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Quantifying the contribution of dyes to the mutagenicity of waters under the influence of textile activities

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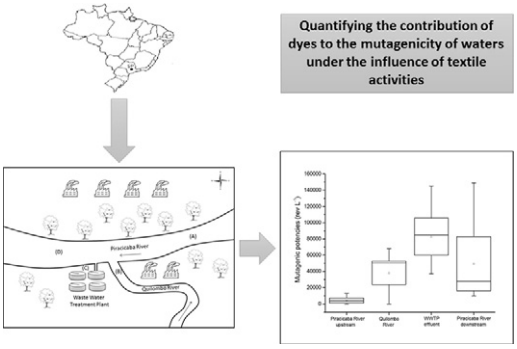
HIGHLIGHTS

- Six disperse dyes were detected in the tested environmental samples.
- Highest mutagenic potency was found in Piracicaba River downstream.
- Disperse dyes contributed up to 44% to the observed mutagenicity.
- Combination of chemical analysis and bioassays identified new priority pollutants.

GRAPHICAL ABSTRACT



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ABSTRACT

The combination of chemical analyses and bioassays allows the identification of potentially mutagenic compounds in different types of samples. Dyes can be considered as emergent contaminants and were detected in waters, under the influence of textile activities. The objective of this study was to evaluate the contribution of 9 azo dyes to the mutagenicity of representative environmental samples. Samples were collected along one year in the largest conglomerate of textile industries of Brazil. We analyzed water samples from an important water body, Piracicaba River, upstream and downstream two main discharges, the effluent of a wastewater treatment plant (WWTP) and the tributary Quilombo River, which receives untreated effluent from local industries. Samples were analyzed using a LC-MS/MS and tested for mutagenicity in the *Salmonella*/microsome microsuspension assay with TA98 and YG1041. Six dyes were detected in the collected samples, Disperse Blue 291, Disperse Blue 373, Disperse Orange 30, Disperse Red 1, Disperse Violet 93, and Disperse Yellow 3. The most sensitive condition for the detection of the mutagenicity was the strain YG1041 with S9. The concentration of dyes and mutagenicity levels varied along time and the dry season represented the worst condition. Disperse Blue 373 and Disperse Violet 93 were the major contributors to the mutagenicity. We conclude that dyes are contributing for the mutagenicity of Piracicaba River water; and both discharges, WWTP effluent and Quilombo River, increase the mutagenicity of Piracicaba River waters in about 10-fold. The combination of chemical analysis and bioassays were key in the identification the main drivers of the water mutagenicity and allows the selection

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of priority compounds to be included in monitoring programs as well for the enforcing actions required to protect the water quality for multiple uses.

1. Introduction

The combination of chemical analyses and bioassays, such as Effect Directed Analysis (EDA) allows the identification of mutagenic compounds in different types of samples (Brack, 2003). This approach is very interesting for water quality monitoring because the biological tools can be selected based on their ability to detect specific effects and their biological significance. This strategy can identify river basin priority pollutants that are not included in monitoring programs (Brack et al., 2016).

Bioassays, such as the *Salmonella*/microsome mutagenicity assay, produce an interesting response to a complex mixture evaluation without prior knowledge of the chemical sample composition (Claxton et al., 2004). Because the test can be performed with strains containing different mutation targets and metabolic capacities it allows the identification of several classes of mutagens which would not be identified by targeted chemical analysis (Umbuzeiro et al., 2011; Umbuzeiro et al., 2016). This assay is also considered an important bioanalytical tool and the responses can be linked to specific adverse outcome pathways when the ultimate goal is to protect the quality of the environment at the population level (Altenburger et al., 2015). Several studies have identified water contaminants when applied the combination of chemical analysis and the *Salmonella* assay (Gallampois et al., 2013; Liu et al., 2015; Muz et al., 2017; Umbuzeiro et al., 2005b).

The most used organic dyes for textiles contain an azo group in their structure (Bafana et al., 2011) and several are genotoxic and mutagenic in mammalian and bacterial tests (Chequer et al., 2009; Josephy et al., 2016; Oliveira et al., 2010; Rajaguru et al., 1999; Tsuboy et al., 2007; Umbuzeiro et al., 2005a). Recently, azo dyes were identified as predominant brominated compounds in house dust and also exhibit mutagenic responses at environmentally relevant concentrations (Peng et al., 2016). Waters containing textile discharges can exhibit genotoxic and mutagenic activity that has been related to the presence of certain dyes and aromatic amines (Oliveira et al., 2007; Umbuzeiro et al., 2005b). However, in the case study of the Cristais River, mutagenic dyes were detected but not quantified and it was not possible to know their contribution in the mutagenicity of the river water (Umbuzeiro et al., 2005b). Considering this, the objective of this study was to identify selected azo dyes in environmental samples following textile discharges and to verify their contribution to the mutagenicity of those samples.

2. Materials and methods

2.1. Study area and sampling

The biggest pole of textile industries of Brazil is located in Americana city, São Paulo state. Piracicaba River, one of the most important rivers in São Paulo, is the main receiving water body for the liquid effluents generated by the industries. At the same time the river quality is protected by law and must be preserved for multiple uses, including aquatic life protection and human consumption. A wastewater treatment plant (WWTP) is responsible for the collection and treatment of several of the industrial effluents from the textile pole. It uses a biological treatment and the final effluent is discharged into the Piracicaba River. Unfortunately, this type of biological treatment alone is not efficient for the removal of disperse dyes (USEPA, 1990), so it is possible that dyes would remain in the final effluent. Furthermore, the capacity of the WWTP is not sufficient to treat all industrial effluents generated in the area, and several textile factories discharge their effluents, without proper treatment, directly to a tributary of Piracicaba River, called Quilombo. Four sampling campaigns were performed in April, June, August and October of 2013. Samples were collected from the WWTP outflow, Quilombo River and Piracicaba River, upstream and downstream the discharges (Fig. 1). Samples (4 l) were collected using amber glass flasks, transported to the laboratory on ice and immediately processed (APHA, 1999).

2.2. Liquid–liquid extraction/concentration procedures

Water samples were liquid-liquid extracted using dichloromethane (DCM) and methanol (2.5:1) as already adopted in other related textile studies (Umbuzeiro et al., 2004). Extracts were rotary evaporated and completely dried with purified nitrogen gas. Extracts were carefully kept frozen and stored in amber vials. For the mutagenicity tests the solvent was exchanged. Adequate volumes of dimethyl sulfoxide (DMSO) were added to the extracts previous diluted in DCM and then DCM was completely evaporated using purified nitrogen gas.

2.3. HPLC-MS/MS analysis

Chemical analyses were performed on the same extracts tested for mutagenicity. Analysis were conducted in a High Performance Liquid Chromatography (HPLC) Agilent 1200 system (Waldbronn, Germany) coupled to an AB Sciex 3200 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer (MS). Extracts were completely dried using purified nitrogen gas and re-suspended in methanol:water (50:50, v/v) containing 0.1% formic acid. Chromatographic separation was performed in Kinetex PFP analytical column (150 mm × 4.6 mm; 5 μm, Phenomenex). As mobile phase water (A) and acetonitrile (B), spiked with 0.1% formic acid, were used at a flow rate of 1.5 mL min^{-1} and a gradient program for water/acetonitrile: 0–1 min, 5% B; 1–5.5 min, 5–9% B; 5.5–6.5 min, 9–25% B; 6.5–9.5 min, 25–40% B; 9.5–11.5 min, 40–45.5% B; 11.5–16.5 min, 45.5–60.5% B; 16.5–18.5 min, 60.5–100% B, 18.5–23 min, 100% B and re-established by 5% B over 7 min. Column temperature was set to 40 °C, injection volume was 20 μL, and total run length was 30 min. The 3200 QTRAP was coupled to the chromatographic apparatus via an electrospray ionization (ESI) source operating in positive ion mode with specific parameters: spray voltage, 5500 V; capillary temperature, 650 °C; the nebulizing gas (nitrogen, 45 psi); the heating gas (nitrogen, 45 psi) and the curtain gas, 15 psi. Selected reaction monitoring (SRM) mode, with two SRM transitions to eliminate false results were used in the identification of the compounds of interest. Fragmentation parameters were optimized by direct infusion of individual compounds solutions at 0.1 mg L^{-1} in methanol/water (50:50, v/v) containing 0.1% formic acid, using a flow of 10 μL min^{-1}. In this step, the following parameters were analyzed: Collision Energy (CE), Declustering Potential (DP), Entrance Potential (EP), Cell Entrance Potential (CEP) and Collision Cell Exit (CXP). All properties and parameters of each compound analyzed are summarized in Table 1 and chromatograms are available at Supplementary Material.

The instrumental limit of detection (LOD) and limit of quantification (LOQ) were defined as the minimum amount of the selected compound analyzed by LC-MS/MS considering the signal-to-noise (S/N) ratio of 3 and a S/N of 10, respectively. The compounds were identified by their retention times and their specific SRM transitions. The validation protocol was adapted based on criteria accepted by different institutions (APHA, 1999; USEPA, 1997). The adaptation of different validation guides was