Investigating the sources of the mutagenic activity found in a river using the *Salmonella* assay and different water extraction procedures

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Abstract

In the routine São Paulo state (Brazil) surface water quality-monitoring program, which includes the *Salmonella* microsome mutagenicity assay as one of its parameters, a river where water is taken and treated for drinking water purposes has repeatedly shown mutagenic activity. A textile dyeing facility employing azo-type dyes was the only identifiable source of mutagenic compounds. We extracted the river and drinking water samples with XAD4 at neutral and acidic pH and with blue rayon, which selectively adsorbs polycyclic compounds. We tested the industrial effluent, raw, and treated water and sediment samples with YG1041 and YG1042 and compared the results with the TA98 and TA100 strains. The elevated mutagenicity detected with YG-strains suggested that nitroaromatics and/or aromatic amines were causing the mutagenicity detected in the samples analyzed. Positive responses for the blue rayon extracts indicated that mutagenic polycyclic compounds were present in the water samples analyzed. The mutagen or mixture of mutagens present in the effluent and water samples cause mainly frameshift mutations and are positive with and without metabolic activation. The *Salmonella* assay combined with different extraction procedures proved to be very useful in the identification of the origin of the pollution and in the identification of the classes of chemical compounds causing the mutagenic activity in the river analyzed.

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1. Introduction

The *Salmonella* mutagenicity assay has been a very sensitive and suitable tool for the detection of mutagens in surface waters (Galassi et al., 1992; Grifoll et al., 1992; Valent et al., 1993; Rehana et al., 1995; White and Rasmussen, 1998; Kataoka et al., 2000; Watanabe et al., 2002; Ohe et al., 2003), and drinking waters (Ringhand et al., 1987; Meier, 1988; Schenck et al., 1998; Monarca et al., 2002) throughout the world. Although this methodology alone cannot identify the contaminants, the total mutagenicity, expressed in revertants per liter (rev/l) can be an indicator of the levels of the genotoxic activity present in those waters. In addition, the response of *Salmonella* strains that are sensitive to different
chemical classes can help in the identification of the class of genotoxicants present in those waters.

Besides being able to help identify the presence and type of mutagens, the assay has facilitated other key observations. White and Rasmussen (1998) studied the mutagenicity of the St. Lawrence River in Montreal and compared it with the genotoxicity found in other rivers in Asia and Europe. They concluded that human activities and river water mutagenicity were strongly related. In Austria, the heterocyclic amines IQ, Trp-P-1 and AzC accounted for 26% of the mutagenicity observed when blue rayon was used to extract primarily polycyclic compounds from the samples (Kataoka et al., 2000). Watanabe et al. (2002) found that 87% of the Japanese river samples analyzed were mutagenic, and 29% showed potencies greater than 500,000 rev/g of blue rayon for YG1024 in the presence of S9. They demonstrated that the novel benzotriazoles (PBTAs), derived from sewage where discharges containing azo dyes are treated, accounted for at least 50% of the mutagenicity. Valent et al. (1993), found that 14% of the rivers sampled in Sao Paulo State, Brazil were positive in Salmonella strain TA98 with potencies ranging from 39 to 3650 rev/l.

Starting in 1998, Sao Paulo state, Brazil, officially included the Salmonella assay in the State Surface Water Quality Monitoring Program at sites where water is taken and treated for drinking water purposes (Umbuzeiro et al., 2001). The water is extracted with XAD4 resin at neutral and acidic pH; the extracts are mixed and tested for mutagenicity with the TA98 and TA100 strains of Salmonella with and without metabolic activation. The surveillance showed that the majority of the samples analyzed bimonthly from the 28 sites (~120 samples/year) showed negative results (CETESB, 2003), indicating a very good quality for Sao Paulo State rivers, at least in relation to this parameter.

Cristais River, located in the Sao Paulo metropolitan region, was an exception, because it showed repeated mutagenic activity. The monitored site showed potencies that ranged from $10^2$ to $10^3$ rev/l for the Salmonella strain TA98 with and without metabolic activation. These values are considered low to moderate according to Umbuzeiro’s river water classification. In this classification, waters with 0–500 rev/l are considered with low mutagenic activity; from 500 to 2500, moderate; from 2500 to 5000, high and with values greater than 5000, extreme (Umbuzeiro et al., 2001).

We conducted this investigation in order to find the sources of the genotoxic contamination observed in Cristais River. Effluents collected from the two industries found upstream from the monitored site were analyzed for mutagenicity along with the corresponding river and drinking water samples. Sediment samples were tested to provide a preliminary indication of the extent of the aquatic contamination.

2. Materials and methods

2.1. Sample collection

We collected industrial effluents, surface and treated water, and sediment in the Cristais area (site 2, Fig. 1) as previously described (APHA, 1998). The Cristais river water is treated for drinking water purposes using a conventional technique that includes prechlorination, flocculation, coagulation and flotation. We also collected raw and treated water samples from the Guarapiranga Reservoir (site 1, Fig. 1), in this work referred to as the control site, because the raw water presented negative results for the Salmonella assay in the same monitoring program; and the corresponding drinking water mutagenicity has been routinely evaluated in another surveillance program.

2.2. Concentration/extraction procedures

2.2.1. Effluent samples

We tested effluents from the only two industries (A and B), located upstream from the river collection site (Fig. 1). The raw and treated effluents were collected as four 6 h composite samples within a 24 h period to obtain a representative sampling of the industrial discharge. Volumes of 1 l were extracted using the liquid/liquid method with a mixture of methanol (Sigma) and methylene chloride (Sigma) as solvents in a proportion of 1:2:5 (Dutka et al., 1981). The extracts were reduced to 2–3 ml using a rotary evaporator, transferred to small vials, evaporated to dryness with a gentle stream of nitrogen just before testing, and then resuspended in dimethylsulfoxide (DMSO, Sigma). The doses tested were 20, 10, 5, 1, and 0.5 ml equivalents per plate.

2.2.2. River and drinking water samples

Volumes of 100 l of raw river and treated drinking water samples were each serially extracted with Amberlite XAD4 (Sigma) (Pellizari et al., 1984) at neutral pH (N) and after acidifying the sample with HCl to pH 2 (A). We used 1 ml of resin per liter of river water and 0.5 ml of resin per liter of drinking water. The elution was performed with 100 ml of methanol and 400 ml of methylene chloride for the neutral pH extract, and with 100 ml of methanol and 400 ml of ethylacetate (Carlo-Erba) for the acidic extract. In the monitoring program described above, the neutral and acidic extracts were tested mixed; in this study, they were tested separately. When the extraction is performed at neutral pH (usually the natural pH of drinking water), compounds like the disinfection by-products MX, chloral hydrate, and halogenated acids will be charged and consequently not adsorbed to the XAD resin, therefore it is necessary to prior acidify the water samples to allow their recovery. Drinking water samples containing mostly halogenated disinfection by-
products usually present negative responses when extracted at neutral pH, only exhibiting mutagenic activity when the extraction is performed at pH 2 (Meier, 1988). We used the same extraction procedure to extract the raw and drinking water samples from the control site. For both river and drinking water samples, the doses tested were 1500, 1000, 500, and 250 ml equivalents per plate.

To verify the possible presence of polycyclic compounds we used blue rayon (Funakoshi Co., Ltd.) to extract the water samples. Compounds with three or more than three rings are preferentially adsorbed by those fibers, although a few two rings compounds can give recoveries greater than 50% (Hayatsu, 1992). For the river water, we used the in situ hanging technique with 15 g of blue rayon for 24 h (Sakamoto and Hayatsu, 1990). For treated water, we employed the blue rayon compacted inside a glass column, as previously described (Kummrow, 2001). The elution was done with methanol in both cases. The doses tested were 0.5, 0.25, 0.1, 0.025 and 0.01 g-equiv of blue rayon per plate. For the river water, we expressed the results in rev/g of blue rayon and rev/l.

2.2.3. Sediment sample

We collected one sediment sample at site 2, ~6 km downstream from the industrial discharges, where the water is taken and treated for drinking water purposes. After drying the sample in the dark at 45 °C, aliquots of 30 g were extracted with methylene chloride and methanol using the ultrasonication method (Grifoll et al., 1990). The eluate was reduced to 2–3 ml using an evaporator, transferred to a small vial, evaporated to dryness with a gentle stream of nitrogen just before testing, and resuspended in DMSO (Sigma). The doses were 100, 50, 25, and 12.5 mg-equiv of sediment per plate.

2.3. Salmonella mutagenicity assay

We employed the standard plate incorporation Salmonella mutagenicity assay (Maron and Ames, 1983; Mortelmans and Zeiger, 2000) with the same strains used in the surface water-monitoring program, TA98 and TA100 with and without S9. The strains were kindly supplied by Dr. B.N. Ames. The S9 mix was freshly prepared before each test using lyophilized Aroclor-1254-induced rat liver S9 fraction (Moltox—Molecular Toxicology Inc., Boone, NC, USA).

In order to determine if arylamines were responsible for the observed mutagenicity, we tested the samples with YG1041 and YG1042 derivatives of the TA98 and TA100 respectively. These strains are more sensitive to
nitroaromatic and aromatic amines than the parental strains. The assays were performed using triplicate plates. Positive controls were 4-nitroquinoline-1-oxide (Sigma) at 0.125 μg per plate, 2-nitrofluorene (Sigma) at 5 μg per plate, without metabolic activation, and 2-aminoanthracene (Sigma) with S9 at 0.03–0.625 μg per plate. We analyzed the results with the Salanal computer program, using the Bernstein model (Bernstein et al., 1982), and potencies were expressed in rev/l-equiv, rev/g of blue rayon or rev/per g-equiv, depending on the sample/extraction procedure.

3. Results and discussion

3.1. Identification of the pollution sources

Upon our investigation of the possible sources of the repeated genotoxic contamination detected in Cristais river (see Table 1), we found two industries, named A and B, ~6 km upstream of the monitored river site (site 2, Fig. 1) discharging treated liquid wastes into the river. The industrial effluents, both before and after treatment, were tested for mutagenicity with strains TA98 and TA100 with and without S9 to determine if they were contributing to the river genotoxicity.

Industry A is a galvanizer facility that chemically treats its effluent. No mutagenic activity was detected for either raw or treated effluent for both TA98 and TA100 strains with and without S9 (Table 2). In addition, the effluent complied with the Brazilian legislation that regulates metals, phenols, pH, BOD, and COD among other parameters (Brazil, 1986).

Industry B is a dye processing plant that dyes polyester and nylon fabrics, and it uses mostly disperse and acid azo-type dyes in its process. Although the final effluent complied with Brazilian legislation (Brazil, 1986), the raw and the treated effluent were mutagenic for TA98 with and without S9 and for TA100 with S9 (Table 2). The treated effluent showed values for TA98 with S9 (1.1 · 10^5 and 3.0 · 10^5 rev/l) considered high according to Houk’s effluent classification (Houk, 1992).

Several other studies had shown that textile-processing plants produce mutagenic effluent (Sanchez et al., 1988; Coelho et al., 1992; Houk, 1992; Claxton, 1997). Comparing the river and the effluent discharge flow rates which are 540 and 15 m^3/h respectively with the levels of mutagenic activity of the industrial discharge and the river samples (Table 2), we observe that the industrial effluent is sufficiently potent to contribute to the genotoxic activity found in the river.

Analyzing the mutagenicity results of the raw and treated effluent of industry B (Table 2), we observe that although the process of treatment (which is an activated sludge system) retained a lot of mutagenic activity; the final effluent was still highly genotoxic. Considering that several azo dyes are mutagenic (Venturini and Tamaro, 1979; Reid et al., 1984; Joachim et al., 1985) and recalcitrant to activated sludge treatment (Pagga and Brown, 1986; Churchley, 1994), the observed mutagenicity could be related to the presence of azo dyes in the final effluent.

Because several azo dyes as well as their breakdown products are arylamines, this class of compound could be responsible for the mutagenicity of the textile dyeing facility that uses azo-type dyes. In order to examine this hypothesis, we tested the raw and treated effluent samples with the nitroreductase and acetyltransferase over-producing strains YG1041 and YG1042, which are highly sensitive to nitroaromatic compounds and aromatic amines. The extracts showed mutagenic activity, both in the presence and absence of S9 for all the strains analyzed, except for the raw effluent for TA100 without S9 (Table 2). The higher responses for those strains compared to their parental strains would indicate that arylamines were causing the mutagenicity of those samples. We can observe that YG1041 potencies are 5–17 times greater than TA98 responses. Although the effluent samples were less mutagenic for TA100, an increase in the YG1042 response was also observed.

Table 1
Results of the repeated mutagenic activity in Salmonella assay found in Cristais river waters during the São Paulo State Water Quality Monitoring Program

<table>
<thead>
<tr>
<th>Sampling dates (month/year)</th>
<th>Number of rev/l for the combined water extracts (neutral and acidic) with TA98 strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−S9</td>
</tr>
<tr>
<td>10/98</td>
<td>240</td>
</tr>
<tr>
<td>01/99</td>
<td>nd</td>
</tr>
<tr>
<td>03/99</td>
<td>nd</td>
</tr>
<tr>
<td>05/99</td>
<td>nd</td>
</tr>
<tr>
<td>07/99</td>
<td>290</td>
</tr>
<tr>
<td>09/99</td>
<td>500</td>
</tr>
<tr>
<td>11/99</td>
<td>820</td>
</tr>
<tr>
<td>01/00</td>
<td>210</td>
</tr>
<tr>
<td>03/00</td>
<td>160</td>
</tr>
<tr>
<td>05/00</td>
<td>210</td>
</tr>
<tr>
<td>07/00</td>
<td>400</td>
</tr>
<tr>
<td>07/00</td>
<td>580</td>
</tr>
<tr>
<td>09/00</td>
<td>164</td>
</tr>
<tr>
<td>09/00</td>
<td>98</td>
</tr>
<tr>
<td>11/00</td>
<td>370</td>
</tr>
<tr>
<td>01/01</td>
<td>nd</td>
</tr>
<tr>
<td>03/01</td>
<td>1600</td>
</tr>
<tr>
<td>05/01</td>
<td>380</td>
</tr>
</tbody>
</table>

nd = no mutagenicity was detected.


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Therefore, we can conclude that direct-acting mutagenic nitroaromatic compounds and/or aromatic amines as well as other indirect-acting compounds, which cause primarily frameshift mutations but also base pair substitution, are likely present in the effluent samples analyzed, and consequently released into the river waters.

3.2. River and drinking water samples analysis

Arylamines can be highly mutagenic or even carcinogenic (IARC, 1975), and some studies have related mutagenic activity in rivers to the presence of aromatic amines (Shiozawa et al., 1999; Ono et al., 2000; Nukaya et al., 2001). Germany bans the use of several azo dyes, which upon cleavage generate some selected aromatic amines considered carcinogens (European Community, 1999). Recent documentation shows that several countries regulate aromatic amines (e.g., aniline, benzidine, m-chloroaniline, 3,3-dichlorobenzidine, and nitrosamines) in surface and drinking waters (United Nations University, 2003).

Azo dyes, when discharged into the river, can remain in the water compartment (Garrison and Hill, 1972; Maguire and Tkacz, 1991) or can be sorbed by the bottom sediment and/or bioconcentrated. Under the anoxic conditions of the sediment, the azo bond can be reduced and the related aromatic amines released into the water column (Weber and Adams, 1995).

Considering that these contaminants could be affecting the quality of the drinking water produced by the water treatment plant located ~6 km downstream of the industrial discharge, river water close to the water treatment plant intake (site 2) and treated water were analyzed for mutagenicity.

To study the mutagenic activity of the raw river and treated water we tested the XAD4 extracts obtained at neutral pH and acidic pH separately with TA98, TA100, YG1041, and YG1042 strains. The mutagenicity results are presented in Table 2. They are also summarized in Fig. 2a.

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample</th>
<th>TA98 +S9</th>
<th>YG1041 +S9</th>
<th>TA100 +S9</th>
<th>YG1042 +S9</th>
</tr>
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<tbody>
<tr>
<td>Galvanizer industry effluent</td>
<td>Raw #1</td>
<td>nda</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>Treated #1</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Dye plant effluent</td>
<td>Raw #1</td>
<td>17 000</td>
<td>340 000</td>
<td>nd</td>
<td>83 000</td>
</tr>
<tr>
<td></td>
<td>Treated #1</td>
<td>34 000</td>
<td>110 000</td>
<td>nd</td>
<td>12 000</td>
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<tr>
<td></td>
<td>Raw #2</td>
<td>23 000</td>
<td>160 000</td>
<td>1 000 000</td>
<td>50 000 000</td>
</tr>
<tr>
<td></td>
<td>Treated #2</td>
<td>100 000</td>
<td>300 000</td>
<td>1 000 000</td>
<td>5 000 000</td>
</tr>
<tr>
<td>Cristais river water</td>
<td>Raw/XAD4/N</td>
<td>77</td>
<td>140</td>
<td>3600</td>
<td>2700</td>
</tr>
<tr>
<td>(site 2)</td>
<td>Raw/XAD4/A</td>
<td>87</td>
<td>67</td>
<td>2100</td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td>Treated/XAD4/N</td>
<td>130</td>
<td>44</td>
<td>2500</td>
<td>1300</td>
</tr>
<tr>
<td></td>
<td>Treated/XAD4/A</td>
<td>150</td>
<td>76</td>
<td>1900</td>
<td>820</td>
</tr>
<tr>
<td></td>
<td>Raw/BR (rev/g)</td>
<td>56</td>
<td>250</td>
<td>18 000</td>
<td>37 000</td>
</tr>
<tr>
<td></td>
<td>Treated/BR (rev/g)</td>
<td>430</td>
<td>320</td>
<td>20 000</td>
<td>11 000</td>
</tr>
<tr>
<td>Cristais river</td>
<td>Sediment</td>
<td>nd</td>
<td>480</td>
<td>2900</td>
<td>51 000</td>
</tr>
<tr>
<td>sediment (site 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control reservoir</td>
<td>Raw/XAD4/N</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>water (site 1)</td>
<td>Raw/XAD4/A</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>Treated/XAD4/N</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>Treated/XAD4/A</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>Raw/BR (rev/g)</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>Treated/BR (rev/g)</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

Samples are raw water samples, treated water samples or sediment samples. When used concentration procedures were either with XAD4 or blue rayon (BR). Please note that the BR results are given as rev/g and the sediment results as rev/g equivalent. The XAD4 extracts consisted of a neutral (N) and acidic (A) fraction. See the text for a full explanation.

\(^a\) Samples are raw water samples, treated water samples or sediment samples. When used concentration procedures were either with XAD4 or blue rayon (BR). Please note that the BR results are given as rev/g and the sediment results as rev/g equivalent. The XAD4 extracts consisted of a neutral (N) and acidic (A) fraction. See the text for a full explanation.

\(^b\) nd = no mutagenicity was detected.

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it seems that nitroaromatic compounds and/or aromatic amines are present in the samples tested, considering the results of the YG-strains.

The above observations become more evident when we compare the results obtained for Cristais River with the results of the Guarapiranga Reservoir, considered in this study as a control site (Fig. 2a and b). The raw water tested did not present mutagenic activity, as expected, since this site is repeatedly negative for the Salmonella assay (CETESB, 2003). Drinking water from the Guarapiranga reservoir showed mutagenic activity only in the acidic extract, for TA100 with and without S9 (Table 2, Fig. 2b). Results for YG1041 were also negative for both raw and treated water and for drinking water; YG1042 detected the same mutagenicity as its parental strain TA100 (Table 2). These mutagenicity results are the ones expected when halogenated disinfection by-products are present (Ringhand et al., 1987; Meier, 1988; Schenck et al., 1998; Monarca et al., 2002). It is important to point out that two additional samples of drinking water from Cristais river were tested (data not shown), and the mutagenicity results were very similar to the ones obtained in this study.

Azo dyes are polycyclic compounds, containing two or more rings. Under anoxic conditions, the azo bond can be reduced, and other polycyclic compounds can be produced (Weber and Adams, 1995; Shiozawa et al., 1999; Nukaya et al., 2001). Additionally, several of those dyes and related aromatic amines can have one or more nitrogroups; an example is the CI disperse blue 79 that is a dinitrobrumozobenzene dye. To corroborate that mutagenic polycyclic compounds were present in the river and in the drinking water, we extracted the samples using blue rayon fibers that selectively adsorb polycyclic compounds. We tested them with the strains TA98, TA100, YG1041, and YG1042. The results presented in Table 2 show that raw and treated river water samples contain direct-acting mutagenic polycyclic nitroaromatic compounds and/or polycyclic aromatic amines. Also indirect-acting compounds of the same class were detected in the raw water samples (Table 2). Samples of raw and treated water from the control site were also analyzed using the blue rayon method, and the extracts showed no mutagenic activity. This indicated that mutagenic polycyclic compounds were not present in detectable amounts under the testing conditions in the control site.

Blue rayon mutagenicity potencies expressed in rev/g of blue rayon (Table 2) for raw and treated water collected in Cristais River were very similar. However, this comparison needs to be made with caution, because the methodology used to extract the raw water was the blue rayon hanging technique and treated water was extracted with blue rayon packed in columns.

In the case of the drinking water samples, we are able to compare the results obtained with XAD4 and blue rayon because both extractions were performed in columns and both results can be expressed in rev/l (Table 3). XAD4 extracts presented higher mutagenicity than that of the blue rayon, indicating that only part of the drinking water mutagenicity could be explained by the presence of polycyclic compounds.

The results presented above suggest that the drinking water from Cristais River contains, besides the expected mutagenic halogenated disinfection by-products, other direct-acting mutagenic compounds that do not seem to be present in the control site. At least part of those compounds has polycyclic structure and belongs to the class of the nitroaromatics and/or aromatic amines. In order to elucidate which compounds are causing this mutagenicity, chemical analysis should be performed to identify the most mutagenic ones and properly regulate them in the aquatic environment. Due to the chemical characteristics of the dyes and their related cleavage products, the analysis should be performed preferably with an HPLC/MS. Besides that, a detailed study of the azo dyes used in the processing plant will be required to define representative chemical standards.

We did a preliminary chemical characterization of the treated effluent, river and drinking water samples from the Cristais River area, using a GC/MS ion trap (Varian Saturn, 2000). We detected 2,6-dichloro-4-nitroaniline (CAS No. 99-30-9), 2-methyl-mercaptoaniline (CAS No. 2987-53-3) in the liquid/liquid extract of the final industrial effluent, and in the XAD4 neutral and acidic pH extracts of raw and treated water and 2,4,5-

<table>
<thead>
<tr>
<th>Strain/metabolization</th>
<th>XAD4 (sum of neutral and acidic extracts)</th>
<th>Blue rayon rev/l</th>
<th>% of mutagenic activity that could account for polycyclic compounds extracted using the blue rayon</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA98 – S9</td>
<td>280</td>
<td>46</td>
<td>16%</td>
</tr>
<tr>
<td>TA98 + S9</td>
<td>120</td>
<td>34</td>
<td>28%</td>
</tr>
<tr>
<td>YG1041 – S9</td>
<td>4400</td>
<td>2150</td>
<td>49%</td>
</tr>
<tr>
<td>YG1041 + S9</td>
<td>2120</td>
<td>1150</td>
<td>54%</td>
</tr>
</tbody>
</table>

Table 3
Comparison of the mutagenic activity of drinking water, extracted with XAD4 and blue rayon, and the contribution of the polycyclic compounds to the total mutagenicity.
trimethylaniline (CAS No. 137-17-7) in the liquid/liquid extract of the treated effluent and in the neutral pH extract of the raw and treated water. In addition, chloral hydrate (CAS No. 302-17-0), 2,2-dichloroacetyl chloride (CAS No. 79-36-7), 1,5-hexadiene, 1,1,2,5,6,6-hexachloro (CAS No. 98141-62-9) were detected, but only in the acidic extract of the drinking water sample, suggesting that chlorinated disinfection by-products were formed. Although preliminary, the results corroborate our previous findings.

3.3. River sediment analysis

The Cristais River sediment sample showed positive results for TA98 only with S9 (480 rev/g-equiv), and for YG1041 with and without S9 (51 000 and 2900 rev/g-equiv respectively) (Table 2). These results suggest that indirect-acting frameshift mutagens from the class of the arylamines are causing the observed mutagenicity. This mutagenic pattern is different from the one observed for the water samples, where mainly direct-acting mutagenic compounds were detected (Fig. 2a).

3.4. Final considerations

From the mutagenicity data and the preliminary chemical analysis we can conclude that the azo dye processing plant discharge was contributing to the mutagenicity found in raw and treated water from a region of the Cristais River and azo-type dyes and/or their related cleavage products appear to be related to that contamination.

There are ways to reduce the mutagenic activity of this type of industrial discharge. Treatment techniques that combine anaerobic with aerobic treatment (Cruz and Buitron, 2001; Ekici et al., 2001) or ozonation (Gahr et al., 1994) have been showing effective results in the treatment of azo dye-containing effluents. Pollution prevention techniques could also be applied based on current efforts in the design and use of non-mutagenic dyes (Freeman et al., 1990).

The sediment of the Cristais River at the site where the water is taken for treatment is contaminated with mutagenic compounds, and even after the reduction of the mutagenic discharges in this area, it can be an important source of water contamination. In the future, the sediment from Cristais River should be monitored and remediated, if necessary. Mutagenicity evaluation and chemical characterization of the sludge produced by the industry and by the water treatment plant would be advantageous, because the samples might contain considerable amounts of mutagenic compounds.

The Salmonella/mammalian microsome assay combined with different extraction procedures and the use of additional strains proved to be very effective in the identification of the origin of the pollution as well as in the indication of the class of compounds that were causing the observed mutagenicity.

Although the levels of the mutagenic activity found in Cristais River are much lower than those ones observed in some rivers from other countries, consideration should be given to reducing the level of mutagens, because pollutants seem to be enhancing the levels of mutagenicity found for chlorinated treated water produced with water from this source.

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