Hair Care Products Manufacturing Wastewaters: Toxicity and test Organism Sensitivity

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Abstract

Wastewater from two (A and B) small-scale hair care product manufacturers located in the state of Minas Gerais were characterized, with regard to physical, chemical and ecotoxicological parameters. Wastewater characteristics typical for this industrial sector were found in three samples collected at each manufacturing plant, with high levels of both particulate and dissolved organic matter (soluble and total COD), oils and grease and acute toxicity to the microcrustacean *Daphnia similis* (EC_{50} < 0.5 %). Strong positive correlations between turbidity, oils and grease and acute toxicity were observed. It is worth mentioning that only one of the manufacturers operates a wastewater treatment plant, while the second discharges untreated wastewater to a local sewer system. Furthermore, neither of the two cities where the enterprises are located has a municipal sewage treatment plant, aggravating environmental impacts of the industrial contributions to sewage discharges in receiving waters. A battery of tests was performed with species from three trophic levels (primary producers and primary and secondary consumers) to select the most sensitive test organisms to the wastewaters evaluated. The microcrustacean *Ceriodaphnia dubia* is indicated for use in routine monitoring, since it exhibited the highest sensitivities to hair care products wastewaters, for both acute and chronic effects.

Highlights

- Hair care products manufacturing generate highly toxic effluents.
- Sensitivity of test organisms to cosmetics industry wastewaters compared.
- Local sewer system not connected to municipal sewage treatment plant.
- Positive correlations between turbidity, oils and acute toxicity observed.
- Ceriodaphnia dubia's highest sensitivities to hair care products wastewaters.

1 Introduction

Water scarcity and rationing in several Brazilian cities has become an alarming reality in recent years. Despite the media's emphasis on this issue, it is not the only water related problems faced by the population. Unplanned urban growth, increasing demands for consumer goods and precarious sanitation conditions all contribute to the degradation of already compromised water supplies. Preservation of aquatic environments should be a goal of all, and both producers and consumers should be aware of their responsibility to minimize environmental degradation arising from production and use of consumer goods.

To assess the real impact of any industrial sector on the aquatic environment, it is essential to characterize the entire production chain, with special attention to wastewater generation points. Wastewater characterization should not be restricted to physical and chemical parameters but should also include ecotoxicological evaluation. Decision-making regarding treatment processes to be implemented should be based on both physical-chemical and ecotoxicological aspects.

Quality control during characterizations is fundamental for reliable data interpretation and is required by legal regulations to certify laboratories issuing reports to environmental agencies. A further requirement in ecotoxicological characterization is the need to evaluate the sensitivity of species from different trophic levels to wastewater samples, since not all organisms respond in the same way to the same concentration of a toxic agent, and it is important to know what the trophic chain levels will be impacted by wastewater discharge to receiving waters.

The Brazilian cosmetics industry is recognized for its economic importance, but little attention has been paid to the environmental impacts associated with wastewater discharge from the hundreds of small-scale manufacturers throughout the country, many of which do not operate wastewater treatment plants (Melo et al. 2013; Melo and Mounteer 2017; Melo et al. 2018). Wastewater composition depends on raw materials and products, but typically is characterize by high chemical oxygen demand (COD) and concentrations of organic compounds with low biodegradability, such as preservatives, surfactants, oils and greases (O & G), dyes and fragrances (Bautista et al. 2007; El-Gohary et al. 2010).

Several studies have already evaluated the comparative sensitivity of test organisms to complex chemicals mixtures, such as industrial effluents (Walsh et al. 1982; Rojieková-Padrtová et al. 1998; Liguoro et al. 2010; Ribeiro 2008; Rosal et al. 2010; among others). However, to the best of our knowledge, no studies have been conducted to select test organisms sensitive to cosmetics industry wastewaters, in particular from hair product factories. Therefore, the goal of this study was to perform physicochemical and ecotoxicological characterization of wastewaters from small-scale hair care product manufacturers and select the most sensitive test organisms for ecotoxicological evaluation of these wastewaters.

2 Material And Methods

2.1 Hair care product manufacturers

After an initial screening of hair care products manufacturers, two representative small-scale hair care products manufacturing plants were selected for this study, hereafter denominated Plant A and Plant B (Table 1), respecting the confidentiality agreement as a condition of their participation. Plant A employs 48 people and has an average monthly water consumption for industrial processes of 50,000 liters. Sanitary sewage and industrial effluents are treated separately. The industrial effluents come from production activities such as reactor washing, packaging and laboratory wastes, together with raw materials and products past their shelf-life, as well from the purge of the compressors and the solutions used in regeneration of ion exchange columns used to prepare the deionized water that is incorporated into products.

At plant A wastewater is treated in a 1300 L stainless steel tank by conventional physicochemical batch process of coagulation, flocculation and sedimentation, followed by passage through an activated carbon filter. Physicochemical sludge is sent to a drying bed and then collected by a third party for incineration. The treated wastewater is discharged to the municipal sanitary system and released without further treatment to the receiving water body.

Factory B has five employees and an average monthly industrial water consumption of 20,000 liters. There is no wastewater treatment system installed in the plant and wastewater is discharged in the municipal sewage system and released without any treatment to receiving waters.

Table 1. Hair care products manufactures at plants A e B

Product	Plant A	Plant B
Shampoo	X	X
Conditioner	X	X
Capillary Mask	X	X
Finisher	X	X
Split end repair	X	X
Keratin	X	X
Extract	X	
Hair ointment	X	
Silicone cream	X	
Oil	X	
Combing Cream		X
Curl Activator Fluid		X

2.2 Wastewaters sampling and physicochemical characterization

Both plants operate batch production processes and therefore grab samples of raw wastewater were collected at each once a month, for three months. Wastewater samples were stored in polyethylene bottles after coarse sieving (0.1 cm sieve) and mixing. Physicochemical characterization was performed according to standard methods (APHA 2017) and included the following parameters: pH (method 4500-H^+ B, Hach HQ40d pHmeter), electrical conductivity (method 2510 B, Tecnopon mCA-150 conductivity meter), turbidity (method 2130 B, TD-300 turbidity meter), total COD (tCOD) and soluble (sCOD) (method 5220 D, Hach DR3800 spectrophotometer), dissolved organic carbon (DOC, method 5310 B, Shimadzu TOC-V CSH) and O&G (method 5520 B). Samples were filtered through 0.45 µm sterile membranes for sCOD and DOC quantification.

All analyzes were performed at the Laboratory of Sanitary and Environmental Engineering (LESA) of the Universidade Federal de Viçosa (UFV), except O&G analyses, which were carried out by a private laboratory.

2.3 Organism maintenance

Toxicity tests were carried out in the ecotoxicology laboratory, Aquatox / LESA / UFV, in which the test organisms *Daphnia similis, Ceriodaphnia dubia*, *Hyalella azteca* and *Raphidocelis subcapitata* were maintained, according to Brazilian technical standards (ABNT NBR 12713: 2016, NBR 13373: 2016, NBR 12648: 2018 and NBR 15470: 2013), which are similar to international standards. The organisms were maintained in water collected at the UFV´s water treatment plant prior to the disinfection process. Quality of each batch of water was monitored by measuring pH, electrical conductivity and total hardness (method 2340 D, APHA 2017). Water was aerated for at least 12 hours before use.

Test organisms were held in beakers under controlled temperature and photoperiod, as specified for each species. The cladocerans *D. similis* and *C. dubia* were transferred to fresh water and fed three times a week with Tetramin® fish meal, yeast (*Saccharomyces cerevisiae*) and a suspension of *R. subcapitata*. The amphipod *H. azteca* water was changed once a week. They were fed daily with a mixture of fish meal, yeast and primrose oil, while solid food consisting of rabbit food (Rói, Guabi, São Paulo) and Tetramin® fish food was supplied three times a week. Nylon screens and *Elodea* were used as substrate for *H. azteca* growth. The green microalga *R. subcapitata* was cultivated in LC Oligo medium under aseptic conditions.

The fish *Danio rerio* was not maintained in the Aquatox laboratory but was obtained from a commercial breeder and transported to the Laboratory of Fish Biology, Department of Veterinary Medicine, UFV, where the necessary structure for testing was made available. Test protocols were duly approved by the UFV animal ethics committee (CEUA), Process 09/2016. After receiving the fish, they were kept for seven days in a 250-liter aerated aquarium, respecting the maximum body mass per volume ratio of water, 1 $g.L^{-1}$. During the seven days adaptation period, fish were fed with Presence Nutripiscis (Campinas, SP), with a minimum content of 450 $g.kg^{-1}$ crude protein. No obvious signs of stress, such as abnormal behavior, bleeding, excessive mucus or lethality greater than 5% of fish during the adaptation period were observed and they were considered fit for toxicity testing.

Sensitivity of the different test organisms to the reference substance sodium chloride (NaCl) was determined periodically, following the methodologies defined in the respective standards, detailed in the following item. Organism sensitivity can be found in supplementary information.

2.4 Toxicity tests

A comparative sensitivity analysis was performed using representative test organisms of three trophic levels, primary producers (*R. subcapitata*), primary consumers (*C. dubia, D. similis, H. azteca*) and secondary consumers *(D. rerio*) in the raw wastewater from the second sample collected at both manufacturing plants.

Before starting all toxicity tests, sample pH was monitored and adjusted to pH 6-8 if necessary. Initial characterization of raw wastewater samples was carried using the static *D. similis* acute immobilization assay (NBR 12713, ABNT 2016). Neonates aged six to 24 hours were exposed to serial dilutions of the samples, for 48 hours, at 22 ± 2 °C under a 12 h light/dark cycle. Four replicates of each dilution and the

control (culture water) were included, each containing 10 mL of test solution and five organisms. The number of immobilized organisms were counted at the end of the assay.

For the semi static *C. dubia* survival and reproduction assay (NBR 13373, ABNT 2016), neonates (6 - 24 hours old) were exposed to serial dilutions of wastewater for eight days at 22 ± 2 °C under a 12 h light/dark cycle. Ten replicates containing one organism and 10 mL test solution were included for each wastewater dilution and the negative control (dilution water). At 48 hours intervals, surviving adults and neonates were counted and the adults were transferred to new test solutions and fed. The number of surviving adults and the cumulative number of neonates in each test solution was tallied after eight days to determine chronic effects. Mortality of adults within the first 48 hours of exposure was quantified as an acute effect.

For the static *H. azteca* survival and growth assay (NBR 15470, ABNT 2013), seven to 14-day-old organisms were exposed to serial dilutions of the samples for 96 hours at 22 ± 2 °C under a 12 h light/dark cycle. Ten replicates containing 20 mL of test solution and one organism were included for each dilution and the control. In each replicate, 0.05 mL of compound food was added at the start of the assay and again after 48 hours. Dead organisms were counted at the end of the assay.

For the *R. subcapitata* growth inhibition assay (NBR 12648, ABNT 2018), test solutions (100 mL sample diluted in LC Oligo medium and inoculum of 10^5 cells.mL^{-1}) were prepared in triplicate under aseptic conditions in 250 mL erlenmeyers capped with a cotton stopper. The erlenmeyers were randomly arranged in shakers (Brand New Ethics, Model 109) and held at 22 ± 2 °C, under constant illumination (4,500 lux) and stirring (100-175 rpm) for 72 hours. At the beginning and at the end of the test the number of algal cells in each test solution was counted.

Acute toxicity to *D. rerio* (NBR 15088, ABNT 2016) was carried using fish with total length of 2.0 ± 1.0 cm in homogeneous lots exposed to serial dilutions of samples for 48 hours at 22 ± 2 °C under a 12 h light/dark cycle. Ten fish were exposed to each dilution and the control (dilution water). At the end of the exposure period dead fish were counted and the survivors were euthanized by immersion in a benzocaine solution.

Acute toxicity assay results (*D. similis C. dubia, D. rerio, H. azteca*) were reported as the half maximal effective or lethal initial concentration (EC_{50} or LC_{50}, %). Chronic toxicity results were reported as the half maximal inhibitory (IC_{50}, %), of cell growth (*R. subcapitata*) or organism reproduction (*C. dubia*). Toxicity indices were quantified by the Trimmed Spearman Karber, Probit, Dunnett or linear interpolation methods, using software made available free of charge by the US Environmental Protection Agency (USEPA, 2006).

Correlations between physical, chemical and ecotoxicological data were determined after confirming the normality of the data by the Shapiro Wilk test, using the Action Stat system (Estatcamp, Campinas, SP).

2.5 Comparative sensitivity of test organisms

Comparison of sensitivity among different organisms was evaluated using the method proposed by Zagatto and Bertoletti (2006), which takes into account the confidence intervals obtained for the EC_{50} or LC_{50} indices of the organisms to be compared. Initially, the G statistic was calculated using Eq. 1:

$$G = \sqrt{\left(log\left(\frac{UL_1}{EC_{50,1}}\right)\right)^2 + \left(log\left(\frac{UL_2}{EC_{50,2}}\right)\right)^2} \quad (1)$$

where: UL_1 = upper confidence interval for test organism 1

UL_2 = upper confidence interval for test organism 2

$EC_{50,1}$ = Effective concentration for test organism 1

$EC_{50,2}$ = Effective concentration for test organism 2

The H and Z values were then calculated using equations 2 and 3:

$$H = 10^G \quad (2)$$

$$Z = \frac{higher\ EC_{50}\ value}{lower\ EC_{50}\ value} \quad (3)$$

and test organisms exhibited significantly different sensitivities to the wastewater when Z > H.

3 Results And Discussion

3.1 Wastewater characterization

All wastewater samples collected at hair care products manufacturing plants A and B presented characteristic odor and were white to lightly colored. The wastewaters contained high levels of organic matter (Table 2), including O & G, typical of wastewaters of this industry (Bautista et al. 2007, El-Gohary et al. 2010). Although total COD was much higher in plant B samples, dissolved organic matter (sCOD and DOC) levels were not as markedly different. High turbidity values in all samples were related to high suspended organic solids contents as evidenced by the differences between tCOD and sCOD values. The higher organic matter contents of plant B samples suggests lower dilution of wastewaters during the washing process.

Sample pH values varied from acid to basic at plant A, which may be a result of purging of the regeneration solution from the process water ion exchange columns.

Table 2
Characteristics of raw wastewaters generated at the two hair products manufacturing plants

Parameter	Plant A	Plant B
pH	8.4 ± 2.8	5.9 ± 0.4
Conductivity ($\mu S.cm^{-1}$)	344 ±180	190 ± 68
Turbidity (NTU)	880 ± 490	1470 ± 58
tCOD ($mg.L^{-1}$)	7180 ± 705	23150 ± 4720
sCOD ($mg.L^{-1}$)	2280 ± 432	2680 ± 787
DOC ($mg.L^{-1}$)	636 ± 143	1230 ± 701
O&G ($mg.L^{-1}$)	1353 ± 695	3544 ± 2480
EC_{50} (%), *D. similis*	0.15–0.33	< 0.02–0.02

All samples exhibited very high acute toxicity to *D. similis*, especially from plant B, in agreement with this effluent´s more concentrated organic content. Cosmetics industry wastewaters are known to contain many substances toxic to aquatic organisms such as surfactants, dyes, preservatives and phenol derivatives (PERDIGÓN-MELÓN et al. 2010) and therefore the high acute toxicity is not surprising, but should certainly be a concern to the manufacturing plant and environmental enforcement agencies.

Only a few significant positive correlations were found among wastewater physicochemical properties (Table 3), notably pH with conductivity, O & G with turbidity, and soluble COD with DOC, while significant negative correlations were only found between soluble COD, DOC and conductivity, DOC and turbidity and total COD and O & G.

Acute toxicity indices had strong negative correlations with turbidity and O & G, that is the higher the values of these latter parameters, the lower the EC_{50} values, and the more toxic the wastewater was. On the other hand, toxicity indices were positively correlated with DOC, meaning the higher the dissolved organic carbon content, the lower the toxicity (higher EC_{50} value).

Table 3
Correlations between wastewater quality parameters (significant correlations in boldface, α = 0.05)

	pH	Conductivity	Turbidity	tCOD	sCOD	DOC	O & G	Toxicity
pH	1.00	-	-	-	-	-	-	-
Conductivity	**0.76**	1.00	-	-	-	-	-	-
Turbidity	-0.10	0.57	1.00	-	-	-	-	-
tCOD	0.80	0.21	-0.68	1.00	-	-	-	-
sCOD	-0.90	**-0.97**	-0.34	-0.45	1.00	-	-	-
DOC	-0.57	**-0.97**	**-0.76**	0.04	**0.87**	1.00	-	-
O & G	-0.21	0.48	**0.99**	**-0.76**	-0.24	-0.68	1.00	-
Toxicity	0.06	-0.60	**-1.00**	0.65	0.38	**0.78**	**-0.99**	1.00

3.2 Comparative sensitivity of test organisms

Test organisms remained healthy and consistently sensitive to the reference toxicant (Supplementary Data), which permitted their use to evaluate toxicity of the hair care products wastewaters (Table 4). Wastewater storage slightly lowered toxicity to *D. similis* (lower EC_{50} in Table 2 than Table 4). However, all tests reported in Table 4 were performed in parallel with wastewater samples stored for the same time period. Wastewater from plant B was more toxic to all organisms, except *C. dubia*, but with a slight difference.

Table 4
Raw wastewater toxicity to different test organisms of samples collected at plants A and B (values in parentheses are 95% confidence intervals)

Effect	Test organism	Index	Plant A (%)	Plant B (%)
Acute	*D. similis*	EC_{50}, 48h	0.49 (0.34–0.69)	0.04 (-)
	C. dubia	EC_{50}, 48h	0.17 (0.13–0.22)	0.04 (0.03–0.05)
	H. azteca	LC_{50}, 96h	0.12 (0.07–0.20)	0.04 (-)
	D. rerio	LC_{50}, 48h	2.67 (1.67–4.28)	2.50 (-)
Chronic	*C. dubia*	IC_{50}, 168h	0.017 (0.014–0.106)	0.018 (0.014–0.033)
	R. subcapitata	IC_{50}, 96h	0.54 (0.46–0.61)	0.08 (0.07–0.09)
(-) no confidence interval could be calculated				

Test organism sensitivity to acute effects varied decreased *C. dubia* and *H. Azteca*, which exhibited equally high sensitivities to the wastewaters (Table 5). Organism sensitivity decreased in the following order: *C. dubia* = *H. azteca* > *D. similis* > *D. rerio*. Greater sensitivity of daphnids than fish has previously been reported for a wide variety of chemicals (Martins et al. 2007) and industrial effluents (Rodgers et al. 1996). Despite the lower sensitivity *D. rerio* compared to the other organisms evaluated, very high toxicities were still observed, especially considering that the effluent from plant B is discharged without undergoing any treatment. Fish occupy the aquatic high trophic levels and are likely to accumulate high levels of substances by bioconcentration (Mackay et al. 2016) and, therefore they useful in environmental monitoring programs because organic fish systems are more histologically and physiologically similar to humans than invertebrates, allowing for more reliable extrapolations.

Table 5
Comparison of acute toxicity indices for different test organisms to hair care products wastewater collected at plant A

Organism 1 x Organism 2	H	Z	Sensitivity
C. dubia x *H. azteca*	1.77	1.42	*C. dubia* = *H. azteca*
D. similis x *C. dubia*	1.53	2.88	*C. dubia* > *D. similis*
D. similis x *H. azteca*	1.85	4.08	*H. azteca* > *D. similis*
D. similis x *D. rerio*	1.79	5.45	*D. similis* > *D. rerio*
C. dubia x *D. rerio*	1.71	15.70	*C. dubia* > *D. rerio*
H. azteca x *D. rerio*	2.00	22.25	*H. azteca* > *D. rerio*

C. dubia was found to be a more sensitive indicator than the alga *R. subcapitata* to wastewaters from both manufacturing plants (Table 6). Geis et al. (2000) cite a review of the US Toxic Substances Control Act databases, in which algae were found to be more sensitive than invertebrates and fish species in 50% of observations and less sensitive in 30%.

Table 6
Comparison of chronic toxicity indices of different test organisms to hair care products wastewaters (plants A and B)

Plant	Organism 1 x Organism 2	H	Z	Sensitivity
A	*C. dubia* x *R. subcapitata*	6.26	31.8	*C. dubia* > *R. subcapitata*
B	*C. dubia* x *R. subcapitata*	1.85	4.44	*C. dubia* > *R. subcapitata*

Controversies exist in the scientific literature regarding the choice of test organisms. Some authors argue that the use of sensitive species is more relevant than the use of native species, but there are suggestions that the use of standardized organisms is more important than the use of sensitive or native species (Rojiecková-Padrtová and Marsalek 1999). In general, conditions must be found that meet the highest

number of requirements in the choice of test organisms, considering data reliability for the greater objective of protecting aquatic life.

All species tested showed high sensitivity to the hair care products manufacturing wastewaters and use of the microcrustaceans *D. similis* and, or *C. dubia* can be recommended for routine analyses of acute and chronic toxicity, respectively, since the methodologies for maintenance and toxicity testing with these test organisms is relatively simple and well established in many ecotoxicology laboratories.

4 Conclusions

Raw wastewaters from two small hair care products manufacturing plants (A and B) presented similar characteristics, with high levels of organic matter, including oils and greases, and acute toxicity to *D. similis*, indicating the need for appropriate treatment prior to discharge, which should be based on techniques capable of reducing both organic matter and toxicity. Wastewater turbidity, oils and grease contents correlated strongly and with increased toxicity.

Test organism sensitivity to the wastewaters was compared and found to decrease in the following order for acute effects: *C. dubia* (cladoceran) = *H. azteca* (amphipod) > D. similis (cladoceran) > *D. rerio* (fish), while *C. dubia* was found more sensitive than the alga *R. subcapitata* for chronic effects. However, although all species tested showed high sensitivity to the wastewaters, with toxicity indices of < 1 to 3%. Use of *C. dubia* for routine monitoring of these industrial wastewaters is recommended since evaluation of the acute and chronic toxicity for this sensitive species will help guarantee the protection of other aquatic organisms.

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