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Performance of the freshwater shrimp *Atyaephyra desmarestii* as indicator of stress imposed by textile effluents

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Textile plants consume large volumes of water and produce a great amount of wastewaters, which can be important sources of toxic discharges in receiving environments. The objective of this study was to evaluate the acute toxicity of textile effluents on the freshwater shrimp *A. desmarestii.* A whole effluent toxicity test procedure was used to determine the aggregate toxicity of three samples taken before and after wastewater treatment in a textile mill. The following LC_{50} – 48 h values (%, v/v) were calculated: Untreated effluent – 29% effluent (sample 1), 22% effluent (sample 2), and 47% (sample 3); Treated effluent – 73% effluent (sample 1), 74% effluent (sample 2), and > 100% (sample 3). Based upon acute toxicity units ($TU_a = 100/LC_{50}$), untreated effluent varied from toxic in samples 1 and 3 ($2.00 \leq TU_a \leq 4.00$) to very toxic in sample 2 ($TU_a > 4.0$), whereas treated effluent varied from no toxic in sample 3 to moderately toxic in samples 1 and 2 ($1.33 \leq TU_a \leq 1.99$). Despite some limitations and constraints related to innate variability of industrial effluents, our results suggested that *A. desmarestii* can be a promising and potential test organism for assessing toxicity of complex chemical mixtures.

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Textile plants are important sources of toxic discharges in the receiving ecosystems. Their effluents may contain organic and inorganic substances that are potentially toxic to aquatic biota, and their identification involves expensive and complex analytical techniques (Gómez et al. 2001). In complex effluents, like those of textile industry, interactions among several pollutants might be synergistic, antagonistic, or simply additive. Consequently, complex mixtures can be considered as non-toxic when some substances are investigated individually but toxic when those mixtures are analysed. Many toxicological investigations involve the study of a single pollutant, which may give a clearer picture of toxicological effects than studies of complex chemical matrix such as textile effluents. However, in natural ecosystems, the biota is usually exposed to a complex and unknown combination of several pollutants (Abel 1998). Therefore, whole effluent toxicity (WET) tests can be more realistic than the study of individual pollutants (Juvonen et al. 2000, Mitchell et al. 2002, Araújo et al. 2005) and are recognized as practical and effective tools for the assessment of combined effects of toxic substances on aquatic ecosystems (Gómez et al. 2001). Though WET tests do not provide indication of the specific cause(s) of toxicity, they can contribute to address the overall effects that a mixture of pollutants might produce on the aquatic environment (EPA 2000, Nieto 2000, Rodriguez et al. 2006). In addition, despite some difficulties related to the interpretation of the results, WET tests can also contribute to protect aquatic biota from potentially detrimental effects imposed by effluent discharges.

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The freshwater shrimp *Atyaephyra desmarestii* was chosen as test organism because it is frequent in many Portuguese watercourses, including rivers, temporary streams, reservoirs, rice fields, and coastal lagoons (Fidalgo and Gerhardt 2003). Moreover, *A. desmarestii* represents an important link in the aquatic food webs (Descouturelle 1980, Fidalgo 1985) and its sensitivity to toxicants still has to be established. To our knowledge, apart from the studies by Abdennour et al. (2000) and Gerhardt et al. (2004), no toxicological studies have been conducted with *A. desmarestii*, what increases its use as a test organism. The use of local species adds the challenge of variability of a wild population, but results of toxicity tests provide a higher ecological relevance than the use of standard toxicity test species from laboratory cultures.

The objective of this study was to evaluate the acute toxicity of textile effluents on *A. desmarestii.* A WET test procedure was used to determine the aggregate toxicity of three different grab samples taken before and after wastewater treatment in a textile mill located in Northern Portugal.

Material and methods

The shrimps were exposed to various concentrations of the two tested effluents and mortality was used as a toxicological endpoint. The shrimps were collected from an area located upstream the reservoir of Crestuma-Lever (41°4′24′′N, 8°29′18′′W), which is ca 20 Km distance from the mouth of the Douro river, NW coast of Portugal. Animals were transported to the laboratory and acclimated during 7 d at least. We used an aerated aquaria supplied with unfiltered water from the shrimp sampling site (oxygen = 9.8 ± 2.2 mg O_2 l^{-1}; pH = 7.7 ± 0.1; conductivity = 268.3 ± 38.6 µS cm^{-1}; ammonia = 0.05 ± 0.05 mg N-NH_4 l^{-1}; total phosphorus = 0.6 ± 0.2 mg P_2O_5 l^{-1}; BOD_5 = 1.5 ± 0.5 mg O_2 l^{-1}; COD = 5.7 ± 2.3 mg O_2 l^{-1}; planktonic chlorophyll a = 8.1 ± 4.9 µg l^{-1}). During the acclimation period, animals were kept at controlled conditions (temperature: 20 ± 1°C; photoperiod: 12 h light/12 h dark) and fed twice a day with tetramin and mud from the shrimp sampling site.

Tested effluents were collected from the homogenization tank (untreated effluent) and the final storage tank (treated effluent) of the wastewater treatment plant of the textile factory on three different dates: sample 1 (12 May 2005), sample 2 (16 June 2005), and sample 3 (4 October 2005). Polyethylene containers previously rinsed with effluent at the collection site were used for taking the samples. The samples were then transported in ice containers to the laboratory and stored in dark at 4°C until processing. Physical and chemical analyses of effluent samples included the in situ measurement of water temperature, dissolved oxygen, pH, and conductivity by means of a Pocket Meter Multiline P4 (WTW, Germany). Determinations of biological oxygen demand (BOD_5) and chemical oxygen demand (COD) were performed in the laboratory according to APHA (1992).

Acute toxicity bioassays were performed in static conditions, i.e. test organisms and tested solutions were placed in the experimental aquaria and kept there during the test without water renewal (APHA 1992, NIWA 1998). The exposures were carried out in 2 l experimental aquaria containing 1.5 l of different concentrations of tested effluents. Unfiltered water from the shrimp sampling site was used as negative control as well as for preparation of dilutions. Triplicate bioassays were done for each treatment, with eight animals per replicate (total body length = 19.1 ± 2.4 mm). Following exploratory tests to determine approximate concentration range to be included in definitive tests, the shrimps were exposed for 48 h to five concentrations of untreated effluent (6.25–50%, v/v) and treated effluent (12.5–100%, v/v). During the tests, aeration was not provided, the animals were not fed, and dead animals were daily counted and removed. Temperature, pH, oxygen, and conductivity were monitored daily. Bioassays were only considered valid when the survival number of individuals in controls was higher than 90% (EPA 2002).

The concentration which is lethal to 50% of the test organisms in the time period prescribed by the test (LC_{50}–48 h values ± confidence limits) were determined using the Trimmed Spearman-Karber method developed by Hamilton (Software program version 1.5) (EPA 2002). Toxicity of samples was also expressed as acute toxicity units (TU_a=100/LC_{50}), which correspond to the reciprocal of the effluent dilution that causes an acute effect by the end of the acute exposure period (CETESB 1987).

Results

The results of the chemical analyses of tested effluents are given in Table 1. A clear improvement of wastewater quality was recorded in the final storage tank as compared to the homogenization tank as a result of the high efficiency of wastewater treatment (COD ≈ 87%, BOD_5 > 92%, TSS ≈ 59%). The samples of treated effluent met the permitted limits established by Portuguese regulations relative to textile effluents released into the freshwater environment (published in D.L. no. 423 of 25 June 1997: pH 5.5–9.0, BOD_5 100 mg O_2 l^{-1}, COD 250 mg O_2 l^{-1}, colour not visible at 1:40 dilution). Treated effluent was also in compliance with some emission limits established in Portuguese regulations concerning general wastewater discharges published in D.L. no. 236 of 1 August 1998: pH 6.0–9.0, BOD_5 40 mg O_2 l^{-1}, COD 150 mg O_2 l^{-1}, TSS 60 mg l^{-1}.

The results of water chemistry variables monitored in experimental aquaria during the exposure period are presented in Table 2. Concerning untreated effluent, mean values of pH were slightly alkaline with a minimum of 7.4 and a maximum of 7.9. In treated effluent samples, pH

Table 1. Basic characteristics of tested effluents (TSS – Total Suspended Solids; COD – Chemical Oxygen Demand; BOD_5 – Biochemical Oxygen Demand).

Parameter	Unit	Untreated Effluent Mean ± SD	Treated Effluent Mean ± SD
Temperature	°C	20.2 ± 2.3	19.2 ± 4.6
pH (25°C)	–	7.5 ± 0.7	7.3 ± 0.4
Conductivity	µS cm^{-1}	649 ± 235	1162 ± 139
Dissolved O_2	mg O_2 l^{-1}	2.62 ± 2.43	6.54 ± 2.93
TSS	mg l^{-1}	295 ± 461	12 ± 4
COD	mg O_2 l^{-1}	525 ± 314	68 ± 28
BOD_5	mg O_2 l^{-1}	183 ± 140	<15
Colour	mg l^{-1} Pt/Co scale	3352 ± 2653	Non visible 1:27 dilution ± 1:79
Detergents	mg l^{-1} lauril sulphate and sodium	45 ± 17	0.20 ± 0.18

values ranged between 7.4 and 8.1. The average values of the treated effluent for conductivity were always higher than those of the untreated effluent. The average values of dissolved oxygen were quite variable during exposure time. This parameter reached the minimum average value of 4.4 mg l^{-1} (untreated effluent sample 2 – very toxic) and the maximum value of 8.8 mg l^{-1} (treated effluent sample 3 – non-toxic).

The results of acute toxicity bioassays performed are presented in Table 3. Values of LC_{50}–48 h corresponding to untreated effluent reached 29% effluent (sample 1), 22% effluent (sample 2), and 47% (sample 3). The following results were obtained for the treated effluent: 73% effluent (sample 1), 74% effluent (sample 2), and >100% (sample 3).

Table 2. Water variables determined during the exposure period.

Effluent sample	Sample	LC_{50} Test (h)	pH mean ± SD	Temperature (°C) mean ± SD	Dissolved Oxygen (mg O_2 l^{-1}) mean ± SD	Conductivity (µS cm^{-1}) mean ± SD
		0	7.39 ± 0.12	20.7 ± 0.8	7.13 ± 1.12	294.5 ± 9.9
Untreated	1	24	7.56 ± 0.15	21.9 ± 0.9	5.10 ± 1.65	298.5 ± 9.7
		48	7.64 ± 0.23	22.0 ± 0.5	6.99 ± 0.61	306.2 ± 11.4
		0	7.77 ± 0.17	24.6 ± 0.3	8.62 ± 1.55	429.5 ± 89.7
	2	24	7.49 ± 0.12	25.9 ± 0.6	4.41 ± 1.33	439.5 ± 93.7
		48	7.70 ± 0.18	25.5 ± 0.1	5.04 ± 0.80	452.3 ± 92.5
		0	7.51 ± 0.12	17.3 ± 1.2	6.71 ± 1.20	358.0 ± 31.4
	3	24	7.80 ± 0.20	22.8 ± 0.2	6.55 ± 1.31	368.3 ± 34.1
		48	7.91 ± 0.17	22.7 ± 0.1	7.27 ± 0.76	379.0 ± 37.1
		0	7.45 ± 0.12	21.5 ± 0.2	8.34 ± 0.70	640.5 ± 320.1
Treated	1	24	7.79 ± 0.05	22.5 ± 0.1	6.92 ± 0.59	649.3 ± 325.5
		48	7.87 ± 0.07	22.2 ± 0.1	7.37 ± 0.76	658.8 ± 330.8
		0	7.99 ± 0.06	18.1 ± 0.1	7.23 ± 3.66	600.7 ± 272.0
	2	24	8.10 ± 0.18	26.2 ± 0.1	5.41 ± 1.06	602.0 ± 267.9
		48	7.99 ± 0.09	25.3 ± 0.3	5.38 ± 1.01	602.2 ± 258.7
		0	7.23 ± 0.20	18.2 ± 2.2	8.78 ± 0.24	724.0 ± 367.7
	3	24	8.12 ± 0.15	22.9 ± 0.1	7.81 ± 0.39	736.5 ± 372.7
		48	8.09 ± 0.04	22.7 ± 0.1	8.02 ± 0.59	752.2 ± 374.9

Table 3. Results of acute toxicity tests for untreated and treated textile effluents expressed as LC_{50}–48 h (%, v/v) and acute toxicity units (TU_a).

Effluent sample	Sample number	LC_{50}–48 h (95% confidence interval)	Acute toxic units (TU_a=100/LC_{50})	Effluent Classification (CETESB 1987)
Untreated	1	29% (26–32)	3.45	Toxic
	2	22% (20–25)	4.55	Very toxic
	3	47% (40–54)	2.13	Toxic
Treated	1	73% (30–180)	1.37	Moderately toxic
	2	74% (68–81)	1.35	Moderately toxic
	3	>100%	–	Non-toxic

Discussion

According to the classification adopted by CETESB (1987) for the characterization of toxicity samples, untreated effluent varied from toxic in samples 1 and 3 ($2.00 \le TU_a \le 4.00$) to very toxic in sample 2 ($TU_a > 4.0$). In turn, treated effluent varied from non-toxic in sample 3 to moderately toxic in samples 1 and 2 ($1.33 \le TU_a \le 1.99$). In other words, results of acute toxicity bioassays changed from date to date. This variability might reflect probable variations of pollution load as a function of the quantity and quality of processed products in the textile mill. In fact, the constituents and the concentrations might vary with the hour of the day, the day of the week, the month of the year, and other conditions such as fashion pressure.

We point out that the effluent quality of treated effluent is not violating the Portuguese discharge limits, but could show toxicity as revealed by acute toxicity bioassays on two sampling dates. The lack of correspondence between the information given by LC_{50} values and basic characteristics of treated effluent shows that Portuguese regulations based upon a set of frequently used chemical variables (pH, BOD_5, COD, and colour) is not enough to evaluate the potential harmful effects of textile discharges on the aquatic biota. For this reason, the existing receiving water discharge standards should be reviewed to include chemical monitoring of potential contaminants jointly with toxicity tests. This would be in agreement with several authors (Villegas-Navarro et al. 1999, Sponza 2002 a, b, 2006, Rodriguez et al. 2006) who emphasized the need of using a comprehensive approach by including bioassays for effluent monitoring in view of a more complete toxicological risk evaluation. Sponza (2006), in a study conducted to evaluate the toxicity of effluents of the treatment plants of the chemical dye production industry in Turkey, advocated the incorporation of toxicity tests into receiving water discharge standards in order to preserve receiving ecosystems. Similarly, Rodriguez et al. (2006), based upon their study carried in the Basque country, recommended the incorporation of biocriteria derived from WET tests in European water regulations as a contribution to the maintenance of the good ecological status of receiving rivers.

Testing effluents from the same textile plant that we studied, Andrade (2004) mentioned that treated effluent was not lethal to the adults of zebra fish *Danio rerio* (LC_{50} >100%), whereas *D. rerio* larvae showed several malformations when exposed to the same effluent. Therefore, based on our results and on those of Andrade (2004), we assume that *A. desmarestii* might be more sensitive than *D. rerio* adults. *A. desmarestii* was recently used for toxicological assessment of an acid mine drainage in Portugal based on behaviour and survival. In this study, Gerhardt et al. (2004) reported that *A. desmarestii* was more sensitive to acid mine drainage than the mosquito fish *Gambusia holbrooki*. Nevertheless, to evaluate *A. desmarestii* sensitivity, some reference toxicants such as potassium dichromate should be tested in future studies. Furthermore, the assessment of toxic effects resulting from textile discharges should include a multispecies toxicity test that include organisms belonging to different trophic levels to maintain the good ecological status of receiving ecosystems. In conclusion, *A. desmarestii* can be considered a promising and potential test organism to evaluate toxicity of complex chemical mixtures, because it is ecologically more relevant to predict the potential effects of contaminants based on native species instead of using only standard toxicity test species from laboratory cultures.

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