) due to their interaction with paraben-polluted water (Błedzka et al., 2014). The persistence of parabens at high concentrations in aquatic environments for extended periods can disrupt the endocrine system of aquatic organisms, affecting their reproductive system and causing animal feminization (Lincho et al., 2021). In addition to the consumption of DW contaminated with parabens, dermal application of PCPs, or ingestion of food containing these contaminants represent remarkable concern for human health (Wei et al., 2021). Parabens are considered endocrine-disrupting compounds with carcinogenic potential. They are also associated with skin irritation and eczematous eruption (Błedzka et al., 2014), obesity (Matwiejczuk et al., 2020), and gestational type 2 diabetes mellitus (Li et al., 2019). However, the long-term effects of parabens at trace concentrations on DW consumers remain to be understood (Wei et al., 2021).

Although the direct impact of parabens, and ECs in general, on human health have been evaluated and described in the literature, their impact on environmental microbial communities, specifically in DW, remains elusive (Gomes et al., 2020a; Wei et al., 2021). A few recent studies reported significant effects of several ECs in DW bacteria: alteration of biofilm formation (Arruda et al., 2022a; Pinto et al., 2023), production of virulence factors, changing their interaction with human cells (Gomes et al., 2018b, 2019a, 2019b), and spread of antimicrobial resistance (Wang et al., 2019). Nevertheless, parabens are part of a group of ECs whose impact on DW microbial communities remains to be understood. Therefore, this work aims to contribute to filling this gap in the scientific knowledge, by assessing for the first time the consequences of the presence of parabens (BP, MP, and PP) on bacteria isolated from DW. For that, single and dual-species biofilms of *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia* were formed on polymeric materials commonly used in plumbing systems (high-density polyethylene (HDPE), polypropylene (PPL) and polyvinyl chloride (PVC)), and were exposed to the parabens selected (MP, PP, BP and a triple combination of all - MIX) for 7 and 26 days. The effects of parabens exposure were evaluated in the biofilm culturability and cell density, bacterial membrane damage, content of EPS, biofilm structure, bacterial motility and production of virulence factors (protease and siderophores production, lipase and gelatinase activity). The results contribute to the prioritization of parabens in DW, anticipating potential microbiological-related public health concerns from the presence of parabens in DW.

2. Materials and methods

2.1. Parabens

MP, PP and BP from Sigma-Aldrich, Germany were selected as ECs representatives from preservatives (parabens class) since they are commonly found in DW (Gomes et al., 2020a). These parabens were tested individually and in combination (MIX formulation), which is a triple formulation composed of MP, BP, and PP (each one at 150 ng/L). The impact of parabens on biofilms was assessed at 150 ng/L which is representative of the range of parabens concentration detected in DW (Gomes et al., 2020a). Parabens were not soluble in water, except MP. Stock solutions of MP were prepared using ultrapure sterile water to avoid chemical interferences (Flasiński et al., 2018). Other parabens stock solutions were prepared using acetone (0.005% (v/v)). Several solvents were screened attending to their impact on biofilm characteristics, and the results showed that acetone at 0.005% (v/v) was the solvent causing the lowest interference in the bacterial biofilms (Supplementary information A). For that reason, acetone at 0.005% (v/v) was the solvent selected to prepare BP and PP solutions.

2.2. Bacteria and culture conditions

Stenotrophomonas maltophilia and *Acinetobacter calcoaceticus,* both Gram-negative bacteria isolated from DW, were used as model DW bacteria (Simões et al., 2007). *A. calcoaceticus* is an opportunistic and emerging pathogen associated with healthcare infections, which has a similar antibiotic resistance profile to *Acinetobacter baumannii* (Glover et al., 2022). *A. calcoaceticus* is often reported for its coaggregation ability, including its bridging function in DW biofilms (Simões et al., 2008). *S. maltophilia* is also an emerging opportunistic pathogen, whose presence in DW has been often reported (Amoli et al., 2017). These strains were cryopreserved at −80 °C, in aliquots of R2A medium growth with 30% (v/v) of glycerol. Both bacteria were grown overnight at 25 °C and under agitation (160 rpm) in R2A broth medium prepared as described by Gomes et al. (2018b).

The number of cells in suspension was adjusted to 1×10^8 colony-forming units per mL (CFU/mL) for both bacteria. For that, bacteria were harvested from overnight bacteria suspension by centrifugation (Eppendorf centrifuge 5810 R) at 3772×*g* for 10 min and resuspended in fresh R2A broth medium. For the assessment of the virulence factors (Section 2.8), bacteria were washed with sterile tap water (STW), prepared as described by Gomes et al. (2019a), and resuspended in the same medium.

2.3. Substratum for biofilm formation

Single and dual-species biofilms were developed in high-density polyethylene (HDPE), polypropylene (PPL), and polyvinyl chloride (PVC) coupons (1 cm × 1 cm) as surface materials representative of DWDS (Gomes et al., 2022; Simões et al., 2007). These polymeric materials have been used as cost-effective replacements for traditional metal plumbing (Momba and Kaleni, 2002; Niquette, 2000). Coupons were acquired from Neves & Neves (Trofa, Portugal). Coupons were cleaned and sterilized before being used for biofilm formation as described by Gomes et al. (2018b).

2.4. Biofilm formation and exposure to parabens

Single-species biofilms were formed in the presence and absence of parabens (MP, BP, PP and MIX) at 150 ng/L for 7 (Arruda et al., 2022a; Pinto et al., 2023) and 26 days (Gomes et al., 2018b) in 48-well microtiter plates, where coupons of PPL, PVC and HDPE were inserted vertically. A volume of 1 mL of bacterial suspension adjusted to 1×10^8 CFU/mL was added in each well of the microtiter plate for biofilm formation. Microtiter plates were incubated for 24 h at 25 °C and under agitation (160 rpm). After 24 h of incubation, colonized coupons were washed in 1 mL/well of STW to remove the weak and non-adherent bacteria (EPA, 2011). Then, biofilms were exposed to each paraben in R2A. After 12 h of incubation, coupons were washed again with STW and exposed to each paraben (prepared in STW, to mimic the low availability of nutrients in DW) until complete 7 days of exposure. For a longer exposure period (26 days), each paraben solution was changed every 2 days to ensure continuous exposure to a constant dose of ECs for 26 days. Biofilms only exposed to STW or acetone at 0.005% (v/v) were used as negative control and solvent control, respectively.

After exposure, the colonized coupons with parabens exposed and non-exposed biofilms were inserted in a 15 mL centrifuge tube containing 5 mL of sterile saline water and vigorously vortexed for 2 min (to ensure the complete removal of adhered bacteria and the dissociation of possible bacterial aggregates without compromising bacterial viability). This biofilm suspension was used for further CFU and cellular density quantification (Section 2.5).

Among the parabens selected, MP was studied in further detail since it was the one that affected more significantly both single-species bacterial biofilms. Therefore, to provide more realistic information dual-species biofilms were also used to assess the impact of MP. Single and dual-species biofilms were formed for 7 days in 24-well microtiter plates (where coupons were displayed horizontally inside each well) and the effect of MP exposure was assessed in bacteria culturability, cellular density, membrane damage (Section 2.5), EPS matrix content (Section 2.6) and, thickness and roughness coefficient (Section 2.7). Moreover, bacteria from single-species biofilms were further characterized in terms of virulence factors (Section 2.8), specifically the motility, siderophores and protease production, and gelatinase and lipase activity. Dual-species biofilms were formed by adding 0.5 mL of each bacterial suspension adjusted to 1×10^8 CFU/mL in each well. The exposure to MP and the preparation of the biofilm suspension followed the procedure previously described for single-species biofilms formed in 48-well microtiter plates.

2.5. Assessment of biofilm culturability, density, and membrane integrity

The culturability of single and dual-species biofilm cells was quantified by plating the biofilm suspensions through appropriate serial dilutions in R2A agar (Merck, Germany). For that, the drop plate method was used in duplicate for three independent assays. The plates were incubated at 25 °C for 24 h, for cell counts. The detection limit (DL) for CFU enumeration was 2 log CFU/cm^2. In dual-species biofilms, bacteria were differentiated from each other by morphological analysis of the colonies.

Firstly, to evaluate the impact of parabens exposure in single-species biofilms formed on coupons in 48-microtiter plates, the number of total cells was determined through 4′,6-diamidino-2-phenylindole (DAPI) staining as described by Gomes et al. (2020b). Biofilm suspensions, after vigorous vortexing for 2 min, were filtered (0.5 mL) using a 0.22 μm black polycarbonate membrane (Nucleopore, UK). The membrane with bacteria was observed by epifluorescence microscopy after staining with 100 μL of DAPI at 0.5 μg/mL. Samples were observed under a LEICA DMLB2 epifluorescence microscope (LEICA Microsystems, Germany) using UV light and the optical 480–500 nm excitation filter and 485 nm emission filter (Chroma 61000-V2 DAPI/FITC/TRITC). The images were acquired with a LEICA DFC300 FX camera and LEICA IM50 Image Manager, Image processing, and archiving software. At least 15 fields were counted for each sample to calculate the total number of cells (cells/cm^2). The total number of cells counted per field of view range from 50 to 200 cells.

In the second phase of this study, the number of total cells and the number of cells with a damaged membrane in MP-exposed and non-exposed biofilms formed in 24-well microtiter plates were assessed through the Live/Dead *Bac*Light bacterial viability kit (Invitrogen Life Technologies, Alfagene, Portugal) assay according to Gomes et al. (2019b). *Bac*Light is composed of two nucleic acid-binding stains: SYTO 9™ and propidium iodide (PI). SYTO 9™ penetrates all bacterial membranes, staining the cells green, while PI only penetrates cells with damaged membranes, binding to single and double-stranded nucleic acids. The combination of these two stains generates red fluorescing cells. According to this method, those cells fluorescing green (without membrane damage) are considered viable, while those fluorescing red (with membrane damage) are considered dead (Ferreira et al., 2011). Biofilm cell suspensions were filtered through a 0.22 μm Nucleopore® (Whatman, UK) black polycarbonate membrane and stained with 250 μL of SYTO 9™ and 50 μL of PI. The samples were incubated for 7 min in the dark at room temperature. After that, the membrane was mounted on *Bac*Light™ mounting oil as described in the manufacturer's instructions. The samples were observed and images were acquired as done for DAPI inspections. The Log cells/cm^2 with damaged and non-damaged membranes were further determined through three independent experiments, with duplicates.

2.6. Assessment of extracellular polymeric substances content

Single and dual-species biofilms were formed in 24-well microtiter plates, as described in Section 2.4. The EPS extraction was performed as described by Frølund et al. (1996). For that, after 7 days of incubation, 8 coupons per condition were placed in 50 mL falcon tubes containing 5 mL of extraction buffer prepared as described by Gomes et al. (2020b). EPS were quantified in terms of proteins and polysaccharides content, after extraction with Dowex® Marathon© resin (NA^+ form, strongly acidic, 20–50 mesh, Sigma-Aldrich, Germany). For that, 0.1 g Dowex® Marathon© resin was added per each mL of biofilm suspension in a 25 mL beaker. The extraction was further performed for 4 h at 400 rpm and 4 °C. This resin is used to remove cations from the EPS matrix, breaking up the aggregates and causing EPS release (Gomes et al., 2020b). After extraction, EPS (in the supernatant) were separated from the cells through centrifugation (3700×*g*, 5 min). The resulting pellet was resuspended in 5 mL of extraction buffer, and both the pellet solution and the supernatant were stored at −20 °C, for further analysis. To increase the efficiency of extraction, EPS in the supernatant were precipitated with 1:4 ice-cold acetone and stored at −20 °C for at least 30 min (Silva et al., 2012). The solution was then centrifuged at 3700×*g* for 10 min and the supernatant was discarded. The pellet (containing extracellular EPS) was resuspended in ultrapure sterile water using the same volume of supernatant discarded.

The total amounts of proteins and polysaccharides were assessed, as these are the major macromolecules present in organic deposits of microbial origin (Decho and Gutierrez, 2017). Polysaccharides were quantified using the phenol-sulphuric acid method using glucose as standard (Dubois et al., 1951). The quantification of proteins was performed according to Lowry et al. (1951) as modified by Gary Peterson (1979), using the Total Protein Kit, Micro Lowry, Peterson's Modification, with bovine serum albumin (BSA) as standard. The detection limit (DL) of polysaccharides and proteins quantification was 0.6 μg/cm^2 and 6.3 μg/cm^2, respectively. This experiment was performed in three independent assays, with duplicates.

2.7. Evaluation of biofilm structure by optical coherence tomography

MP-exposed and non-exposed 7 days-old single and dual-species biofilms were characterized in terms of biofilm thickness (μm) and roughness coefficient using optical coherence tomography (OCT). Coupons containing biofilms were carefully immersed in STW to remove suspended cells and to keep the biofilm hydrated. Image acquisition was performed using a Thorlabs Ganymede spectral domain system (Thorlabs GmbH, Dachau, Germany) with a central wavelength of 930 nm. The visualization field was 3.66 × 2.98 mm^3 at the X–Z section corresponding to 1024 pixels × 1024 pixels. Since biofilms are mainly composed of water (Bakke et al., 2001), the refractive index was set to 1.4, to be closer to the refractive index of water (1.33) (Narciso et al., 2022). For each biofilm, 2D imaging was performed with 3 fields to ensure the accuracy and reproducibility of the results obtained. Biofilm Imaging and Structure Classification Automatic Processor (BISCAP) was used to automatically process 2D OCT biofilm images as described by Narciso et al. (2022). OCT images were converted to binary black-and-white images, and objects not connected to the bottom were rejected from the biofilm structure. Three horizontal bounds were defined: a lower bound where all biofilm pixels must be below $z^{bot}(x)$; a substratum bound where all substratum pixels must be below this bound

and an upper bound where all biofilm pixels must be above this bound $z^{top}(x)$. Then, the mean biofilm thickness and roughness coefficient were automatically calculated by BISCAP software. Biofilm thickness $L_F(x)$ is the total length between the top and bottom interfaces delimiting the biofilm structure as defined in Equation (1) (Narciso et al., 2022).

$$L_F(x) = \left(z^{bot}(x) - \min(z^{top}(x) - 1)px_{len}, x = 0, 1, \ldots, N_x - 1 \right) \quad (1)$$

Biofilm thickness is calculated as the total number of pixels between the two interfaces and then converted to distance units, via the pixel length constant ($px_{len,}$). The average value of this series is presented by software ($\overline{L_F}$) (Narciso et al., 2022).

Biofilm roughness was analyzed by the determination of the roughness coefficient (R^*_α) as this parameter allows comparing the structure of different biofilms, across different studies as well as across scales, as this value is normalized by the mean biofilm thickness as described in Equation (2) (Narciso et al., 2022).

$$R^*_\alpha = \frac{1}{N_x} \times \sum_{i=0}^{N_x - 1} \frac{(L_F(i) - \overline{L_F})}{\overline{L_F}} \quad (2)$$

Where N_x is the number of thickness measurements, $L_F(i)$ is a local biofilm thickness measurement at location i and $\overline{L_F}$ is the mean biofilm thickness. Higher roughness coefficient values are associated with more robust biofilms. This experiment was performed in three independent assays, with duplicates, and at least eight 2D OCT images were acquired for each condition.

2.8. Bacterial motility and virulence factors production

Bacteria from 7-day-old MP-exposed single-species biofilms were inoculated in R2A broth medium and incubated overnight at 25 °C under agitation (160 rpm). Then, the bacterial cell density was adjusted to 10^8 CFU/mL in STW for further evaluation of motility and virulence factors production.

Three types of motilities (swimming, swarming, and twitching) were characterized using different concentrations of agar. The assays were performed as described by Gomes et al. (2016). A volume of 15 μL of bacterial suspension was dropped in the center of the agar plates prepared with tryptone (Merck, Germany) at 10 g/L, NaCl (VWR, Belgium) at 2.5 g/L and agar (VWR, Belgium) at 3 g/L, 7 g/L, and 15 g/L for swimming motility, swarming motility, and twitching motility, respectively. Then, the plates were incubated at 25 °C for 72 h and the colony growth halo was measured after 24, 48 and 72 h of incubation. Three independent assays in triplicate were tested for each condition.

The production of virulent factors by bacteria from MP-exposed and non-exposed biofilms was evaluated in terms of siderophores, protease and lipase production as well as gelatinase activity. Siderophores production was evaluated using chrome azurol S (CAS) plates prepared in R2A agar (Merck, Germany). CAS-iron dye solution was prepared by adding 60.7 mg of CAS (Fluka, Switzerland) in 50 mL of distilled water and 10 mL of Iron III solution (VWR, Germany). Then, the previous solution was mixed with 40 mL of a solution of hexadecyltrimethylammonium bromide (HDTMA) (Merck, Hohenbrunn, Germany) containing 72.4 mg of HDTMA in distilled water. Briefly, 25 mL of a sterilized solution of CAS-iron dye prepared according to Neilands (1987) was added to 250 mL R2A agar. A volume of 20 μL of bacterial suspension was placed in the center of each plate of CAS agar. Plates were incubated for 48 h at 25 °C and siderophores production was evaluated by the formation of an orange halo that was measured (mm) after the incubation period. The phenotypic assay of gelatinase activity was performed as described by Lopes et al. (2006). A drop (10 μL) of bacterial suspension was deposited on a plate containing gelatin agar consisting of 5 g/L of peptone (Merck, Darmstadt, Germany), 3 g/L of yeast extract (Merck, Darmstadt, Germany), 30 g/L of gelatin (Oxoid, UK), and 15 g/L agar (VWR, Leuven, Belgium) at pH 7. After incubation at 25 °C for 48 h, the plates were flooded with a saturated solution of ammonium sulphate (2.84 M) (VWR, Leuven, Belgium). If gelatin precipitates, a transparent halo around cells will appear in gelatinase producers. The transparent halo and the bacterial growth halo were measured at the end of the incubation period. The production of lipases was assayed in LB agar (Sigma-Aldrich, Steinheim, Germany) supplemented with 2.0 g/L of $CaCL_2$ (Merck, Darmstadt, Germany), 15.0 g/L of agar (VWR, Leuven, Belgium), and 10 g/L of Tween-80 (VWR, Ohio, USA). A volume of 15 μL of bacterial suspension was placed on each plate and a clear halo was measured after 72 h of incubation. A positive reaction was indicated by a light-yellow clear halo around the colonies, after 72 h (Meheissen et al., 2022). For the assessment of protease activity, bacterial suspensions (10 μL) were placed on Petri dishes (in three different positions) containing plate count agar (PCA) (VWR, Leuven, Belgium) supplemented with 10 g/L skim milk powder (Merck, Darmstadt, Germany) and incubated at 37 °C for 72 h. The clearance zones formed (indicating protease production by bacteria) were measured at the end of the incubation time (Abdelmoteleb et al., 2017).

2.9. Statistical analysis

Data were analyzed using the statistical program GraphPad Prism 5.0 (GraphPad Software, La Jolla California, USA). The mean and standard deviations (SDs) within samples were calculated for all cases based on a minimum of three independent assays, with duplicates. Data were evaluated in terms of normality (Gaussian distribution) using D'Agostino and Pearson omnibus normality test. Then, for parametric data, the differences between data were obtained by the application of ANOVA with Tukey's post-test (for more than two groups), and the unpaired *t*-test to analyze the differences between the two groups. Statistical calculations were based on a confidence level of ≥95% ($P < 0.05$ was considered a statistically significant difference).

3. Results

3.1. Effect of parabens on bacterial culturability and cellular density from 7 to 26-days-old single-species biofilms

The bacterial culturability of single-species biofilms of different ages (7 and 26-days) formed in the presence and absence of parabens was evaluated (Fig. 1). *A. calcoaceticus* and *S. maltophilia* bacteria were able to form biofilms on the polymeric surfaces selected with comparable CFU/cm^2 ($P > 0.05$).

A. calcoaceticus biofilms developed on HDPE were not significantly affected by exposure to the parabens in the 7 days ($P > 0.05$). However, when increasing the exposure time to 26 days, an increase in the number of culturable *A. calcoaceticus* cells was observed for BP (230%) and MIX (170%) ($P < 0.05$), in comparison to non-exposed *A. calcoaceticus* biofilms. An increase of 85% and a decrease of 67% in the number of *S. maltophilia* culturable cells was observed for MP and MIX-exposed *S. maltophilia* 7-days-old biofilms formed on HDPE, respectively ($P < 0.05$). On the other hand, the culturability of 26-day-old *S. maltophilia* biofilms formed on HDPE was not affected by exposure to parabens ($P > 0.05$).

Regarding single-species biofilms formed on PPL, no significant differences were found in the culturability of parabens-exposed and non-exposed *A. calcoaceticus* biofilms, for both exposure times tested ($P > 0.05$). However, for 7-day-old *S. maltophilia* biofilms exposed to MP in PPL, an increase of 121% in the number of CFU/cm^2 was noticed in comparison to the non-exposed counterparts ($P < 0.05$). A similar trend of culturability increase was observed for the 26-day-old biofilms exposed to MP, however, this increase was not statistically significant when compared to the control ($P > 0.05$). All the other conditions tested did not alter significantly the culturability of 7-day-old and 26-day-old parabens-exposed *S. maltophilia* biofilms ($P > 0.05$). The number of culturable cells from biofilms formed on PVC was less affected by the

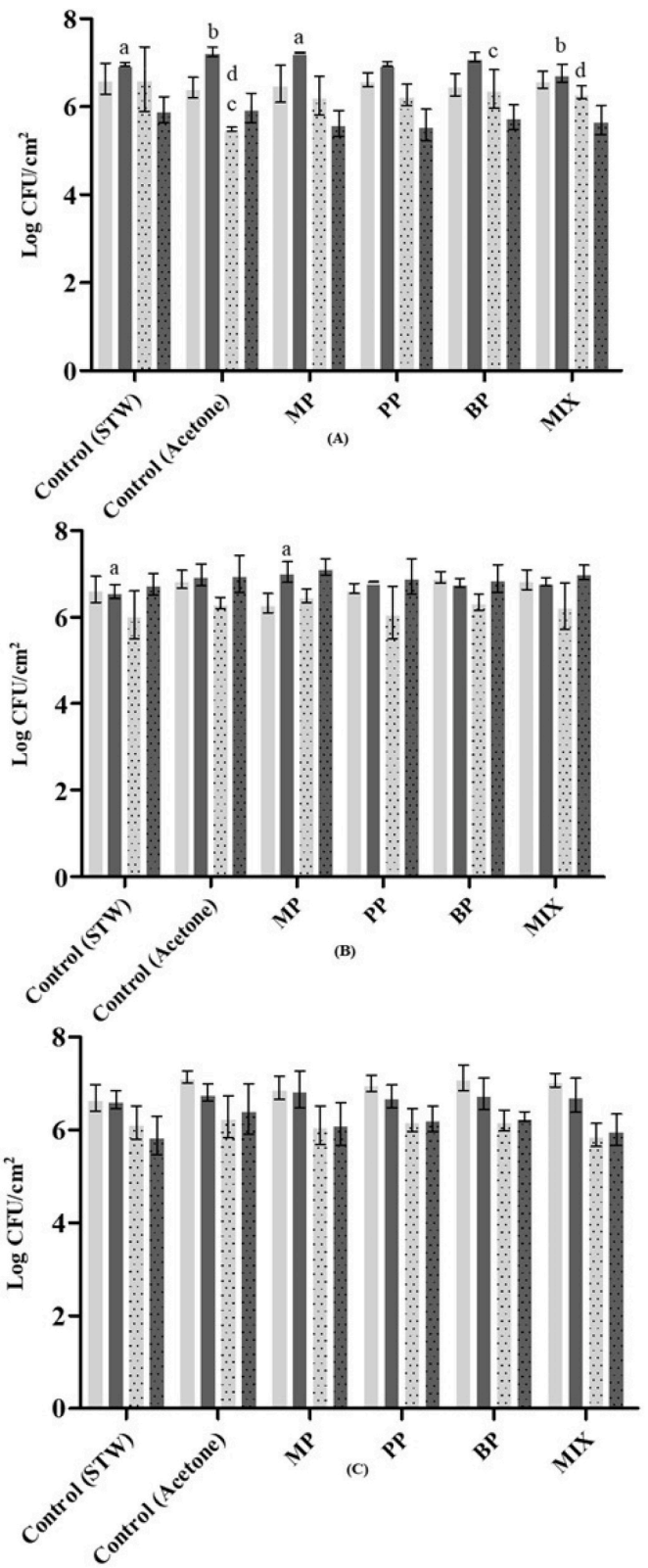

Fig. 1. Culturability of biofilm cells (Log CFU/cm^2) of 7-days-old (*A. calcoaceticus* *S. maltophilia*) and 26-days-old (*A. calcoaceticus* *S. maltophilia*) single-species biofilms formed on (A) HDPE, (B) PPL and (C) PVC exposed and non-exposed to parabens (150 ng/L). The results presented are the mean of three independent assays and the respective standard deviation. $^{a, b, c, d}$ - samples were statistically different from non-exposed biofilms (t-test and ANOVA $P < 0.05$).

exposure to the parabens, independently of the exposure time and bacterial species. Despite that, 7-day-old MP-exposed biofilms showed an increase in the numbers of CFU/cm^{2,} in comparison to the non-exposed biofilms.

The cellular density of parabens-exposed biofilms was assessed by DAPI staining (Fig. 2). *A. calcoaceticus* biofilms formed on HDPE coupons and exposed to BP for 7 days revealed higher cellular density (total number of cells per cm^2) than the non-exposed counterparts ($P < 0.05$). The cellular density of *A. calcoaceticus* biofilms was 73% higher for BP-exposed biofilms (for 7 days) than for the non-exposed biofilms. MIX-exposed *S. maltophilia* biofilms formed on the HDPE for 7 days had a lower number of total cells than these non-exposed ($P < 0.05$). Exposure to MIX caused a reduction of about 73% in 7-day-old *S. maltophilia* biofilms. All the other conditions tested did not cause a significant impact in both 7-day-old bacterial biofilms formed on HDPE coupons ($P > 0.05$). However, for a longer exposure period (26 days), a significant increase in the number of total cells was observed for MP, PP, and BP-exposed *S. maltophilia* biofilms formed on HDPE ($P < 0.05$). Moreover, MP exposure for 26 days increased by 85% the total cellular density of *A. calcoaceticus* biofilms ($P < 0.05$).

The total number of *A. calcoaceticus* and *S. maltophilia* cells from single-species biofilms formed on PPL and PVC coupons for 7 and 26 days was not affected by the exposure to parabens ($P > 0.05$). The exception was *S. maltophilia* biofilms formed on PPL coupons, where an increase in the number of cells (about 115%) was observed when exposed to MP for 26 days ($P < 0.05$).

3.2. Effect of MP on the culturability, cellular density, and membrane damage of bacteria from 7-days-old single and dual-species biofilms

The impact of MP exposure on 7 days on single- and dual-species biofilms is presented in Fig. 3. MP exposure for 7 days did not affect the culturability of bacteria from single-species biofilms formed on HDPE and PVC coupons ($P > 0.05$). The number of culturable *A. calcoaceticus* and *S. maltophilia* cells from MP-exposed 7-day-old single-species biofilms formed on PPL increased by 233% and 75%, respectively ($P < 0.05$). MP exposure favoured the proliferation of culturable cells in *A. calcoaceticus* biofilms to a higher extent than in *S. maltophilia* biofilms ($P < 0.05$). These results follow the same trend observed for the culturability of 7-days-old single-species biofilms formed in 48-microtiter plates in the presence and absence of MP (Section 3.1), demonstrating that the platform used, and the position of coupons do not seem to affect the biofilm characteristics.

The dual-species biofilms were colonized at a higher extent by *S. maltophilia* ($P < 0.05$) (Fig. 3). In general, *S. maltophilia* represents more than 70% of the total cells in dual-species biofilms. The culturability of dual-species biofilms formed on all the surface materials was not affected by MP exposure for 7 days ($P > 0.05$).

Fig. 4 presents the number of membrane damaged and total cells in MP-exposed and non-exposed single-species biofilms. In general, for all single-species biofilms developed in all surface materials, the number of bacteria with non-damaged membranes was higher than the bacteria with membrane damage, for both non-exposed and MP-exposed biofilms ($P < 0.05$). The exposure of both bacterial biofilms formed on PVC to MP affected membrane integrity, increasing the number of *A. calcoaceticus* and *S. maltophilia* cells with membrane damage by 232% (0.52 log CFU/cm^2) and 179% (0.45 log CFU/cm^2), respectively ($P < 0.00.5$). In biofilms formed on HDPE, only *S. maltophilia* revealed an increase in bacterial damage of circa 228% (0.52 log CFU/cm^2) when exposed to MP ($P < 0.05$). On the other hand, MP-exposed *A. calcoaceticus* biofilms formed on PPL had an increase of 91% in the number of cells with membrane damage ($P < 0.05$).

Regarding the total number of cells, no significant differences were found between MP-exposed and non-exposed single-species biofilms formed on PVC ($P > 0.05$). Using HDPE coupons, both single-species biofilms had increased cellular density when exposed to MP ($P <$

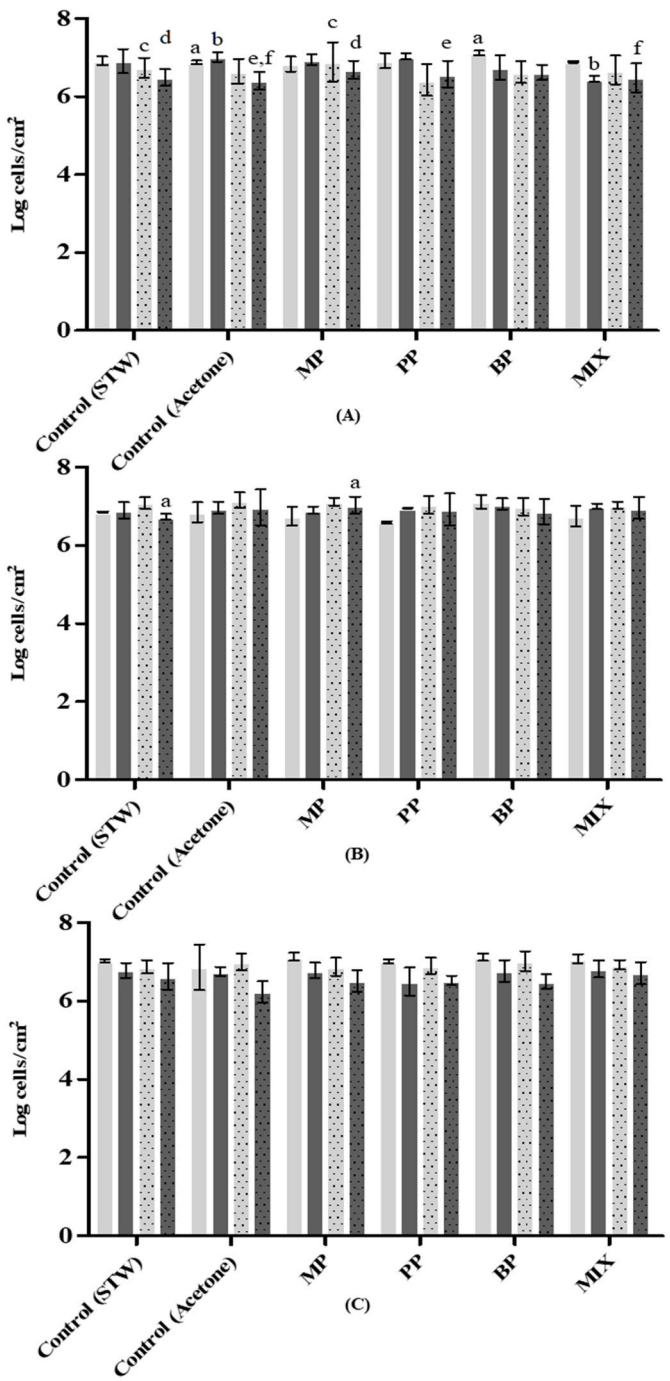

Fig. 2. Cellular density of biofilm cells (Log cells/cm^2) of 7-days-old (*A. calcoaceticus* *S. maltophilia*) and 26-days-old (*A. calcoaceticus* *S. maltophilia*) single-species biofilms formed on (A) HDPE, (B) PPL and (C) PVC exposed and non-exposed to parabens (150 ng/L). The results presented are the mean of three independent assays and the respective standard deviation. $^{a, b, c, d, e}$ - samples were statistically different from non-exposed biofilms (t-test and ANOVA $P < 0.05$).

0.05). More specifically, MP-exposed *A. calcoaceticus* and *S. maltophilia* biofilms on HDPE showed an increase in cellular density of 150% and 62%, respectively. In addition, *A. calcoaceticus* biofilms formed on PPL coupons had a total number of cells 54% higher than that of non-exposed biofilms formed on the same material ($P < 0.05$).

Fig. 5 presents the results of the viability and density of MP-exposed and non-exposed dual-species biofilms. The number of *S. maltophilia* cells/cm^2 in all the surface materials was higher (representing 80% of the total biofilm cells) than the number of *A. calcoaceticus* cells/cm^2 for both non-exposed and MP-exposed biofilms ($P < 0.05$) (Fig. 5). In addition, for dual-species biofilms developed on HDPE, PPL and PVC coupons, the percentage of bacterial biofilm cells with the intact bacterial membrane represents 90% of the total cells. This is consistent with the results obtained for single-species biofilms.

The exposure to MP did not cause significant bacterial membrane damage to dual-species biofilm cells developed on HDPE, and did not provoke the potentiation of biofilm growth, for all the surface materials ($P > 0.05$). However, for dual-species biofilms formed on PPL, the exposure to MP promoted an increase in the number of bacteria with membrane damage (200%) in comparison to the non-exposed biofilms ($P < 0.05$). More specifically, this alteration is translated in an increase of 444% and 30% of *S. maltophilia* and *A. calcoaceticus* biofilm cells with membrane damage, respectively. Regarding the dual-species biofilms developed on PVC, the exposure to MP revealed an increase of 166% in the number of cells with membrane damage ($P < 0.05$). *S. maltophilia* from dual-species biofilms formed on PVC were the most affected by MP ($P < 0.05$). The number of *S. maltophilia* cells with damaged membrane increased by 404% in comparison to *S. maltophilia* from non-exposed dual-species biofilms.

3.3. Effect of MP on the production of EPS by single and dual-species biofilms

The EPS content was assessed by the quantification of extracellular polysaccharides and proteins for both single and dual-species biofilms. No significant differences were found in the EPS content of MP-exposed single and dual-species biofilms in comparison to the non-exposed counterparts ($P > 0.05$) (Supplementary material - Table B1). Data shows that the content of extracellular polysaccharides was higher than that of proteins ($P < 0.05$) for MP-exposed *A. calcoaceticus* biofilms. However, for *S. maltophilia* and dual-species biofilms, the extracellular content of polysaccharides and proteins was comparable, which is translated in polysaccharides/proteins ratios close to 1. Moreover, the production of extracellular EPS in dual-species biofilms was higher than in *S. maltophilia* biofilms ($P < 0.05$).

It is important to highlight an increasing trend (80%) in polysaccharides content for MP-exposed *A. calcoaceticus* biofilms formed on PVC and for MP-exposed *S. maltophilia* biofilms formed on HDPE, even if not statistically different ($P > 0.05$). Moreover, a decreasing trend in polysaccharides content was observed for *S. maltophilia* biofilms exposed to MP and formed on PPL (36%) and PVC (56%) ($P > 0.05$). For dual-species biofilms formed on all surface materials, a downward trend in the production of EPS was observed when biofilms were exposed to MP, even if not statistically significance ($P > 0.05$).

3.4. Effect of MP on the biofilm structure

Table 1 presents the values of the thickness and roughness coefficient for MP-exposed and non-exposed single and dual-species biofilms. It is possible to note that *A. calcoaceticus* biofilm thickness and roughness coefficient were not affected by MP exposure ($P > 0.05$). Moreover, *S. maltophilia* biofilms formed on HDPE and PVC were not possible to be analyzed through OCT because these were very thin, which made unfeasible the observation and analysis of their structure using this equipment. Nevertheless, it was possible to analyze *S. maltophilia* biofilms formed on PPL coupons (Supplementary Information – Figure C3). MP-exposed *S. maltophilia* biofilms formed on PPL were thicker (an increase of thickness in 44%) than the non-exposed counterparts ($P < 0.05$). The roughness coefficient was similar for both MP-exposed and non-exposed biofilms ($P > 0.05$). In general, *S. maltophilia* biofilms were thinner than these of *A. calcoaceticus* ($P < 0.05$).

Dual-species biofilms seem to be thinner than any of these single-species ($P < 0.05$) and MP-exposed dual-species biofilms were thicker

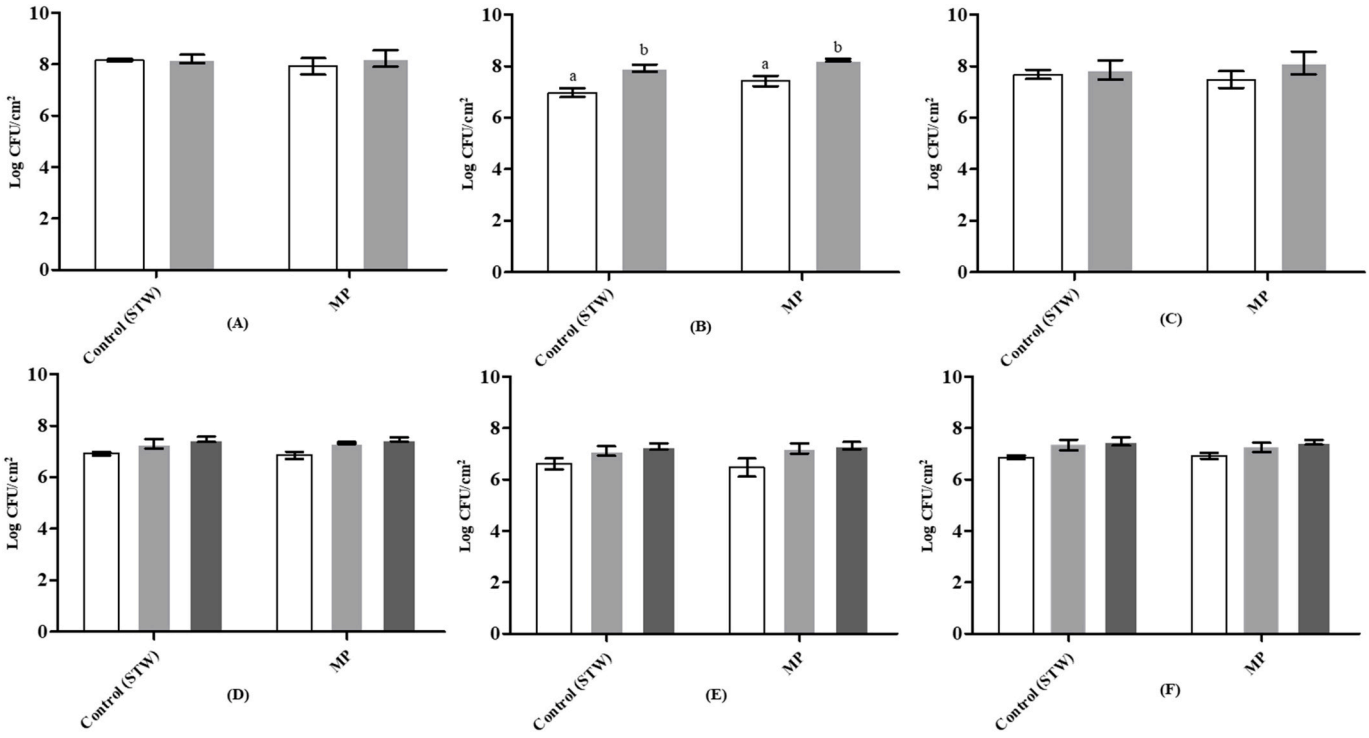

Fig. 3. Culturability of biofilm cells (Log CFU/cm$_2$) of 7-days-old single-species biofilms (A, B and C) and dual-species biofilms (D, E and F) (□*A. calcoaceticus* ■*S. maltophilia* ■dual-species biofilms *A. calcoaceticus* + *S. maltophilia*) formed on HDPE (A and D), PPL (B and E) and PVC (C and F) exposed and non-exposed to MP (150 ng/L). The results presented are the mean of three independent assays and the respective standard deviation. [a, b] - samples were statistically different from non-exposed biofilms (t-test, $P < 0.05$).

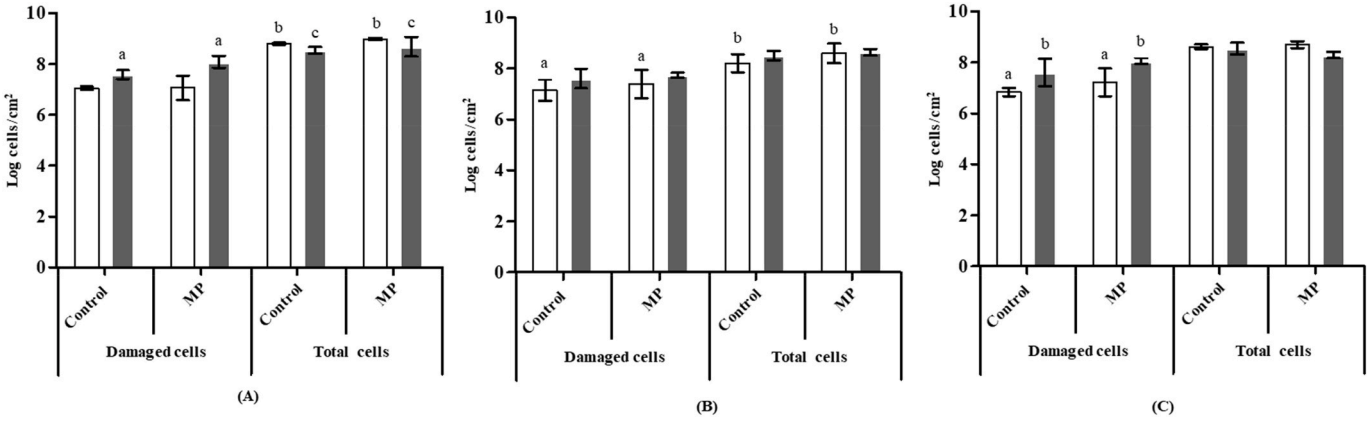

Fig. 4. Cellular density (damaged cells; total cells) of 7-days-old single-species biofilms (□*A. calcoaceticus* ■*S. maltophilia*) formed on (A) HDPE, (B) PPL and (C) PVC exposed and non-exposed to MP (150 ng/L). The results presented are the mean of three independent assays and the respective standard deviation. [a, b, c] - samples were statistically different from non-exposed biofilms (t-test, $P < 0.05$).

than the non-exposed counterparts (Table 1). The exposure to MP caused an increase in dual-species biofilm thickness equal to 224% (circa 30 μm) and 81% (circa 13 μm) for dual-species biofilms formed on HDPE and PPL, respectively ($P < 0.05$). OCT images of these biofilms are presented in Supplementary Information C. The roughness coefficient obtained for MP-exposed and non-exposed dual-species biofilms was similar, regardless of the surface material used ($P > 0.05$).

3.5. Effect of MP on the production of virulence factors

A. calcoaceticus and *S. maltophilia* from biofilms developed on the selected surface materials and exposed for 7 days to MP can produce siderophores (Table 2). The diameter of the orange halo formed around the growing colony was similar for MP-exposed and non-exposed cells ($P > 0.05$). No significant impact in virulence factors production and motility was observed for *A. calcoaceticus* bacteria derived from MP-exposed biofilms ($P > 0.05$). However, an increase in the protease activity of *S. maltophilia* was found in MP-exposed biofilms formed on PPL and PVC ($P < 0.05$). Moreover, the gelatinase activity was more pronounced in *S. maltophilia* from MP-exposed biofilms formed on HDPE than in the non-exposed counterparts, causing an increase of 73% in the gelatinase activity of MP-exposed *S. maltophilia* relative to the non-exposed counterpart ($P < 0.05$). For the other virulence factors, no significant impact of MP exposure was detected in *S. maltophilia* derived from biofilms ($P > 0.05$). However, the results showed that MP exposure has an impact on bacterial swimming motility, promoting an increase in

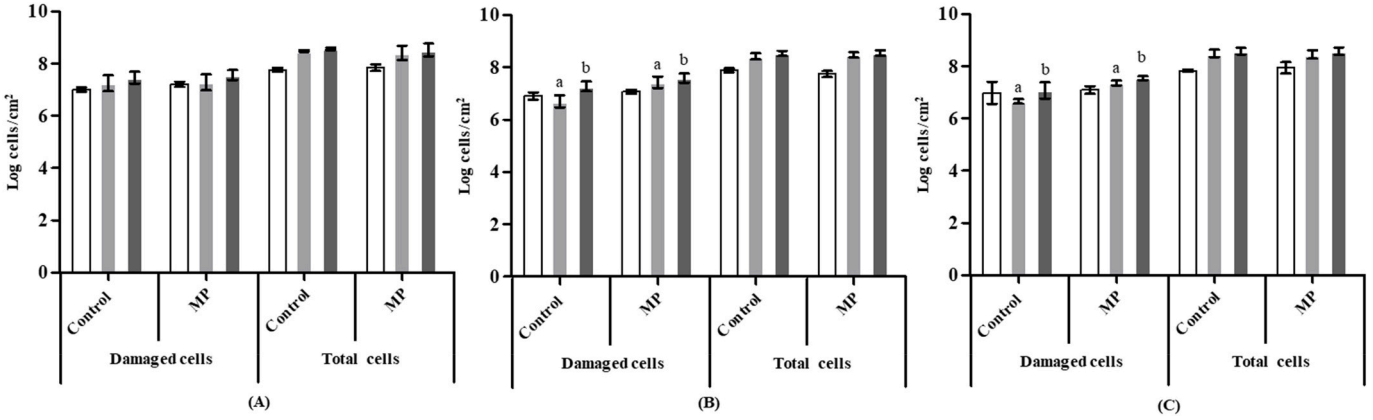

Fig. 5. Cellular density (damaged cells; total cells) of 7-days-old dual-species biofilms (□*A. calcoaceticus* ■*S. maltophilia* ▒dual-species biofilms *A. calcoaceticus* + *S. maltophilia*) formed on (A) HDPE, (B) PPL and (C) PVC exposed and non-exposed to MP (150 ng/L). The results presented are the mean of three independent assays and the respective standard deviation. [a, b] - samples were statistically different from non-exposed biofilms (t-test, $P < 0.05$).

Table 1
Biofilm thickness (μm) and roughness coefficient obtained for 7-days-old single and dual-species biofilms formed on HDPE, PPL and PVC, exposed and non-exposed to MP (150 ng/L). The results are the mean of three independent assays and the respective standard deviation. nd – not possible to analyze. *- samples were statistically different from non-exposed biofilms (*t*-test, $P < 0.05$).

		A. calcoaceticus			*S. maltophilia*			Dual-species		
		HDPE	PPL	PVC	HDPE	PPL	PVC	HDPE	PPL	PVC
Thickness (μm)	**Control**	40 ± 17	49 ± 16	34 ± 15	nd	46 ± 9.0*	nd	12 ± 3.1*	17 ± 2.3*	25 ± 6.1
	MP	48 ± 12	54 ± 37	23 ± 7.1	nd	66 ± 27*	nd	39 ± 2.9*	30 ± 6.6*	30 ± 12
Roughness coefficient	**Control**	0.4 ± 0.04	0.4 ± 0.1	0.3 ± 0.1	nd	0.5 ± 0.2	nd	0.7 ± 0.05	0.7 ± 0.2	0.5 ± 0.1
	MP	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.3	nd	0.4 ± 0.2	nd	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1

Table 2
Virulence factors and motility values obtained for 7-days-old bacteria from biofilms formed on HDPE, PPL and PVC exposed and non-exposed to MP (150 ng/L). The average diameter of the initial drop was 7.1 ± 0.5 mm* - samples were statistically different from non-exposed bacteria.

		Colony growth halo (mm)					
		HDPE		PPL		PVC	
		Control	**MP**	**Control**	**MP**	**Control**	**MP**
A. calcoaceticus	**Siderophores**	16 ± 0.4	16 ± 1.4	15 ± 1.1	16 ± 1.8	15 ± 2.1	17 ± 1.8
	Protease	25 ± 0.4	23 ± 0.7	29 ± 2.5	27 ± 0.7	26 ± 1.8	26 ± 1.8
	Gelatinase	22 ± 1.1	18 ± 0.4	19 ± 1.8	18 ± 2.1	19 ± 0.5	19 ± 1.2
	Lipase	21 ± 1.1	23 ± 0.7	23 ± 2.5	22 ± 0.4	22 ± 0.7	22 ± 1.4
	Swimming	20 ± 2.7	14 ± 0.4	18 ± 4.8	14 ± 0.7	20 ± 2.1	14 ± 1.5
	Swarming	12 ± 1.6	11 ± 0.4	10 ± 0.7	13 ± 1.8	11 ± 0.0	11 ± 0.4
	Twitching	13 ± 1.1	12 ± 0.5	11 ± 0.5	12 ± 0.7	12 ± 0.0	11 ± 0.2
S. maltophilia	**Siderophores**	16 ± 1.6	14 ± 2.1	14 ± 1.6	13 ± 0.6	14 ± 1.1	13 ± 0.7
	Protease	24 ± 1.4	24 ± 2.1	**24 ± 4.2***	**34 ± 2.9***	**28 ± 0.5***	**37 ± 2.6***
	Gelatinase	**16 ± 1.4***	**28 ± 0.4***	16 ± 1.7	20 ± 3.2	20 ± 0.0	18 ± 3.2
	Lipase	22 ± 0.7	22 ± 0.6	22 ± 4.7	25 ± 5.1	22 ± 0.7	22 ± 2.8
	Swimming	**17 ± 3.7***	**38 ± 4.2***	**15 ± 3.7***	**36 ± 9.1***	**16 ± 3.4***	**38 ± 5.7***
	Swarming	9.5 ± 1.4	14 ± 1.1	10 ± 1.9	12 ± 2.0	9.3 ± 0.4	13 ± 1.2
	Twitching	10 ± 1.8	11 ± 1.3	10 ± 1.3	11 ± 0.9	11 ± 0.4	11 ± 0.0

S. maltophilia from MP-exposed biofilms formed on HDPE, PPL and PVC ($P < 0.05$). An increase of the halo spread of 125%, 131% and 141% was observed for *S. maltophilia* derived from MP-exposed biofilms formed on HDPE, PPL and PVC, respectively, in comparison to the non-exposed counterparts ($P < 0.05$). No differences were found in *S. maltophilia* swarming and twitching motilities between MP-exposed biofilms and non-exposed, regardless of the surface material ($P > 0.05$).

In general, swimming motility values obtained for both bacteria were higher than those for swarming and twitching ($P < 0.05$). Swarming and twitching motility values were similar ($P > 0.05$). In addition, *A. calcoaceticus* and *S. maltophilia* swarming and twitching motilities were not affected by parabens exposure ($P > 0.05$).

4. Discussion

The absence of environmental contaminants and microorganisms along DWDS is utopic (Chan et al., 2019). The impact of emerging contaminants, particularly parabens, in the DW microbiome remains to be understood. The present study provides pioneer data on the impact of selected parabens in single and dual-species biofilms formed by bacteria isolated from DW. This work further considers the influence of polymeric surface materials commonly found in DWDS and exposure time to parabens (7 and 26 days). Both selected exposure times have already been used to evaluate the impact of ECs exposure on bacterial biofilms and, for both, some changes in biofilm characteristics were observed

(Arruda et al., 2022a; Gomes et al., 2018b; Pinto et al., 2023). In this study, more impactful alterations in the culturability and the total number of cells were found for 7-day-old biofilms exposed to parabens than for 26-day-old biofilms. This suggests that the impact of parabens on biofilms is dependent on the exposure time.

Parabens (MP, PP, BP and MIX) exposure altered the characteristics of single-species biofilms, being PP the paraben causing the lowest impact. On the other hand, MP caused the most significant impact on single-species biofilms by increasing the number of culturable and total cells in biofilms formed on HDPE and PPL. Thereby, HDPE and PPL seem to be critical surface materials for use in plumbing systems contaminated with parabens, as biofilm proliferation seems to be favoured on these materials. On the other hand, PVC seems to be the most inert and safest material in terms of the potentiation of parabens' impact on bacterial proliferation. It is important to note that the type of surface material can affect biofilm development, originating biofilms with different characteristics and structures, as already demonstrated by several authors (Arruda et al., 2022a, 2022b; Berry et al., 2006). Consequently, it is expected that for different bacterial biofilms formed on different materials, the interactions between biofilms and parabens will also vary.

The increase in cell culturability and density in single biofilms exposed to parabens, particularly MP, proposes that bacteria can adapt and/or metabolize parabens in long-term exposure. This result corroborates Juárez-Jiménez et al. (2019) who reported microbial adaptation to MP and BP by biodegradation, revealing an increase in the number of culturable bacteria (higher after MP exposure) and an increase in bacterial proliferation from parabens exposure. Previous studies have also reported MP degradation under aerobic and anaerobic conditions (activated sludge process) (Li et al., 2015). However, in the present work, the exposure to MIX for 7 days reduced the culturability and density of *S. maltophilia* biofilms formed on HDPE. Lee et al. (2018) reported an increase of 40% in toxicity against *Aliivibro fischeri* when MP and PP were used in combination, in comparison to the effect of each paraben alone. Therefore, the antimicrobial activity of parabens seems to be higher when they are used in combination, as proposed by Al-Halaseh et al. (2022).

Most of the biofilm biomass present in DWDS is located at the pipe walls, however, the detachment of biofilms to the bulk water may occur (Zhu et al., 2020). Thereby, the increase in biofilm proliferation due to exposure to MP may potentiate the detachment of biofilms from plumbing systems, which will compromise the DW quality and safety as the detached biofilm portion can reach the consumers' tap. However, dual-species biofilm cell culturability and density were not affected by MP exposure. This stability of dual-species biofilms to parabens exposure may result from their higher complexity and stability, as a result of the bacterial heterogeneity (Simões et al., 2010; Zhu et al., 2020). Moreover, in this study, dual-species biofilms produced a higher content of EPS than these single-species. This higher production of EPS is a result of the synergistic interspecies bacterial interactions (Gomes et al., 2016, 2018a, 2019c), which protects the biofilm bacteria from environmental stresses (Wang et al., 2019). In this study, it seems that the higher EPS content in dual-species biofilms may constitute a defensive synergistic response to MP exposure, helping to explain their lower susceptibility to MP.

MP was able to disturb the integrity of the bacteria by increasing the number of cells with membrane damage in single-species biofilms formed on PVC. The same occurred in dual-species biofilms formed on PPL and PVC. In this case, the increase in the number of damaged cells was more evident for *S. maltophilia* from dual-species biofilms. Therefore, despite *S. maltophilia* being prevalent in dual-species biofilms, this bacterium is the most affected in the membrane integrity by MP. This may occur as a consequence of the lower ability of *S. maltophilia* biofilm cells to produce EPS than these of *A. calcoaceticus*. Thereby, since EPS hinders molecular diffusion through the biofilm structure, the MP interaction with bacterial cells may be facilitated in biofilms with lower EPS content (Wang et al., 2019). However, it was not possible to predict these results from the alterations found in single-biofilms cells, since *A. calcoaceticus* from biofilms formed on PPL and PVC were the most affected in cell membrane damage. Although the mechanism of antimicrobial action of parabens is not well explored (Flasiński et al., 2018), these results may be relevant to understand how parabens, specifically MP may impact DW bacteria by demonstrating their direct action with the bacterial membrane (Neri et al., 2022). Nevertheless, the results of this study also demonstrate that some bacterial cells with damaged membranes may be culturable or viable, which suggests that membrane perturbations caused by trace concentrations of MP are not sufficient to affect bacterial cell viability or culturability.

Flasiński et al. (2016, 2018) studied the interaction between parabens (0.001–1 mM) and bacterial membranes components (phosphatidylglycerol and cardiolipin) and found that the impact of parabens on biofilms depends on their chemical structure, concentration, and class of lipids. These authors found that parabens (MP, EP, PP and BP) induce some surface film modifications, being this effect higher for more hydrophobic parabens (Flasiński et al., 2018). In fact, BP strongly affected the bacterial monolayer characteristics, leading to its disruption (Flasiński et al., 2016).

There is also a lack of information on how parabens interact with EPS and biofilm components or even with the materials where they are formed. Preliminary evidence suggests the possible adsorption of parabens either to biofilms (by EPS components) or to the surface material (Dong et al., 2019). These interactions may result in chemical changes on the surface of the material and may affect the biofilm formation process. This will lead to the formation of biofilms with different characteristics not only at the cellular and EPS levels but also at the structural level. Therefore, the changes induced by MP exposure on biofilm characteristics may be explained in part by the different mechanisms and extent of MP interaction with biofilm bacteria and with the surface materials where biofilms are formed. The presence of biofilms was found to enhance the adsorption of ECs (namely carbendazim, carbamazepine, and diclofenac) and other toxic contaminants (perfluorooctane sulfonate and lead), which increased bioaccumulation (Ateia et al., 2020; Bhagwat et al., 2021; Zhang et al., 2022). MP-exposed and non-exposed single and dual-species biofilms did not differ in EPS content, which proposes that MP did not interact (adsorption or reaction) with the biofilm EPS to a significant extent. Other authors already found that the adsorption of parabens to activated sludge (mainly composed of bacteria, EPS, and water, similar to biofilms) is not significant (Lu et al., 2018). Although Wang et al. (2019) reported an increase in EPS proteins caused by exposure to trace levels of sulfadiazine and ciprofloxacin, the type of EC will certainly promote a distinct effect on the biofilm structure. Additionally, the impact of ECs on microbial communities may be influenced by other environmental factors (*i.e.* hydrodynamic stress, oxygen and nutrient availability, temperature, pH), which also constitutes a challenge to understand the real impact of ECs on biofilms (Pinto et al., 2022).

The biofilm structure analyzed by OCT did not reveal a significant impact of MP in *A. calcoaceticus* biofilms for all the surface materials but showed an increase in biofilm thickness for single *S. maltophilia* biofilms formed on PPL coupons, and for dual-species biofilms formed on HDPE and PPL. Dual-species biofilms seem to have a structure highly influenced by the presence of *S. maltophilia,* as already described by Gomes et al. (2018a). MP-exposed dual-species biofilms formed on HDPE and PPL had cellular densities and EPS content comparable to the non-exposed counterparts. However, these were thicker than the non-exposed biofilms. Therefore, this alteration in thickness is not related to major changes in the biofilm composition (EPS or cellular content) but it may suggest that MP exposure cause significant alterations in the biofilm structure. Kenchenten (2017) has already shown that the exposure of *Pseudomonas* sp. biofilms to MP at 1000 ng/L promoted an increase in the biofilm thickness.

The selective increase of biofilm thickness on HDPE and PPL suggests

that a lower interaction between MP and HDPE and PPL can occur, leaving MP available to interact with the biofilm cells. On the contrary, if MP is preferentially adsorbed by PVC, the concentration of MP available to interact with the biofilm cells will be lower. A recent study found a higher adsorption ability of PVC in comparison to polyethylene (Dong et al., 2019). Amin et al. (2012) studied the interaction of MP and PP with low-density polyethylene (LDPE) and PPL, and showed a higher PP adsorption on LDPE, corroborating the findings of Bahal and Romansky (2001).

Behind the direct impact of MP on the biofilms formed, the results also describe the impact of MP on bacterial motility and the ability to produce virulence factors (Warrier et al., 2021). The production of virulence factors, including fimbriae (pili), enzymes and iron chelators are crucial in the inactivation of the immune system by degrading serum and tissue proteins, which contributes to increased pathogenicity (Bitrian et al., 2012; Meheissen et al., 2022). Although the expression of these factors can be changed in response to fluctuations in environmental conditions, no work ever studied the impact of parabens on the production of these virulence factors. Several types of bacterial motility were already described, including swimming, swarming, and twitching (Wadhwa and Berg, 2022). In this study, *S. maltophilia* revealed a great ability for swimming motility after being exposed to MP, independently of the material where the exposure took place. This means that exposure to MP may stimulate *S. maltophilia* individual movement in liquid environments, which may result in a positive effect on flagellum synthesis, as reported by Arruda et al. (2022a). Another study reported an inhibitory effect on the motility of *Paramecium* in the presence of erythromycin, at concentrations commonly found in DW (Shunmugam et al., 2021). Both bacteria tested have less motility in denser media, therefore, swarming and twitching motility values are lower when compared with that for swimming. These two types of motilities were not affected by MP, suggesting that proteins related to bacterial motility in denser media are not over or under-expressed in the presence of MP. Nevertheless, MP exposure seems to have a higher impact on individual movements controlled by flagella and not on collective movements (swarming) or movements controlled by pili retraction, as in the case of twitching (Wadhwa and Berg, 2022).

Siderophores production for iron acquisition by both bacterial strains was not affected by the exposure to MP. Gomes et al. (2019b) also did not report changes in siderophores production after *S. maltophilia* exposure to clofibric acid. No differences in the production of protease, gelatinase, and lipase by MP-exposed *A. calcoaceticus* were also observed in comparison to non-exposed bacteria. However, protease production was potentiated for *S. maltophilia* from biofilms exposed to MP in PPL and PVC coupons. Wang et al. (2019) have already reported that the presence of sulfadiazine and ciprofloxacin at trace levels in DWDS increased protease activity. Another author showed that protease activity of *Stenotrophomonas rhizophila* was potentiated in the presence of Ca^{2+}, Mg^{2+}, Co^{2+} and K^{+} ions and with acetone and hexane solvents (Lich et al., 2022). However, a decreasing trend in bacterial protease activity after di-n-butylphatalate and metallic nanoparticles exposure was also reported (Wan et al., 2022; Wang et al., 2022). In this study, *S. maltophilia* also had an increase in gelatinase activity after exposure to MP in biofilms formed on HDPE. Elgendy et al. (2017) had already reported an increase in gelatinase activity for bacteria in an aquatic environment polluted by heavy metals (Cr, Pb and Mn). The potentiation of virulence factors production observed for *S. maltophilia* from DW in the presence of MP increases the concern about the potential risks of parabens contaminated DW for consumers.

5. Conclusions

This study highlights that the presence of parabens in DW should not be disregarded as these contaminants, particularly MP, may cause significant alterations in bacterial behaviour and their biofilms. The presence of MP potentiates biofilm formation by increasing the culturability and cellular density of single-species biofilms up to 233 and 150%, respectively. This effect was more evident for biofilms formed on HDPE and PPL. The interaction of parabens with biofilms differs according to the surface materials where they are formed, the bacterial species, and the exposure time. An exposure time of 7 days to parabens was sufficient to interfere with biofilms including the increase in the number of cells with membrane damage. The biofilm EPS production was not impacted by the presence of MP. Although dual-species biofilms were less affected by MP exposure in terms of cell density and culturability, their biofilm structure was significantly altered. MP presence caused an increase in the biofilm thickness of the single *S. maltophilia* biofilms formed on PPL coupons (44%), and of dual-species biofilms formed on HDPE (224%) and PPL (81%). This highlights PVC as a more inert material, which application in DWDS can lead to the formation of biofilms less susceptible to the presence of parabens. From a public health perspective and trying to understand the impact of parabens on the virulence of DW bacteria, it was found that exposure to MP potentiated the virulence of *S. maltophilia* through the increase of swimming motility (141%), and gelatinase (41%) and protease production (73%). This work further demonstrates the importance of studying the effects of parabens and other ECs on DW bacterial communities, not only in the biofilm characteristics but also in bacterial virulence.

Author contributions

Ana Rita Pereira – methodology, investigation, data curation, writing—original draft preparation, Inês Gomes – conceptualization, methodology, investigation, writing—review and editing, supervision, Manuel Simões - conceptualization, resources, writing—review and editing, supervision, project administration, funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.117413.

References

Abdelmoteleb, A., Troncoso-Rojas, R., Gonzalez-Soto, T., González-Mendoza, D., 2017. Antifungical activity of autochthonous *Bacillus subtilis* isolated from *Prosopis juliflora* against phytopathogenic fungi. MYCOBIOLOGY 45, 385–391. https://doi.org/10.5941/MYCO.2017.45.4.385.

Al-Halaseh, L.K., Al-Adaileh, S., Mbaideen, A., Hajleh, M.N.A., Al-Samydai, A., Zakaraya, Z.Z., Dayyih, W.A., 2022. Implication of parabens in cosmetics and cosmeceuticals: advantages and limitations. J. Cosmet. Dermatol. 21, 3265–3271. https://doi.org/10.1111/jocd.14775.

Amin, A., Chauhan, S., Dare, M., Bansal, A.K., 2012. Sorption of antimicrobial agents in blow-fill-seal packs. Pharmaceut. Dev. Technol. 17, 84–93. https://doi.org/10.3109/10837450.2010.516438.

Amoli, R.I., Nowroozi, J., Sabokbar, A., Fattahi, S., Amirbozorgi, G., 2017. Isolation of *Stenotrophomonas maltophilia* from water and water tap. Biomed. Res. 28, 8750–8754.

Arruda, V., Simões, M., Gomes, I., 2022a. The impact of synthetic musk compounds in biofilms from drinking water bacteria. J. Hazard Mater., 129185 https://doi.org/10.1016/j.jhazmat.2022.129185.

Arruda, V., Simões, M., Gomes, I., 2022b. Synthetic musk fragrances in water systems and their impact on microbial communities. Water 14, 692. https://doi.org/10.3390/w14050692.

Ateia, M., Zheng, T., Calace, S., Tharayil, N., Pilla, S., Karanfil, T., 2020. Sorption behavior of real microplastics (MPs): insights for organic micropollutants adsorption on a large set of well-characterized MPs. Sci. Total Environ. 720, 137634 https://doi.org/10.1016/j.scitotenv.2020.137634.

Bahal, S.M., Romansky, J.M., 2001. Sorption of parabens by flexible tubings. Pharmaceut. Dev. Technol. 6, 431–440. https://doi.org/10.1081/PDT-100002251.

Berry, D., Xi, C., Raskin, L., 2006. Microbial ecology of drinking water distribution systems. Curr. Opin. Biotechnol. 17, 297–302. https://doi.org/10.1016/j.copbio.2006.05.007.

Bhagwat, G., Tran, T.K.A., Lamb, D., Senathirajah, K., Grainge, I., O'Connor, W., Juhasz, A., Palanisami, T., 2021. Biofilms enhance the adsorption of toxic contaminants on plastic microfibers under environmentally relevant conditions. Environ. Sci. Technol. 55, 8877–8887. https://doi.org/10.1021/acs.est.1c02012.

Bitrian, M., Solari, C.M., González, R.H., Nudel, C.B., 2012. Identification of virulence markers in clinically relevant strains of Acinetobacter genospecies. Int. Microbiol. 15, 79–88. https://doi.org/10.2436/20.1501.01.161.

Błedzka, D., Gromadzińska, J., Wasowicz, W., 2014. Parabens. From environmental studies to human health. Environ. Int. 67, 27–42. https://doi.org/10.1016/j.envint.2014.02.007.

Bolujoko, N.B., Ogunlaja, O.O., Alfred, M.O., Okewole, D.M., Ogunlaja, A., Olukanni, O. D., Msagati, T.A.M., Unuabonah, E.I., 2022. Occurrence and human exposure assessment of parabens in water sources in Osun State, Nigeria. Sci. Total Environ. 814, 152448 https://doi.org/10.1016/j.scitotenv.2021.152448.

Chan, S., Pullerits, K., Keucken, A., Persson, K.M., Paul, C.J., 2019. Bacterial release from pipe biofilm in a full-scale drinking water distribution system. NPJ Biofilms Microbiomes 5, 9. https://doi.org/10.1038/s41522-019-0082-9.

Decho, A.W., Gutierrez, T., 2017. Microbial extracellular polymeric substances (EPSs) in ocean systems. Front. Microbiol. 8, 922. https://doi.org/10.3389/fmicb.2017.00922.

Dong, X., Zheng, M., Qu, L., Shi, L., Wang, L., Zhang, Y., Liu, X., Qiu, Y., Zhu, H., 2019. Sorption of tonalide, musk xylene, galaxolide, and musk ketone by microplastics of polyethylene and polyvinyl chloride. Mar. Pollut. Bull. 144, 129–133. https://doi.org/10.1016/j.marpolbul.2019.04.046.

Dubois, M., Gilles, K., Hamilton, J.K., Rebers, P.A., Smith, F., 1951. A colorimetric method for the determination of sugars. Dreywood, R., Indust. Enq. Chem., Anal. Ed 18, 476.

Elgendy, M.Y., Soliman, W.S., Abbas, W.T., Ibrahim, T.B., Younes, A.M., Omara, S.T., 2017. Investigation of some virulence determents in *Aeromonas hydrophila* strains obtained from different polluted aquatic environments. JJBS 10, 265–272.

Epa, U., Homeland Security Research Center, N., 2011. Development and Testing of Methods to Decontaminate a Building's Plumbing System Impacted by a Water Contamination Event: DECONTAMINATION of BACILLUS SPORES. EPA.

Ferreira, C., Pereira, A.M., Pereira, M.C., Melo, L.F., Simões, M., 2011. Physiological changes induced by the quaternary ammonium compound benzyldimethyldodecylammonium chloride on *Pseudomonas fluorescens*. J. Antimicrob. Chemother. 66, 1036–1043. https://doi.org/10.1093/jac/dkr028.

Flasiński, M., Gawryś, M., Broniatowski, M., Wydro, P., 2016. Studies on the interactions between parabens and lipid membrane components in monolayers at the air/aqueous solution interface. Biochim. Biophys. Acta Biomembr. 1858, 836–844. https://doi.org/10.1016/j.bbamem.2016.01.002.

Flasiński, M., Kowal, S., Broniatowski, M., Wydro, P., 2018. Influence of parabens on bacteria and fungi cellular membranes: studies in model two-dimensional lipid systems. J. Phys. Chem. B 122, 2332–2340. https://doi.org/10.1021/acs.jpcb.7b10152.

Gary Peterson, R.L., 1979. Review of the folin phenol protein quantitation method of Lowry, rosebrough, farr and randall. Anal. Biochem. 100, 201–220. https://doi.org/10.1016/0003-2697(79)90222-7.

Glover, J.S., Ticer, T.D., Engevik, M.A., 2022. Profiling antibiotic resistance in *Acinetobacter calcoaceticus*. Antibiotics 11. https://doi.org/10.3390/antibiotics11070978.

Gomes, I., Lemos, M., Mathieu, L., Simões, M., Simões, L.C., 2018a. The action of chemical and mechanical stresses on single and dual species biofilm removal of drinking water bacteria. Sci. Total Environ. 631–632, 987–993. https://doi.org/10.1016/j.scitotenv.2018.03.042.

Gomes, I., Madureira, D., Simões, L.C., Simões, M., 2019a. The effects of pharmaceutical and personal care products on the behavior of *Burkholderia cepacia* isolated from drinking water. Int. Biodeterior. Biodegrad. 141, 87–93. https://doi.org/10.1016/j.ibiod.2018.03.018.

Gomes, I., Maillard, J.-Y., Simões, L.C., Simões, M., 2020a. Emerging contaminants affect the microbiome of water systems—strategies for their mitigation. NPJ Clean Water 3, 39. https://doi.org/10.1038/s41545-020-00086-y.

Gomes, I., Pereira, M.A., Simões, L.C., Simões, M., 2022. Influence of surface materials on biofilm formation. In: Viruses, Bacteria and Fungi in the Built Environment. Elsevier, pp. 45–63. https://doi.org/10.1016/B978-0-323-85206-7.00013-7.

Gomes, I., Querido, M.M., Teixeira, J.P., Pereira, C.C., Simões, L.C., Simões, M., 2019b. Prolonged exposure of *Stenotrophomonas maltophilia* biofilms to trace levels of clofibric acid alters antimicrobial tolerance and virulence. Chemosphere 235, 327–335. https://doi.org/10.1016/j.chemosphere.2019.06.184.

Gomes, I., Simões, L., Simões, M., 2020b. Influence of surface copper content on *Stenotrophomonas maltophilia* biofilm control using chlorine and mechanical stress. Biofouling 36, 1–13. https://doi.org/10.1080/08927014.2019.1708334.

Gomes, I., Simões, L.C., Simões, M., 2019c. The role of surface copper content on biofilm formation by drinking water bacteria. RSC Adv. 9, 32184–32196. https://doi.org/10.1039/C9RA05880J.

Gomes, I., Simões, L.C., Simões, M., 2018b. The effects of emerging environmental contaminants on *Stenotrophomonas maltophilia* isolated from drinking water in planktonic and sessile states. Sci. Total Environ. 643, 1348–1356. https://doi.org/10.1016/j.scitotenv.2018.06.263.

Gomes, I., Simões, M., Simões, L.C., 2016. The effects of sodium hypochlorite against selected drinking water-isolated bacteria in planktonic and sessile states. Sci. Total Environ. 65, 40–48. https://doi.org/10.1016/j.scitotenv.2016.04.136.

Haddaoui, I., Mateo-Sagasta, J., 2021. A review on occurrence of emerging pollutants in waters of the MENA region. Environ. Sci. Pollut. Res. 28, 68090–68110. https://doi.org/10.1007/s11356-021-16558-8.

Juárez-Jiménez, B., Pesciaroli, C., Maza-Márquez, P., López-Martínez, S., Vílchez-Quero, J.L., Zafra-Gómez, A., 2019. Biodegradation of methyl and butylparaben by bacterial strains isolated from amended and non-amended agricultural soil. Identification, behavior and enzyme activities of microorganisms. J. Environ. Manag. 245, 245–254. https://doi.org/10.1016/j.jenvman.2019.05.122.

Kenchenten, K., 2017. Interaction between Micropollutants and Degradative Biofilm Communities: Basis for a Biomimetic Approach to Water Treatment.

Le, T.M., Pham, P.T., Nguyen, Truong Quang, Nguyen, Trung Quang, Bui, M.Q., Nguyen, H.Q., Vu, N.D., Kannan, K., Tran, T.M., 2022. A survey of parabens in aquatic environments in Hanoi, Vietnam and its implications for human exposure and ecological risk. Environ. Sci. Pollut. Res. 29, 46767–46777. https://doi.org/10.1007/s11356-022-19254-3.

Lee, J., Bang, S.H., Kim, Y.-H., Min, J., 2018. Toxicities of four parabens and their mixtures to *Daphnia magna* and *Aliivibrio fischeri*. Environ Health Toxicol 33, e2018018. https://doi.org/10.5620/eht.e2018018.

Li, W., Shi, Y., Gao, L., Liu, J., Cai, Y., 2015. Occurrence, fate and risk assessment of parabens and their chlorinated derivatives in an advanced wastewater treatment plant. J. Hazard Mater. 300, 29–38. https://doi.org/10.1016/j.jhazmat.2015.06.060.

Li, Ying, Xu, S., Li, Yuanyuan, Zhang, B., Huo, W., Zhu, Y., Wan, Y., Zheng, T., Zhou, A., Chen, Z., Huang, Z., Hu, J., Zhang, W., Wang, X., Ye, D., Xia, W., 2019. Association between urinary parabens and gestational diabetes mellitus across prepregnancy body mass index categories. Environ. Res. 170, 151–159. https://doi.org/10.1016/j.envres.2018.12.028.

Lich, N.Q., Thao, T.T.P., Huy, N.D., 2022. Characterization of extracellular protease from *Stenotrophomonas rhizophila* mt1 isolated from aquaculture sludge waste. Appl. Ecol. Environ. Res. 20, 2409–2423. https://doi.org/10.15666/aeer/2003_24092423.

Lincho, J., Martins, R.C., Gomes, J., 2021. Paraben compounds—part i: an overview of their characteristics, detection, and impacts. Appl. Sci. 5, 2307. https://doi.org/10.3390/app11052307.

Lopes, M. de F.S., Simões, A.P., Tenreiro, R., Marques, J.J.F., Crespo, M.T.B., 2006. Activity and expression of a virulence factor, gelatinase, in dairy enterococci. Int. J. Food Microbiol. 112, 208–214. https://doi.org/10.1016/j.ijfoodmicro.2006.09.004.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Lu, J., Li, H., Tu, Y., Yang, Z., 2018. Biodegradation of four selected parabens with aerobic activated sludge and their transesterification product. Ecotoxicol. Environ. Saf. 156, 48–55. https://doi.org/10.1016/j.ecoenv.2018.02.078.

Matwiejczuk, N., Galicka, A., Brzóska, M.M., 2020. Review of the safety of application of cosmetic products containing parabens. J. Appl. Toxicol. 40, 176–210. https://doi.org/10.1002/jat.3917.

Meheissen, M.A., Morsi, M.G., Hamad, M.A., Raouf, M.M., Ahmed Meheissen, M., 2022. A one year single-center experience on *Stenotrophomonas maltophilia* strains in Alexandria, Egypt. J. Med. Microbiol. 31, 69–76. https://doi.org/10.21608/EJMM.2022.228827.

Momba, M.N.B., Kaleni, P., 2002. Regrowth and survival of indicator microorganisms on the surfaces of household containers used for the storage of drinking water in rural communities of South Africa. Water Res. 36, 3023–3028. https://doi.org/10.1016/s0043-1354(02)00011-8.

Narciso, DiA.C., Pereira, A., Dias, N.O., Melo, L.F., Martins, F.G., 2022. Characterization of biofilm structure and properties via processing of 2D optical coherence tomography images in BISCAP. Bioinformatics 38, 1708–1715. https://doi.org/10.1093/bioinformatics/btac002.

Neilands, B.B., 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160, 47–56. https://doi.org/10.1016/0003-2697(87)90612-9.

Neri, I., Laneri, S., di Lorenzo, R., Dini, I., Russo, G., Grumetto, L., 2022. Parabens permeation through biological membranes: a comparative study using franz cell diffusion system and biomimetic liquid chromatography. Molecules 27, 4263. https://doi.org/10.3390/molecules27134263.

Niquette, P., 2000. Impacts of pipe materials on densities of fixed bacterial biomass in a drinking water distribution system. Water Res. 34, 1952. https://doi.org/10.1016/S0043-1354(99)00307-3. –1956.

Pinto, I., Simões, M., Gomes, I., 2022. An overview of the impact of pharmaceuticals on aquatic microbial communities. Antibiotics 11, 1700. https://doi.org/10.3390/antibiotics11121700.

Pinto, I., Simões, M., Gomes, I., 2023. The effects of emerging contaminants on the behaviour of *Acinetobacter calcoaceticus* derived from biofilms. Environ Sci Water Res Technol 9, 74–85. https://doi.org/10.1039/D2EW00246A.

Rasheed, T., Ahmad, N., Ali, J., Hassan, A.A., Sher, F., Rizwan, K., Iqbal, H.M.N., Bilal, M., 2021. Nano and micro architectured cues as smart materials to mitigate recalcitrant pharmaceutical pollutants from wastewater. Chemosphere 274, 129785. https://doi.org/10.1016/j.chemosphere.2021.129785.

Sadutto, D., Andreu, V., Ilo, T., Akkanen, J., Picó, Y., 2021. Pharmaceuticals and personal care products in a Mediterranean coastal wetland: impact of anthropogenic and spatial factors and environmental risk assessment. Environ. Pollut. 271, 116353 https://doi.org/10.1016/j.envpol.2020.116353.

Shunmugam, A.P., Subramanian, G., Fernandez, J.G., 2021. Measurements of the swimming speeds of motile microorganisms using object tracking and their correlation with water pollution and rheology levels. Sci. Rep. 11, 11821 https://doi.org/10.1038/s41598-021-91134-1.

Silva, A.F., Carvalho, G., Soares, R., Coelho, A.v., Crespo, M.T.B., 2012. Step-by-step strategy for protein enrichment and proteome characterisation of extracellular polymeric substances in wastewater treatment systems. Appl. Microbiol. Biotechnol. 95, 767–776. https://doi.org/10.1007/s00253-012-4157-2.

Simões, L.C., Simões, M., Oliveira, R., Vieira, M.J., 2007. Potential of the adhesion of bacteria isolated from drinking water to materials. J. Basic Microbiol. 47, 174–183. https://doi.org/10.1002/jobm.200610224.

Simões, L.C., Simões, M., Vieira, M.J., 2010. Influence of the diversity of bacterial isolates from drinking water on resistance of biofilms to disinfection. Appl. Environ. Microbiol. 76, 6673–6679. https://doi.org/10.1128/AEM.00872-10.

Simões, L.C., Simões, M., Vieira, M.J., 2008. Intergeneric coaggregation among drinking water bacteria: evidence of a role for *Acinetobacter calcoaceticus* as a bridging bacterium. Appl. Environ. Microbiol. 74, 1259–1263. https://doi.org/10.1128/AEM.01747-07.

Wadhwa, N., Berg, H.C., 2022. Bacterial motility: machinery and mechanisms. Nat. Rev. Microbiol. 20, 161–173. https://doi.org/10.1038/s41579-021-00626-4.

Wan, J., Hu, L., Zhang, C., Cheng, M., Xiong, W., Zhou, C., 2022. Response of microorganisms to phosphate nanoparticles in Pb polluted sediment: implications of Pb bioavailability, enzyme activities and bacterial community. Chemosphere 286, 131643. https://doi.org/10.1016/j.chemosphere.2021.131643.

Wang, H., Hu, C., Shen, Y., Shi, B., Zhao, D., Xing, X., 2019. Response of microorganisms in biofilm to sulfadiazine and ciprofloxacin in drinking water distribution systems. Chemosphere 218, 197–204. https://doi.org/10.1016/j.chemosphere.2018.11.106.

Wang, P., Ma, J., Wang, Z., Jin, D., Pan, Y., Su, Y., Sun, Y., Cernava, T., Wang, Q., 2022. Di-n-butyl phthalate negatively affects humic acid conversion and microbial enzymatic dynamics during composting. J. Hazard Mater. 436, 129306 https://doi.org/10.1016/j.jhazmat.2022.129306.

Warrier, A., Satyamoorthy, K., Murali, T.S., 2021. Quorum-sensing regulation of virulence factors in bacterial biofilm. Future Microbiol. 16, 1003–1021. https://doi.org/10.2217/fmb-2020-0301.

Wei, F., Mortimer, M., Cheng, H., Sang, N., Guo, L.H., 2021. Parabens as chemicals of emerging concern in the environment and humans: a review. Sci. Total Environ. 778, 146150 https://doi.org/10.1016/j.scitotenv.2021.146150.

Zhang, H.Y., Zhang, C.Y., Rao, W.L., Zhang, H., Liang, G.H., Deng, X., Zhao, J.L., Guan, Y.F., Ying, G.G., 2022. Influence of biofilms on the adsorption behavior of nine organic emerging contaminants on microplastics in field-laboratory exposure experiments. J. Hazard Mater. 434, 128895 https://doi.org/10.1016/j.jhazmat.2022.128895.

Zhu, Z., Shan, L., Li, X., Hu, F., Yuan, Y., Zhong, D., Zhang, J., 2020. Effects of interspecific interactions on biofilm formation potential and chlorine resistance: evaluation of dual-species biofilm observed in drinking water distribution systems. J. Water Proc. Eng. 38, 101564 https://doi.org/10.1016/j.jwpe.2020.101564.