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RESEARCH ARTICLE

Emerging pollutants in textile wastewater: an ecotoxicological assessment focusing on surfactants

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Abstract
Water and several chemicals, including dyestuffs, surfactants, acids, and salts, are required during textile dyeing processes. Surfactants are harmful to the aquatic environment and induce several negative biological effects in exposed biota. In this context, the present study aimed to assess acute effects of five surfactants, comprising anionic and nonionic classes, and other auxiliary products used in fiber dyeing processes to aquatic organisms *Vibrio fischeri* (bacteria) and *Daphnia similis* (cladocerans). The toxicities of binary surfactant mixtures containing the anionic surfactant dodecylbenzene sulfonate + nonionic fatty alcohol ethoxylate and dodecylbenzene sulfonate + nonionic alkylene oxide were also evaluated. Nonionic surfactants were more toxic than anionic compounds for both organisms. Acute nonionic toxicity ranged from 1.3 mg/L (fatty alcohol ethoxylate surfactant) to 2.6 mg/L (ethoxylate surfactant) for *V. fischeri* and from 1.9 mg/L (alkylene oxide surfactant) to 12.5 mg/L (alkyl aryl ethoxylated and aromatic sulfonate surfactant) for *D. similis*, while the anionic dodecylbenzene sulfonate EC50s were determined as 66.2 mg/L and 19.7 mg/L, respectively. Both mixtures were very toxic for the exposed organisms: the EC50 average in the anionic + fatty alcohol ethoxylate mixture was of 1.0 mg/L ± 0.11 for *V. fischeri* and 4.09 mg/L ± 0.69 for *D. similis*. While the anionic + alkylene oxide mixture, EC50 of 3.34 mg/L for *D. similis* and 3.60 mg/L for *V. fischeri*. These toxicity data suggested that the concentration addition was the best model to explain the action that is more likely to occur for mixture for the dodecylbenzene sulfonate and alkylene oxide mixtures in both organisms. Our findings also suggest that textile wastewater surfactants may interact and produce different responses in aquatic organisms, such as synergism and antagonism. Ecotoxicological assays provide relevant information concerning hazardous pollutants, which may then be adequately treated and suitably managed to reduce toxic loads, associated to suitable management plans.

Keywords Acute toxicity · Anionic surfactant · *Daphnia similis* · Nonionic surfactant · Textile wastewater · *Vibrio fischeri*

Introduction

Industrial development and aquatic resources are strictly related. The textile sector is one of the most expressive in the world's economy, with a market share around 2000 billion USD worldwide (Kishor et al. 2021). In Brazil, textile revenues were over 36 billion USD in 2021, directly contributing to 1.34 million employments (ABIT 2023). Despite the economic importance of textile manufacturing, the dyeing process demands high water volumes, generating equally high wastewater volumes containing several hazardous pollutants. About 200 L of water is used to produce 1 kg of textiles, and the water volume required by the cotton dyeing process ranges from 60.500 to 447.200 L/1000 kg of products (Ghaly et al. 2014). About 1 to 10 million liters of wastewater are discharged per day by textile industries (Kishor et al. 2021), and besides, about 200,000 tons of wastewater dyes are lost each year, containing about 10–45% textile dyes, which are not fixed to the fiber during the dyeing process (Islam et al. 2023).

Textile wastewater represents significant aquatic pollution source in many countries. The wide variety of compounds identified as chemically hazardous in these residues include dyes, surfactants, salts, peroxides, additives, and other persistent organic pollutants (Garcia et al. 2020; Tkaczyk et al.

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2020). About 72 different types of highly toxic pollutants are present in textile wastewater, part of them resistant to microbial degradation, leading to environmental and human health consequences. This type of discharge has induced critical changes in different aquatic ecosystems worldwide, including in Brazil, China, India, and USA, which represent approximately 60% of global textile production (Heydebreck et al. 2015; Mcdonough et al. 2016; Vacchi et al. 2017; Xue et al. 2019).

Textile wastewater toxicity towards photosynthetic producers includes morphological and physiological alterations and photosynthetic activity reduction (Alkimin et al. 2020; Cai et al. 2020), while effects in fishes include hepatic histopathological damage (Zhang et al. 2021), oxidative stress and antioxidant enzyme alterations (Bhattacharya et al. 2022), as well as gill histopathology (Mustapha and Bawa-Allah 2020), and degenerated hepatocytes (Shukla and Trivedi 2018). Red dyes contained in textile effluents, for instance, have been noted as leading to dye deposition in daphnid eggs and consequent malformations (Garcia et al. 2020), while Congo red dye has been reported as inducing yolk sac edema and skeletal deformities and inhibiting larvae hatching in zebrafish embryos (Hernández-Zamora and Martínez-Jerónimo 2019). Furthermore, both acute and sublethal effects in several aquatic organisms, such as *Daphnia similis* (crustacean), *Biomphalaria glabrata* (snail), and *Danio rerio* (fish), have been reported, including reproductive and cellular damages, egg and embryo-larval malformations, and abnormal development (Meireles et al. 2018; Hernández-Zamora and Martínez-Jerónimo 2019; Garcia et al. 2021).

Surfactants are examples of critical textile wastewater compounds due to their persistence, high organic matter content, and potential for negative biological effects. The dyeing processes of polyamide, polyester, and cotton fibers require 1 g/L of surfactant for each 1 kg of fiber. In addition, many other auxiliary products, such as dispersants and sequestering and humectant agents, also contain surfactants (Rosa et al. 2019; Garcia et al. 2020).

González et al. (2008) carried out screening and confirmation of several surfactants present in textile wastewater samples (without any pre-treatment) from a producer of home textiles (Serbia). Some surfactants detected were alcohol ethoxylates (AEOs), nonylphenol ethoxylates (NPEOs), linear alkylbenzene sulfonates (LAS), and alkyl ether sulfate (AES). In the final textile wastewater were found concentrations ranging from 5.16 to 5.74 mg/L for alcohol ethoxylate and 0.56–5.68 mg/L for nonylphenol ethoxylates. Araújo et al. (2022) reported in real textile wastewater, stored in an equalization tank of the reactors (biologic treatment: anaerobic–aerobic system) and treated by a physicochemical process, surfactant final concentration up to 5.9 mg/L of linear alkylbenzene sulfonate. While when applied biological treatment in wastewater samples from a fabric printing factory located in a textile district (Italy), it was obtained a residual surfactant concentration of around 5 mg/L and after ozone integration up to 3.5 mg/L (Lotito et al. 2012).

The surfactants in water generally reduce surface tension of water, which can lead to foam formation. Surfactant foam reduces the biodegradation capacity of waterways, decreases dissolved oxygen, and contributes to algal blooms. Furthermore, surfactants increase the solubility of organic contaminants in aqueous, which increases the depreciation of water quality (Badmus et al. 2021; Nunes and Teixeira 2022). Other parameters such as dissolved solids, turbidity, chemical oxygen demand, and biological oxygen demand are also altered by surfactants (Sasi et al. 2021; Nunes and Teixeira 2022). Nonetheless these numbers may vary widely according to daily processing, for instance, textile wastewater could have BOD ranging from 430 to 1200 mg/L and total solids about 6500 mg/L (Islam et al. 2022). COD values between 341 and 430 mg/L were determined in textile effluent containing nonionic surfactants (ethoxylated class) (Garcia et al. 2020).

Surfactants are considered emerging pollutants (EPs) for aquatic environment and usually display the ability to cause adverse ecological and human health effects, with risk assessments being highly recommended (Geissen et al. 2015). In terms of ecotoxicological effects, surfactants, even at relatively low concentrations, may induce deleterious effects in aquatic organisms from distinct trophic levels, such as cell damages, biochemical and physiological effects, and reproduction and growth alterations (Zanoletti et al. 2017; Garcia et al. 2020; Nunes and Teixeira 2022).

Regarding acute effects of surfactants, we may summarize: *Vibrio fischeri* bacteria exposed to fatty alcohol ethoxylate: EC50 between 1.24 and 4.75 mg/L (Jurado et al. 2009); *Clarias gariepinus* fish exposed to alcohol ethoxylate: EC50 was 16.88 mg/L (Mustapha and Bawa-Allah 2020). In daphnids, EC50 = 6.31 mg/L when exposed to linear alkylbenzene sulfonate—LAS (Sobrino-Figueroa 2018); ethoxylate—EC50 value was 8.3 mg/L (Roberts et al. 2007). Other effects observed include changes in the histopathology in gills of fish, *Clarias gariepinus*, exposed to LAS (Mustapha and Bawa-Allah 2020), hepatic and renal dysfunction, and electrolyte imbalance in fish when exposed to sodium dodecyl sulfate (Sayed and Authman 2018). In *Potamogeton perfoliatus* aquatic plant exposed to LAS, physiological parameters and growth were significantly affected, hindering the activity of enzymes (Zhou et al. 2018).

The present study aimed to assess ecotoxicological effects of five surfactants (anionic and nonionic classes) to aquatic organisms: *Vibrio fischeri* (bacteria) and *Daphnia similis* (cladocerans). These surfactants were selected for the study due to their use in many industrial activities, including textiles. Showing and discussing data related to surfactants ecotoxicity may be one way for developing greener products and/or possible replacement for less toxic surfactants, when performing similar function. Since several surfactants can be used in the textile process, the presence of more than one type of surfactant probably are in the final effluents. Therefore, the toxicities of binary surfactant mixtures containing the anionic surfactant dodecylbenzene sulfonate + nonionic fatty alcohol ethoxylate and dodecylbenzene sulfonate + nonionic alkylene oxide were also evaluated herein. Furthermore, to confirm that surfactants are indeed one of the critical compound classes in effluents containing them, our study also evaluated the acute toxicity of other auxiliary products used in fiber (cotton, polyamide, and polyester) dyeing processes.

Material and methods

The surfactants and other auxiliaries selected for this study are the same used during the bleaching stage of the polyamide, polyester, and cotton fiber dyeing process (Rosa et al. 2019; Rodrigues et al. 2020). The nonionic surfactants alkylene oxide, fatty alcohol ethoxylate, ethoxylate, alkyl aryl ethoxylated and aromatic sulfonate, and the anionic dodecylbenzene sulfonate were evaluated herein. Besides other auxiliary products, that comprise a humectant agent and nonionic and anionic dispersant/sequestering compounds. The surfactants and auxiliaries were supplied by Golden Technology and Clariant S.A. and were used with no further purification (purity > 99.0%). All these compounds were subjected to ecotoxicological assays with *Vibrio fischeri* and *Daphnia similis*.

Acute toxicity assays

The *Daphnia similis* (Claus, 1876) crustacean assays were conducted following Brazilian Standard Methods (ABNT NBR 12713: 2016). The natural water was used for maintaining the organisms applied for ecotoxicity assays. The same natural water (filtered and 44 ± 2 hardness-adjusted) was used for dilutions of tested samples and for the negative control. Twenty juveniles (6–24 h) were exposed to each sample concentration and to a negative control (filtered natural water) for 48 h, at a controlled temperature (20 °C ± 2), without food. The observed effect was immobility (endpoint). All assays were carried out in triplicate, totaling 60 organisms per concentration.

Surfactant and other auxiliary product concentrations were established after preliminary assays, as follows: alkylene oxide (1.0, 2.5, 5.0, 10, and 25 mg/L), alkyl aryl ethoxylated and aromatic sulfonate (1.0, 2.5, 5.0, 10, and 25 mg/L), fatty alcohol ethoxylate (1.0, 2.5, 5.0, 7.5, and 10 mg/L), ethoxylate (1.0, 2.5, 5.0, 7.5, and 10 mg/L), dodecylbenzene sulfonate (2.5, 5.0, 10, 25, and 50 mg/L), dispersant/sequestering anionic compound (200, 300, 500, 750, and 1000 mg/L), dispersant/sequestering nonionic auxiliary compound (150, 250, 300, 500, and 700 mg/L), and humectant agent (5.0, 12.5, 25, 37.5, and 50 mg/L).

The acute toxicities of two binary mixtures were also evaluated: anionic dodecylbenzene sulfonate + nonionic fatty alcohol ethoxylate and anionic dodecylbenzene sulfonate + nonionic alkylene oxide (1/1 ratio, w/w). All binary assays were also performed in triplicate (60 organisms exposed to each concentration) at dodecylbenzene sulfonate + fatty alcohol ethoxylate mixture concentrations of 1.0, 5.0, 10.0, 25, and 50 mg/L and dodecylbenzene sulfonate + alkylene oxide concentrations of 0.5, 1.0, 5.0, 10, and 20 mg/L. The surfactants for the mixtures were selected according to the results of toxicity assays for each single surfactant. In this case, it was decided to evaluate two different mixtures: anionic with nonionic from the alkylene class and anionic with one from the ethoxylated nonionic class. Here, the fatty alcohol ethoxylated was selected because it is the most toxic of the class of ethoxylates evaluated in this study for both organisms. Furthermore, in textile dyeing, mainly nonionic/anionic surfactant mixtures are applied during the fiber process (Sarayu and Sandhya 2012).

The *Vibrio fischeri* (Beijerinck, 1889) bacteria assays were performed employing a Microtox®, M-500, Microbics system (temperature = 3 °C ± 0.2), reconstituted in a reactivation solution before assays. Bacteria luminescence was evaluated before and after 15 min of sample exposure and toxicity was evidenced by loss of luminescence (endpoint). The methodology followed Brazilian Technical Standard Methods (ABNT NBR 15411:2012).

The surfactant and other auxiliary compound concentrations were established after preliminary assays, as follows: alkylene oxide (0.5, 1.0, 2.0, and 4.0 mg/L), alkyl aryl ethoxylated and aromatic sulfonate (1.0, 2.0, 4.0, and 8.0 mg/L), fatty alcohol ethoxylate (0.5, 1.0, 2.0, and 4.0 mg/L), ethoxylate (2.5, 5.0, 10, and 20 mg/L) and dodecylbenzene sulfonate (25, 50, 100, and 200 mg/L), anionic dispersant/sequestering compound (102.3, 204.4, 409.5, and 819 mg/L), nonionic dispersant/sequestering compound (102.3, 204.4, 409.5, and 819 mg/L), and humectant agent (5.1, 10.2, 20.4, and 40.9 mg/L). The negative control, a diluent solution (NaCl 2%), and a positive control (phenol) were also performed. All assays were carried out in triplicate.

The binary surfactant mixture (1/1 ratio, w/w) assays were also performed in triplicate at 2.55, 5.11, 10.23, and 20.47 mg/L for dodecylbenzene sulfonate + fatty alcohol ethoxylate and 5.11, 10.23, 20.46, and 40.95 mg/L for dodecylbenzene sulfonate + alkylene oxide. These concentrations were established after preliminary assays.

Toxicity assay data analysis

The toxicity data for both organisms were expressed as EC50, comprising the median concentration necessary to observe an effect for 50% of all exposed organisms. The EC50 is an inversely proportional parameter, which means that the lower the EC50 values, the higher the toxicity of the tested compound. Statistics were applied according to Brazilian ABNT standard methods, with EC50 values obtained by the trimmed Spearman-Karber method for *D. similis* (Hamilton et al. 1977) and by linear regressions for *V. fischeri*, based on sample concentrations and gamma values (γ). The gamma effect was calculated from the ratio between the lost and remaining luminescence.

An analysis of variance (ANOVA) was applied to verify the significance of differences between controls and experimental treatments values. A post hoc Dunnett's test was performed when significant differences ($p < 0.05$) were observed.

The toxicity assessment of the binary mixtures was performed using the toxic unit (TU_i) model (Eq. 1), as previously described (Ríos et al. 2017; Di Toro and McGrath 2000; Altenburger et al. 2003; EC 2012). The toxic unit of a surfactant mixture (TU_{mix}) corresponds to the sum of the TU_i of single compounds, calculated by Eq. 2 for binary mixtures (TU_{mix}).

$$TU_i = {}^{C_i}/_{EC_{50i}} \tag{1}$$

$$TU_{mix} = TU_A + TU_B \tag{2}$$

where C_i represents the ratio between a chemical concentration in a mixture, EC_{50i} is the toxicological acute endpoint, and TU_A and TU_B are the toxic units of two surfactants in the mixture (A and B).

If the chemicals in a mixture display the same mode of action, the dose/concentration addition principle can be assumed to quantify the mixture toxicity, where the TU_{mix} should be equal to 1.0 ± 0.2. In case of synergism or antagonism, TU_{mix} takes on < 1.0 and > 1.0 values, respectively.

The response addition model can be applied when the chemicals in the mixture act independently from each other through different modes of action, where an organism's response to the first surfactant is the same regardless of the presence of the second. Thus, the addition effect comprises the sum of biological responses. In this model, TU (TU_r) was calculated according to Eqs. 3 and 4.

$$\text{If} TU_A > TU_B; TU_r = 1 + TU_B/TU_A \tag{3}$$

$$\text{If} TU_A < TU_B; TU_r = 1 + TU_A/TU_B \tag{4}$$

Employing this concept, the TU_r depends on the TU_i of each compound relative to the other, which can be expressed as a proportion of the total TU for the mixture. Therefore, the EC50 obtained for any binary mixture of compounds that operates via response addition will be mostly determined by the component with the highest proportionate value of TU = 1 (Ríos et al. 2017; Hodges et al. 2006).

Results and discussion

Acute toxicity of surfactants towards *Daphnia similis* and *Vibrio fischeri* are summarized in Fig. 1. *Vibrio fischeri* bioluminescence inhibition (%) data at four concentrations and the EC50 (average of the triplicate assay) for surfactants are reported in Fig. 1A, while *D. similis* immobility (%) data at five concentrations and the EC50 (average of the triplicate assay) for the same compounds are reported in Fig. 1B.

Vibrio fischeri was more sensitive than *D. similis* to the nonionic surfactants, displaying EC50 values between 1.3 and 2.6 mg/L and from 1.9 to 12.5 mg/L, respectively. The opposite was observed for the anionic surfactant, with EC50 values of 66.2 mg/L for *V. fischeri* and 19.7 mg/L for *D. similis*.

Bioluminescence inhibition (%) for *V. fischeri* (Fig. 1A) was higher with increasing surfactant concentrations. Toxic effects were obtained from 0.5 mg/L for alkylene oxide and fatty alcohol ethoxylate, while about 100% bioluminescence inhibition was noted at 4 mg/L. Nonionic surfactants (alkyl aryl ethoxylated and aromatic sulfonate, and ethoxylate) effects were observed from 2.0 mg/L for both, and about 100% bioluminescence inhibition was obtained at 8.0 mg/L for alkyl aryl ethoxylated and aromatic sulfonate and 5.0 mg/L for ethoxylate. The EC50 was higher for the anionic surfactants compared to the nonionic compounds to bacteria, indicating lower toxicity, with acute effects observed from 50 mg/L ($p < 0.05$) (Fig. 1A).

The same pattern was observed for *D. similis* (Fig. 1B) with a higher acute effect (immobility, %) observed with increasing surfactant concentrations, although higher toxicity was noted for the nonionic surfactants compared to the anionic surfactants. Ethoxylate and fatty alcohol ethoxylate did not significantly induce toxic effects (*D. similis* immobility) compared to the controls up to 2.5 mg/L, while about 100% immobility was noted at 10 mg/L ($p < 0.05$—Dunnett's test). For alkylene oxide, acute effects were observed from 2.5 mg/L, increasing to 10 mg/L for alkyl aryl ethoxylated and aromatic sulfonate ($p < 0.05$). For the anionic surfactant, toxic effects were observed from 10 mg/L, while 100% immobility was obtained at 50 mg/L ($p < 0.05$).

Concerning the auxiliary compounds used in textile dyeing processes, the humectant agent was toxic for both organisms, with an EC50 < 16 mg/L (*D. similis* = 13.0 mg/L ± 0.81

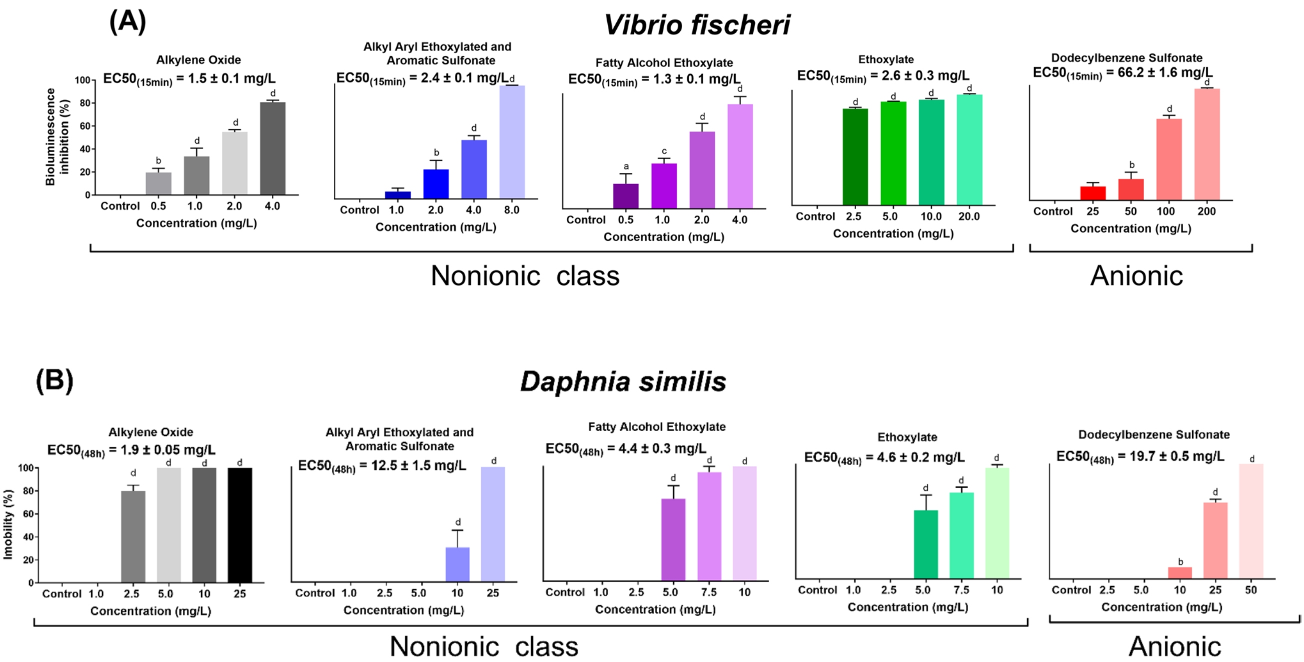

Fig. 1 **A** Bioluminescence inhibition (%) in *V. fischeri* at four surfactant concentrations and their respective EC50 (triplicate assay—average). **B** *D. similis* immobility (%) at five surfactant concentrations and their respective EC50 (triplicate assay—average). Letters indicate significant differences compared to the control (a, $p<0.05$, b, $p<0.01$, c, $p<0.001$, d, $p<0.001$)

and *V. fischeri* = 15.16 mg/L ± 1.24). Regarding the dispersant/sequestering compounds, EC50 values for *V. fischeri* were 289.16 mg/L ± 52.94 for the anionic agent and 320.23 mg/L ± 44.35 for the nonionic agent, and for *D. similis*, 812.4 mg/L ± 60.18 anionic and 604.5 mg/L ± 93.7, respectively (Supplementary Information, Table SI1; Figure SI1). These data indicate that the surfactants were more toxic to the assessed aquatic organisms than the humectant and dispersant/sequestrant agents. As previously mentioned, the acute toxicity was expressed by EC50, which is a parameter inversely proportional to toxicity. Lower EC50 values indicate that lower concentrations of samples are required to achieve effective median concentrations (EC50%), evidencing higher effects. In the present study, the EC50s to nonionic surfactants, for both organisms, ranged to 1.3–12.5 mg/L, which evidenced that these surfactants were the most toxic when compared to other analyzed auxiliary chemicals.

Table 1 (nonionic class) and Table 2 (anionic class) summarize literature toxicity data in comparison to the toxic effects of the investigated surfactants in this study. The results reported herein confirm the high toxicity of surfactants to aquatic organisms belonging to distinct trophic levels, although acute effects depend on the type of surfactant and organism interactions.

Comparing data from Table 1, the worst effects of nonionic surfactants were noted for *V. fischeri* (bacteria), followed by *Daphnia similis* (crustacean), *Daphnia magna* (crustacean), *Clarias gariepinus* (fish), and *Selenastrum capricornutum* (algae) (EC50 = 0.35–155.02 mg/L). While the anionic (Table 2), for example, linear alkylbenzene sulfonate (LAS), *Hyallela azteca* (amphipod) was the most sensitive, followed by *Pseudokirchneriella subcapitata* (algae), *Daphnia magna* (crustacean), *Danio rerio* (fish), *Daphnia similis* (crustacean), *Clarias gariepinus* (fish), and *Channa punctatus* (fish) (EC50 = 0.91–34.40 mg/L).

A relationship between surfactant structure and toxicity was very well established by Wong et al. (1997), where toxicity in daphnids and fish tends to increase with increasing alkyl chain lengths and also increase with decreasing ethylene oxide groups. Concerning ethoxylated surfactants, toxicity is higher for formulas with lower alkyl chain lengths. Ethoxylated fatty alcohols generally consist of a mixture of several homologs differing in alkyl chain lengths and degree of ethoxylation, which can influence their toxicity (Lechuga et al. 2016). Therefore, surfactant toxicity is clearly influenced by chemical structure, surface activity, properties, concentration, exposure time, and exposed organism (Han et al. 2020; Nunes and Teixeira 2022). Moreover, effect severity is associated to a surfactant's capability of absorbing and penetrating cellular membranes (Rathi et al. 2021).

In the present study, the toxicity values for ethoxylated fatty alcohol were 4.2 mg/L ± 0.3 for *D. similis* and 1.3 mg/L ± 0.1 for *V. fischeri*. The same pattern was reported for *V. fischeri* by Jurado et al. (2009) with an EC50 = 1.24–4.76 mg/L (Table 1) and previously by Farré

Table 1 Acute toxicity of several nonionic surfactants to different aquatic organisms

Surfactant/class	Aquatic species/trophic level	E(L)C50 (mg/L)	Reference
Polyoxyethylene alkyl ether	*Clarias gariepinus* (fish)	22.61	Mustapha and Bawa-Allah 2020
Alcohol ethoxylate		16.88	Mustapha and Bawa-Allah 2020
Myristyl dimethyl amine oxide	*Daphnia magna* (crustacean)	15.46	Fernández Serrano et al. 2014
Lauryl dimethyl amine oxide		27.14	Fernández Serrano et al. 2014
Ethoxylate		0.3–8.3	Roberts et al. 2007
Ethoxylate	*Daphnia similis* (crustacean)	4.6	Present study
Fatty alcohol ethoxylate		4.4	Present study
Alkyl aryl ethoxylated and aromatic sulfonate		12.5	Present study
Alkylene oxide		1.9	Present study
Myristyl dimethyl amine oxide	*Vibrio fischeri* (bacteria)	3.39	Fernández Serrano et al. 2014
Lauryl dimethyl amine oxide		0.35	Fernández Serrano et al. 2014
Alkylene oxide		1.5	Present study
Fatty-alcohol ethoxylate ($C_{10}E_6$)		4.76	Jurado et al. 2009
Fatty-alcohol ethoxylate ($C_{10}E_3$)		2.01	Jurado et al. 2009
Fatty-alcohol ethoxylate ($C_{12–14}E_4$)		1.24	Jurado et al. 2009
Fatty alcohol ethoxylate		1.3	Present study
Ethoxylate		2.6	Present study
Alkyl aryl ethoxylated and aromatic sulfonate		2.4	Present study
Myristyl dimethyl amine oxide	*Selenastrum capricornutum* (algae)	57.77	Fernández Serrano et al. 2014
Lauryl dimethyl amine oxide		155.02	Fernández Serrano et al. 2014

Table 2 Acute toxicity of several anionic surfactants to different aquatic organisms

Surfactant	Aquatic species/trophic level	E(L)C50 (mg/L)	Reference
Sodium dodecyl sulfate	*Cyprinus carpio* (fish)	3.45	Bhattacharya et al. 2022
Sodium Laureth Sulfate		6.90	Bhattacharya et al. 2022
Isopropylamine dodecylbenzene sulfonate	*Carassius auratus gibelio* (fish)	19.85	Zhang et al. 2021
Alkyl ether sulfates	*Clarias gariepinus* (fish)	10.57	Mustapha and Bawa-Allah 2020
Linear alkylbenzene sulfonate		15.16	Mustapha and Bawa-Allah 2020
Linear alkylbenzene sulfonate	*Channa punctatus* (fish)	34.40	Shukla and Trivedi 2018
Linear alkylbenzene sulfonate	*Danio rerio* (fish)	6.6	Sobrino-Figueroa 2018
Linear alkylbenzene sulfonate	*Daphnia magna* (crustacean)	6.31	Sobrino-Figueroa 2018
Linear alkylbenzene sulfonate	*Daphnia similis* (crustacean)	14.17	Coelho and Rocha 2010
Laureth-4 carboxylic acid	*Daphnia magna* (crustacean)	3.47	Fernández Serrano et al. 2014
Capryleth-6 carboxylic acid		29.87	Lechuga et al. 2016
Dodecylbenzene sulfonate	*Daphnia similis* (crustacean)	19.70	Present study
Linear alkylbenzene sulfonate	*Hyallela azteca* (amphipod)	0.918	Sobrino-Figueroa 2018
Sodium lauryl ether sulfate	*Vibrio fischeri* (bacteria)	4.0	Mariani et al. 2020
Laureth-4 carboxylic acid		3.58	Fernández Serrano et al. 2014
Capryleth-6 carboxylic acid		32.71	Lechuga et al. 2016
Docusate sodium		74.5	Rosal et al. 2010
Dodecylbenzene sulfonate		66.20	Present study
Linear alkylbenzene sulfonate	*Pseudokirchneriella subcapitata* (algae)	2.1	Sobrino-Figueroa 2018
Docusate sodium		39.5	Rosal et al. 2010
Laureth-4 carboxylic acid	*Selenastrum capricornutum* (algae)	7.08	Fernández Serrano et al. 2014
Capryleth-6 carboxylic acid		76.42	Lechuga et al. 2016

et al. (2001), with an EC50 of 0.67 mg/L. In final textile wastewater were verified values for alcohol ethoxylates surfactant ranged 5.16–5.74 mg/L (González et al. 2008). These values in wastewater are higher than our data and the literature for EC50s, confirming that the final concentrations of surfactants in textile effluents are toxic to several aquatic organisms.

Concerning the ethoxylated surfactant, the EC50 values reported herein were 4.4 mg/L ± 0.2 for *D. similis* and 2.6 mg/L ± 0.3 for *V. fischeri*. Similar data for daphnids were described by Roberts et al. (2007), with the EC50 values ranging between 0.3 and 8.3 mg/L for nine types of ethoxylated surfactants, suggesting that the acute toxicity of ethoxylated surfactants is associated with degree of ethoxylation. For example, acute toxicity to *Daphnia magna* was noted for surfactants containing two ethoxylate units (2EO), at an EC50 of 0.4 mg/L, while surfactants with 10 EO units resulted in EC50 values between 2.9 and 3.2 mg/L (Roberts et al. 2007).

Other nonionic surfactants effects to aquatic biota have been described in the literature, including histopathological alterations and metabolic hepatic system transformation in several fish species exposed to alcohol ethoxylate (Cowan-Ellsberry et al. 2008; Bejarano and Wheeler 2021). The degradation products of nonionic surfactant alkylphenol ethoxylates have been considered endocrine disruptors in some biological systems (i.e., mammalians, fishes, and amphibians) (Neamţu and Frimmel 2006; Borghi et al. 2011; Jardak et al. 2016). Nonylphenol polyethoxylate degradation products are, in fact, frequently more toxic, persistent, and bioaccumulative than the primary compounds to aquatic ecosystems (Murdoch and Sanin 2016).

The acute toxicity results for the anionic surfactant were EC50 = 66.2 mg/L ± 1.6 for bacteria and 19.7 mg/L ± 0.5 for daphnids (Fig. 1A, B, Table 1). Previously published data reported EC50 values between 3.58 and 74.5 mg/ L for *V. fischeri* (Fernández Serrano et al. 2014; Rosal et al. 2010) and from 6.31 to 29.87 mg/L for daphnids (Sobrino-Figueroa. 2018; Lechuga et al. 2016) (Table 2).

In addition to acute effects, sublethal effects due to LAS anionic surfactant have also been reported, such as biochemical, physiological, and metabolic alterations, also reducing the resistance of several species against environmental stress, affecting reproduction, and growth processes (Hampel et al. 2012; Petrie et al. 2015; Badmus et al. 2021). Mustapha and Bawa-Allah (2020) evidenced gill histopathology changes and significantly reduced liver function enzymes activities in fish due to LAS, while hepatotoxicity was evidenced in bullfrog tadpoles (*Lithobates catesbeianus*), as well as morphometric, metabolic, and histopathological effects and homeostasis alterations (Franco-Belussi et al. 2021).

The present study also evaluated the toxicity of two binary surfactant mixtures (Table 3). Both mixtures were very toxic for the exposed organisms, where the EC50 average in the anionic + fatty alcohol ethoxylate mixture (triplicate assay) was of 1.0 mg/L ± 0.11 for *V. fischeri* and 4.09 ± 0.69 for *D. similis*. Similar toxicity values were observed for the anionic + alkylene oxide mixture, with an EC50 of 3.34 mg/L for *D. similis* and 3.60 mg/L for *V. fischeri*. In addition, both mixtures exhibited similar EC50 values for the individual nonionic surfactants (Fig. 1A, B), demonstrating that these compounds are highly toxic, even in mixtures containing anionic surfactants.

Literature reported that surfactant mixtures are present in real textile wastewater. González et al. (2008) evaluated textile effluent samples and some examples of surfactant mixture detected were linear alkylbenzene sulfonates (LAS), alkylsulfate (AS), alkyl ether sulfate (AES), dihexylsulfosuccinate (DHSS), alcohol ethoxylates (AEOs), coconut diethanolamide (CDEA), and nonylphenol ethoxylates (NPEOs), besides to degradation products of some surfactants, e.g., NPEO. In the final textile wastewater were found surfactants concentrations from to 5.74 mg/L. Furthermore, in wastewater textile samples of fabric in the textile district was founded nonionic, cationic, and anionic surfactant mixture. After biological treatment, it was obtained a final surfactant concentration of 5 mg/L (Lotito et al 2012). These concentrations in final wastewater demonstrate how hazardous are surfactants to aquatic biota, once our toxicity data in mixtures, expressed by EC50, were less than 4.0 mg/L.

Regarding mixture dose responses (Fig. 2), the same pattern was observed for both mixtures, where acute effects (*D. similis* immobility and *V. fischeri* bioluminescence inhibition) were proportional to increasing concentration. For *D. similis*, no effects were noted for both mixtures compared to control at 1 mg/L, while 100% immobility was obtained from 10 mg/L ($p < 0.05$—Dunnett's test). For *V. fischeri*, exposure to 2.55 mg/L of the anionic + fatty alcohol ethoxylate mixture resulted in over 70% bioluminescence inhibition, while 10.23 mg/L led to over 69% inhibition and 20.47 mg/L resulted in 93.8% bioluminescence inhibition ($p < 0.05$). Concerning the anionic + alkylene oxide mixture, exposure to 5.11 mg/L resulted in 57.7% bioluminescence inhibition, while 10.23 mg/L led to 70.2% and 40.95 mg/L, to 86.3% inhibition ($p < 0.05$).

Our study also evaluated the toxicity of binary mixtures employing the toxic unit model. In the concentration addition (CA) model, the compounds in a mixture display the same mechanism of action for a specific response and act at the same action site; in this model, the mixture effect is the sum of the relative toxicities of individual compounds. In the independent action model (IA), the compounds affect the organisms through different mechanisms of action, showed statistically independent effects from each other without interaction (Kar and Leszczynski 2019). In present study, concentration addition model was the best model for explaining the mode of action ($TU_{mix} = 1 \pm 0.2$).

Furthermore, the dodecylbenzene sulfonate + fatty alcohol ethoxylate mixtures resulted in lower EC50 values than the pure surfactant aqueous solutions for both investigated organisms (Table 3). The results for the dodecylbenzene sulfonate + alkylene oxide mixture suggest synergism ($TU_{mix} < 1.0$) for both organisms. In contrast, the dodecylbenzene sulfonate + alkylene oxide presents synergism for *D. similis* ($TU_{mix} < 1.0$) and antagonism for *V. fischeri* ($TU_{mix} > 1.0$), reinforcing the importance of evaluating organisms for different trophic levels.

Jurado et al. (2012) and Fernández Serrano et al. (2014) highlighted that the least toxic surfactants in binary mixtures exhibit lower individual toxicity. Synergism increases with the degree of surfactant charge difference. Thus, the synergism between anionic/anionic or nonionic/nonionic surfactants was lower than between anionic/nonionic compounds (Kume et al. 2008; Fernández Serrano et al. 2014). It is worth highlighting that in the textile dyeing process, several classes of nonionic and mixed nonionic/anionic surfactants are applied during fiber scouring and bleaching, and these mixtures are compound the final effluent (Sarayu and Sandhya 2012).

The present study confirmed that nonionic surfactants are harmful to aquatic biota (with the lowest EC50 values obtained herein), and the data obtained concerning the surfactant mixtures and conditions applied herein indicate prevailing high nonionic compound toxicity. Further studies concerning surfactant mixtures are needed, including their toxicity in aquatic matrices, to adequately determine surfactant effects in aquatic biota. Mixtures frequently incorporate nonionic surfactants, as these compounds do not produce ions in aqueous solutions, making them compatible with other types of ionic surfactants, thus leading to their widespread use in many commercial products and industrial wastewaters (Fernández Serrano et al. 2014; Ríos et al. 2017).

Our findings suggest that the high toxicity of textile effluents to aquatic species may be also associated to surfactants and compound mixtures. As textile effluents comprise a complex mixture, chemical interactions/transformations also contribute towards high toxicity. The binary surfactant mixtures resulted in more toxic effects than anionic surfactants and auxiliary chemicals.

Conclusion

Textile wastewaters represent mixture of hazardous pollutants, many of which are considered emerging pollutants to aquatic ecosystem. As surfactants are of environmental

Table 3 Acute toxicity and EC50 data, for binary anionic and nonionic surfactant mixtures (1:1 ratio)

	Surfactant A	Surfactant B	Surfactant A (EC_{50} mg/L)	Surfactant B (EC_{50} mg/L)	Mixture A + B (EC_{50} mg/L)	TU_A	TU_B	TU_{mix}	TU_f	Type of action
D. similis	Dodecylbenzene sulfonate	Fatty alcohol ethoxylate	19.70 ± 0.5	4.4	4.09 ± 0.69	0.10	0.46	0.57	1.3	–
	Dodecylbenzene sulfonate	Alkylene oxide	19.70 ± 0.5	1.9	3.34 ± 0.85	0.08	0.88	0.96	1.4	Concentration addition
V. fischeri	Dodecylbenzene sulfonate	Fatty alcohol ethoxylate	66.20 ± 1.6	1.3	1.0 ± 0.11	0.01	0.38	0.39	1.0	–
	Dodecylbenzene sulfonate	Alkylene oxide	66.20 ± 1.6	1.5	3.60 ± 0.40	0.03	1.20	1.23	1.7	Concentration addition

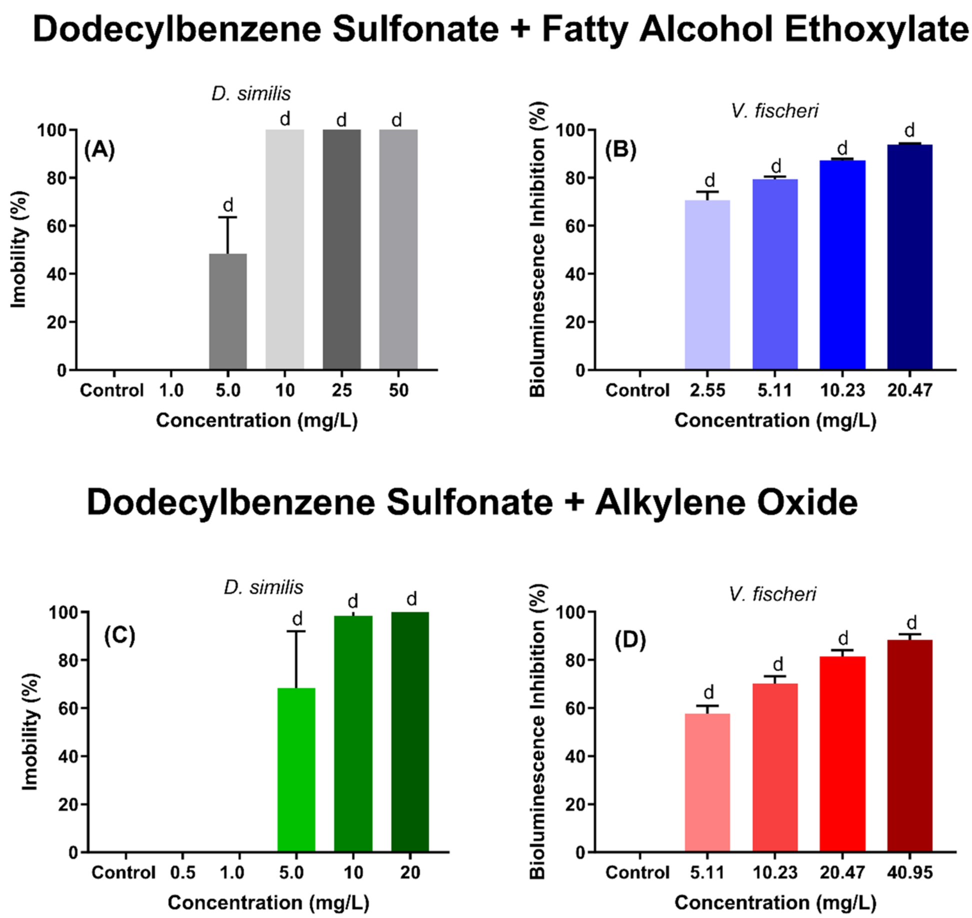

Fig. 2 *Daphnia similis* immobility (%) at five concentrations of **A** the anionic + fatty alcohol ethoxylate mixture and **C** anionic + alkylene oxide mixture (triplicate assay—average) and *V. fischeri* bioluminescence inhibition (%) at four concentrations of **B** the anionic + fatty alcohol ethoxylate mixture and **D** anionic + alkylene oxide mixture (triplicate assay—average). Letters indicate significant differences compared to the control (a, $p < 0.05$, b, $p < 0.01$, c, $p < 0.001$, d, $p < 0.001$)

concern, the present study evaluated the toxicity of two binary surfactant mixtures alongside single surfactant effects. Both mixtures were very toxic for exposed organisms, with the anionic + fatty alcohol ethoxylate presenting an EC50 average of 1.0 mg/L ± 0.11 for *V. fischeri* and of 4.09 ± 0.69 for *D. similis*. Very similar data were noted for the anionic + alkylene oxide mixture, with an EC50 of 3.34 mg/L for *D. similis* and of 3.60 mg/L for *V. fischeri*. Concerning the dodecylbenzene sulfonate + alkylene oxide mixture, the findings suggest that the concentration addition was the best model to explain the action that is more likely to occur for mixture. Higher acute toxicity values to both aquatic organisms were noted for the nonionic surfactants, EC50 ranging from 1.3 to 12.5 mg/L, that evidenced the nonionic surfactants were the most toxic when compared to other analyzed compounds evaluated in this study. These data indicate that the assessed surfactants are very toxic, demonstrating the need for suitable monitoring programs and preventive actions. The ecotoxicological approach applied herein provides an assessment of the impact of emerging pollutants on the aquatic environment and can significantly contribute to pollution control, aquatic protection, and mitigation strategies. Moreover, assessing the environmental behavior of these emerging pollutants is paramount importance for the improvement of environmental risk assessments.

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Author contribution All authors contributed to the study conception and design. Material preparation, investigation, and formal analysis were performed by Vanessa Silva Granadeiro Garcia, Flávio Kiyoshi Tominaga, and Jorge Marcos Rosa. Project administration, resources, and supervision were performed by Sueli Ivone Borrely. The first draft of the manuscript was written by Vanessa Silva Granadeiro Garcia and Sueli Ivone Borrely and all authors commented on previous versions of the manuscript. All authors (Vanessa Silva Granadeiro Garcia, Flávio Kiyoshi Tominaga, Jorge Marcos Rosa, and Sueli Ivone Borrely) read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethical approval Not applicable.

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Consent for publication Not applicable.

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