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Comparison of the mutagenic activity of XAD4 and blue rayon extracts of surface water and related drinking water samples

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Abstract

The combination of mutagenicity tests and selective extraction methodologies can be useful to indicate the possible classes of genotoxic organic contaminants in water samples. Treated and source water samples from two sites were analyzed: a river under the influence of an azo dye-processing plant discharge and a reservoir not directly impacted with industrial discharges, but contaminated with untreated domestic sewage. Organic extraction was performed in columns packed with XAD4 resin, that adsorbs a broad class of mutagenic compounds like polycyclic aromatic hydrocarbons (PAHs), arylamines, nitrocompounds, quinolines, antraquinones, etc., including the halogenated disinfection by-products; and with blue rayon that selectively adsorbs polycyclic planar structures. The organic extracts were tested for mutagenicity with the Salmonella assay using TA98 and TA100 strains and the potencies were compared. A protocol for cleaning the blue rayon fibers was developed and the efficiency of the reused fibers was analyzed with spiked samples. For the river water samples under the influence of the azo-type dye-processing plant, the mutagenicity was much higher for both blue rayon and XAD4 extracts when compared to the water from the reservoir not directly impacted with industrial discharges. For the drinking water samples, although both sites showed mutagenic responses with XAD4, only samples from the site under the influence of the industrial discharge showed mutagenic activity with the blue rayon extraction, suggesting the presence of polycyclic compounds in those samples. As expected, negative results were found with the blue rayon extracts of the drinking water collected from the reservoir not contaminated with industrial discharges. In this case, it appears that using the blue rayon to extract drinking water samples and comparing the results with the XAD resin extracts we were able to distinguish the mutagenicity caused by industrial contaminants from the halogenated disinfection by-products generated during water treatment.

Keywords: River water; Drinking water; Blue rayon; XAD; Mutagenicity; *Salmonella* assay

1. Introduction

Many studies have shown the presence of different organic mutagenic and carcinogenic compounds in drinking water, and epidemiological studies have highlighted some cancer hazard in populations using

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treated drinking water [1]. These genotoxic substances are derived from industrial, agricultural and urban pollution, and also from the disinfection treatment used for drinking water production, particularly when water is obtained from surface sources and then chlorinated [2]. Chlorination has long been used as a simple, effective and economic method for disinfection of water for drinking water purposes, but genotoxic products can be formed by the interaction of chlorine and organic components, such as fulvic and humic acids, which are naturally present in raw waters [3].

The main genotoxic disinfection by-products as well as pesticides, phenols, polycyclic aromatic hydrocarbons (PAHs), metals are regulated in drinking water in several countries [4,5] but other mutagenic compounds from the classes of the nitrosoamines, antraquinones, quinolines, substituted PAHs, mono and polycyclic aromatic amines and many others are not, and they can also be present in drinking waters. From those classes of compounds only aminopyridine, aniline, azobenzene, benzidine, chloro-*o*-toluidine, dichlorobemzidine and *N*,nitroso-dimethylamine are regulated in two states of Canada [5]. The detection and chemical identification of the genotoxic compounds present in drinking water is considered an essential step to predict the effects of the consumption of this complex mixture on human health [6]. The *Salmonella* assay can be a useful tool in the quantification of the genotoxic activity of those complex mixtures and the different responses of the several *Salmonella* strains can help in the identification of the classes of genotoxicants present in those samples [7,8].

The water samples must be concentrated, and proper extraction of the mutagenic compounds is required for both the chemical analysis and *Salmonella* test.

Different strategies can be used to extract organic genotoxicants from water samples and the Amberlite XAD resins have been the most applied method [3,9–11]. The XAD-resins allow the recovery of a wide range of chemicals and are very efficient to extract all the polar and the apolar chemicals potentially effective in toxicity and genotoxicity assays [12–14]. The blue rayon, more recently developed [15], is a group of fibers of rayon covalently linked to a copper phthalocyanine that selectively adsorbs substances with planar polycyclic molecular structure present in water samples [14–16].

The XAD resins have been widely used to concentrate genotoxicants present in surface waters and positive results were observed when those extracts were analyzed in different short-term tests [17–22]. The blue rayon hanging (using in situ) method has also been used to concentrate mutagenic polycyclic compounds from freshwater and seawater [23,24]. This method was employed to concentrate a new class of mutagens in river waters, the 2-phenylbenzotriazoles (PBTAs). Those compounds are generated by cyclization of the azo bond of some dyes in a reductive environment after chlorination [25]. They showed to be responsible for at least 50% of the mutagenic activity of several Japanese rivers [6,25–27]. Ohe et al. [28], using the same hanging technique, analyzed rivers from the US and Canada, and some presented positive results for the *Salmonella* assay, although with lower potencies than the ones observed in Japan.

To drinking waters, the mutagenicity is usually related to the presence of the halogenated disinfection by-products like MX, chloral hydrate and halogenated acids which are mutagenic for the *Salmonella* assay. They are efficiently extracted by the XAD resins but only when the sample is acidified to pH 2 [10,11,29–31]. Because halogenated disinfection by-products are usually aliphatic compounds, they are not expected to be adsorbed by the copper phtalocyanine, and that could explain the negative results for mutagenicity obtained by some authors [32] when drinking water samples were extracted with blue rayon. With this concentration method, only when mutagenic polycyclic compounds are present in the sample we expect positive responses for the *Salmonella* test.

The comparison of the results obtained by both methods, XAD and blue rayon, is difficult to be performed because the blue rayon extraction method was developed as an in situ procedure and the results can only be expressed per gram of fibers and not per volume of water concentrated.

The objectives of this study were: (1) to establish the conditions to use the blue rayon packed in columns to be able to compare the results with XAD4 extraction; (2) to define a suitable procedure to clean and reuse the blue rayon fibers for surface and chlorinated drinking water extraction; (3) to compare the raw and drinking water mutagenicity obtained using both concentration methods of two

different sites contaminated with different types of discharges; and (4) to analyze drinking water with the blue rayon end XAD to verify whether it is possible to distinguish the mutagenicity of industrial contaminants from the regular halogenated disinfection by-products.

2. Materials and methods

2.1. Sampling

We selected two sampling sites in the metropolitan region of São Paulo, SP, Brazil where water is routinely tested for mutagenicity using the *Salmonella* assay in the São Paulo State Surface Water Quality Monitoring Program, performed by the São Paulo Environmental Protection Agency (CETESB), in which the *Salmonella*/microsome assay was officially included in 1998 [22]. These raw waters are treated for drinking water purposes using a conventional treatment process that includes flocculation, filtration, and chlorination.

2.1.1. Site 1

Site 1 is a river under the influence of discharges of an azo-type dye-processing plant. Water samples from this site usually presents mutagenic activity when extracted with XAD4 and in is presumed to be contaminated with azo dyes that are compounds with polycyclic structures.

2.1.2. Site 2

Site 2 is a water reservoir not directly affected by industrial discharges, although contaminated with untreated domestic sewage. XAD4 extracts from the water samples collected in this site present usually negative results for the *Salmonella* assay.

Volumes of 120 l of raw and treated water were collected as described [33]. Volumes of 60 l per sample were extracted using columns of 2.5 cm diameter packed with XAD4 (Sigma) or blue rayon (Sigma). Sample collections were performed twice at each site. Sampling 1 was performed in November, 2000 and sampling 2 in April and May, 2001. The levels of free chlorine of the treated water samples were measured just after the sample collection and ranged from 1.8–2.5 ppm.

2.2. Washing procedures of blue rayon

According to Hayatsu [15], blue rayon may be reused, and for that purpose a washing procedure was standardized and optimized in this study. Ultra-pure water was poured over blue rayon in a beaker and stirred for 5 min. The procedure was repeated four times. The excess of water was removed with a paper towel. The blue rayon was immersed in a methanol (Merck)/ammonia (Merck) (50:1 v/v) solution and agitated for 1 h, twice. Blue rayon was left to settle in the methanol/ammonia solution overnight and washed with methanol for 1 h, stirring it occasionally. The methanol was removed and reduced to 2–3 ml using a rotary evaporator transferred to a small vial and evaporated to dryness with a gentle stream of nitrogen. Dimethylsulfoxide (DMSO; Sigma) was added, and the *Salmonella* mutagenicity assay was performed to verify the effectiveness of the washing procedure in the removal of mutagenic contaminants.

This washing procedure was efficient for the purpose of this work, and 93% of the blue rayon fibers washed once showed negative results for the *Salmonella* test. When necessary a second washing procedure was performed, and this additional washing was 100% effective to clean blue rayon. A blue rayon batch was ready for use only when the *Salmonella* assay showed negative results.

2.3. XAD4 extraction method

The water samples and the spiked sample were serially extracted at natural pH (N) and acidic pH (H) by adding HCl (Merck) until pH 2. The XAD4 resin, is a copolymer of styrene divinyl benzene, and was washed before use [32,34]. For raw water we used 1 ml resin/liter and for treated water, 0.5 ml resin/liter, both at a flow rate of 100 ml/min. For the natural pH extraction the elution was performed using 1 ml of methanol and 4 ml of methylene chloride per milliliter of resin. For the acidic extraction, 1 ml of methanol and 4 ml of ethyl acetate were used. In both cases the elution speed was 2 ml/min. Eluates were reduced to 2–3 ml using an evaporator, transferred to small vials, evaporated to dryness with a gentle stream of nitrogen and resuspended in DMSO before testing. When we extract drinking water samples at their natural pH, which is usually between 6–9.5, non-polar compounds

are preferentially adsorbed. Disinfection by-products like MX, chloral hydrate and halogenated acids due to their higher polarity, are preferentially adsorbed to the XAD resin when the pH of the sample is 2 [31]. Drinking waters containing mostly disinfection by-products are negative when extracted at neutral pH, exhibiting their mutagenic activity only when the extraction is performed at pH 2 [3].

2.4. Blue rayon extraction method

The blue rayon extraction was performed only at the natural pH of the sample, because the adsorption capacity of the blue rayon is not related to the polarity of the molecules but to their planar and polycyclic structure [15].

Blue rayon was packed carefully in a glass column [35], and the water flow rate was 50 ml/min. We used 0.5 g blue rayon per liter of raw water and 0.25 g of blue rayon per liter of treated water.

The elution was performed with methanol/ammonia solution (50:1, v/v) [15,16]. For the elution, the blue rayon was carefully withdrawn from the columns and transferred to a beaker to be washed with ultra-pure water in order to remove the non-adsorbed components and suspended solids. The blue rayon was then dried with a paper towel, transferred to a flask containing methanol/ammonia (50:1, v/v) [15,16], and then agitated. All eluates were reduced to 2–3 ml using an evaporator, transferred to small vials, evaporated to dryness with a gentle stream of nitrogen just before testing, and then resuspended in DMSO.

2.5. Performance of the reused blue rayon using spiked samples

In order to analyze the ability of the blue rayon to adsorb mutagenic compounds after being used several times with different types of samples, we compared the mutagenic response of a sample spiked with 2-aminoantracene, known to be adsorbed by those fibers, due to its planar polycyclic structure. That sample was extracted using new and reused blue rayon and also with XAD4. Volumes of 5 l of ultra-pure water were spiked with 100 μg of 2-aminoantracene and extracted as regular samples. The recovered mutagenic activity of 2-aminoanthracene was analyzed using the TA98 strain of *Salmonella* with metabolic activation.

2.6. Salmonella mammalian microsome mutagenicity assay

The *Salmonella* assay was conducted according to Maron and Ames [36], using TA98 and TA100 strains with and without S9 mix containing 4% (v/v) lyophilized aroclor-1254-induced rat liver S9 fraction (Moltox Inc.) and cofactors. The environmental samples were tested at four to six different doses ranging from 50 to 1500 ml equivalents of water per plate. The assay were performed using triplicate plates. DMSO was used as negative control. Positive controls were 4-nitroquinoline-1-oxide without metabolic activation, and 2-aminoanthracene with S9, at concentrations of 0.5 and 2.5 μg per plate, respectively. The background was carefully evaluated for toxicity using a stereomicroscope. We analyzed the results performing an ANOVA test followed by a linear regression analysis with the Salanal computer program applying the Bernstein model [37] and the potencies were expressed in revertants per liter equivalent of water. A sample was considered positive according the criteria describe by Valent [21].

3. Results and discussion

3.1. Performance of the blue rayon compared to XAD4 using spiked samples

The results demonstrated that both new and reused blue rayon showed similar results in the recovery of the genotoxic activity of 2-aminoanthracene (2-aminoanthracene) for strain TA98 with S9 when compared to XAD4 (Fig. 1). However, when the spiked water was extracted with blue rayon that was used previously to extract only treated water (BR3), a relative lower recovery of 2-aminoanthracene was observed (Fig. 1). According to Hayatsu [15], the free chlorine present in treated water could attack the copper phthalocyanine trisulphonate of the blue rayon, which can be seen by the loss of its color, reducing its lifetime. In these conditions, we can suggest that blue rayon used four times to extract drinking water samples should be further evaluated or discarded because of the apparent chlorine attack. Blue rayon used only to extract non-chlorinated water samples can be reused more times. The XAD4 acid extract

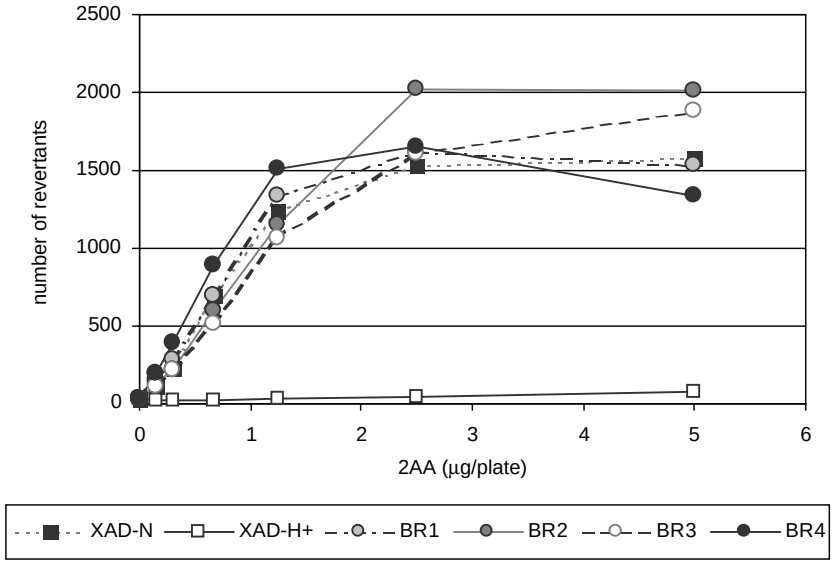

Fig. 1. Comparison of the mutagenic activity expressed in number of revertens per plate for TA98 with S9 with different doses of the extracts of water samples spiked with 2-aminoanthracene (2AA) and extracted using new and reused blue rayon and XAD4 at natural pH (N) and acidic pH (H). BR1: used for both raw and treated water samples (more than 10 times); BR2: used only for raw water samples (seven times); BR3: used only for treated water samples (five times), BR4: new blue rayon.

showed low mutagenic activity as expected, because the spiked water was first concentrated at neutral pH, where the 2-aminoanthracene would not be charged, therefore almost completely adsorbed.

3.2. Mutagenicity results

The results obtained in this study are presented in Table 1. The summarized results can be seen in Table 2 and Fig. 2. In general, the extracts obtained with blue rayon gave lower or negative results when compared to the results obtained with the XAD4 resin. This can be explained due to its selectivity to polycyclic compounds.

Raw water samples collected from the river under the influence of an azo dye-processing plant (site 1), showed genotoxic activity mainly with TA98 with and without S9 both with XAD4 and blue rayon. Those results are expected considering that azo dyes are polycyclic compounds, and several can exhibit mutagenic activity in the absence of S9 and in the presence of S9 [38–41]. Genotoxic activity of the treated water samples from this site was detected with strains TA98 and TA100 with and without S9 in the natural and acidic pH XAD4 extracts, but only with TA98 with and without S9 in the blue rayon extracts. Those data suggest the presence of mutagenic compounds with polycyclic planar characteristics in addition to the disinfection by-products (DBP) in the drinking water samples from site 1. The majority of the disinfection by-products are direct-acting mutagens that are extracted mostly at acidic pH and detected with TA98 without S9 and TA100 with and without S9 [10,11,29,42–44], these are not expected to be adsorbed by blue rayon fibers [32].

Raw water samples collected from site 2 presented only low genotoxic activity for TA98 with and without S9 for the first sampling, and negative results for the second sampling, for both extraction methods. Although the results of the raw samples of that reservoir are usually negative for *Salmonella* mammalian microsome assay, this low mutagenicity observed could be explained by the presence of mutagenic heterocyclic amines derived from untreated sludge discharges [20,24], or other polycyclic compounds adsorbed by blue rayon and XAD4.

As expected, drinking water samples from this site showed genotoxic activity in only the acidic pH XAD4

Table 1
Mean of the revertants per plate for each dose tested with TA98 and TA100 strains with and without S9 mix for the raw and treated waters extracted with XAD4 and blue rayon

Milliliter equiv/plate	Blue rayon				XAD4							
	TA98		TA100		Natural pH (N)				Acidic pH (H)			
	−S9	+S9	−S9	+S9	TA98		TA100		TA98		TA100	
					−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Site 1, sampling 1												
Raw water												
0	22	21	70	91	22	27	147	109	27	27	147	109
67.5	35	29	67	75	–	–	–	–	–	–	–	–
125	33	29	67	101	–	–	–	–	–	–	–	–
250	40	–	73	88	37	52	130	149	61	69	124	137
500	53	–	79	89	31	65	161	151	84	100	110	167
1000	–	–	–	–	41	99	152	174	93	124	113	188
1250	54	70	114	82	–	–	–	–	–	–	–	–
1500	–	–	–	–	46	106	197	266	92	108	119	141
Treated water												
0	27	27	147	109	25	28	147	109	27	27	105	104
250	32	28	131	133	53	47	–	–	–	–	134	113
500	33	37	129	131	68	45	–	–	–	–	184	138
1000	46	38	129	137	84	57	135	119	154	78	220	145
1500	49	45	131	123	91	67	131	130	193	123	315	177
Site 1, sampling 2												
Raw water												
0	33	27	111	109	20	21	102	95	20	21	78	104
50	–	27	–	–	–	–	–	–	–	–	–	–
100	–	33	–	–	–	–	–	–	–	–	–	–
250	44	61	113	111	62	74	108	118	50	57	80	120
500	59	88	116	108	85	149	109	125	86	73	91	106
1000	69	199	113	123	94	152	113	127	101	96	87	106
1500	98	–	111	132	146	281	107	135	141	108	104	116
Treated water												
0	23	28	78	104	20	21	102	95	20	21	102	95
250	30	27	80	80	39	27	125	120	59	36	154	123
500	24	33	82	82	44	36	120	134	80	48	177	144
1000	–	43	79	78	64	59	122	162	102	77	226	161
1500	57	84	79	86	93	49	138	184	81	99	281	158
Site 2, sampling 1												
Raw water												
0	25	28	121	109	18	17	99	90	21	20	107	108
250	25	30	140	121	26	18	110	114	29	30	119	117
500	26	36	99	89	17	23	89	100	36	29	108	118
1000	33	51	81	90	19	20	119	118	47	32	105	99
1500	38	42	94	89	21	27	111	121	51	40	106	114
Treated water												
0	15	17	99	90	38	29	120	98	21	29	120	98
250	15	14	114	119	39	23	93	80	44	27	156	108
500	16	16	97	101	44	18	83	83	65	34	241	119
1000	17	19	100	105	43	20	81	88	97	37	258	120
1500	15	20	101	102	35	19	96	102	89	36	281	132

Table 1 (*Continued*)

Milliliter equiv/plate	Blue rayon				XAD4							
	TA98		TA100		Natural pH (N)				Acidic pH (H)			
	−S9	+S9	−S9	+S9	TA98		TA100		TA98		TA100	
					−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Site 2, sampling 2												
Raw water												
0	23	27	116	119	20	21	116	119	23	27	116	119
250	22	24	128	123	17	21	142	133	20	28	145	131
500	25	27	127	130	19	27	126	131	30	32	129	118
1000	24	28	111	125	18	19	116	106	25	25	126	132
1500	28	29	111	122	25	24	120	116	25	36	134	121
Treated water												
0	27	24	93	88	23	28	93	88	23	28	93	119
250	27	23	99	89	32	–	104	101	39	29	182	146
500	35	34	101	86	28	29	95	97	49	28	178	139
1000	34	37	96	108	24	28	93	94	49	31	171	154
1500	32	30	104	118	23	28	95	90	52	28	342	162

extracts without S9, suggesting the presence of the halogenated disinfection by-products. The negative results obtained for the blue rayon extracts corroborate the findings of other authors, that blue cotton is not efficient to adsorb those classes of compounds [32]. It seems that in the first sampling the treatment removed or reduced the mutagenicity of the raw water present at levels not detected in our experiments.

Table 2
Summarized results obtained for the raw and treated water samples with XAD4 and blue rayon extraction methods using the *Salmonella* mammalian microsome mutagenicity assay with TA98 and TA100 strains

Site and sampling	Strain	Potency in revertants per liter							
		Raw water				Treated water			
		XAD4			Blue rayon	XAD4			Blue rayon
		N	H	Total		N	H	Total	
Site 1, sampling 1	TA98 − S9	16	118	134	64	100	117	217	15
	TA98 + S9	74	151	225	40	26	58	84	12
	TA100 − S9	–	–	–	–	–	130	130	–
	TA100 + S9	87	89	176	–	–	–	–	–
Site 1, sampling 2	TA98 − S9	142	127	269	41	47	94	141	21
	TA98 + S9	238	114	352	128	24	54	78	30
	TA100 − S9	–	–	–	–	–	122	122	–
	TA100 + S9	–	–	–	–	61	48	109	–
Site 2, sampling 1	TA98 − S9	–	22	22	10	–	81	81	–
	TA98 + S9	–	13	13	12	–	–	–	–
	TA100 − S9	–	–	–	–	–	150	150	–
	TA100 + S9	–	–	–	–	–	–	–	–
Site 2, sampling 2	TA98 − S9	–	–	–	–	–	29	29	–
	TA98 + S9	–	–	–	–	–	–	–	–
	TA100 − S9	–	–	–	–	–	125	125	–
	TA100 + S9	–	–	–	–	–	–	–	–

(–): Mutagenic activity not detected; N: natural pH extract; H: acidic pH extract.

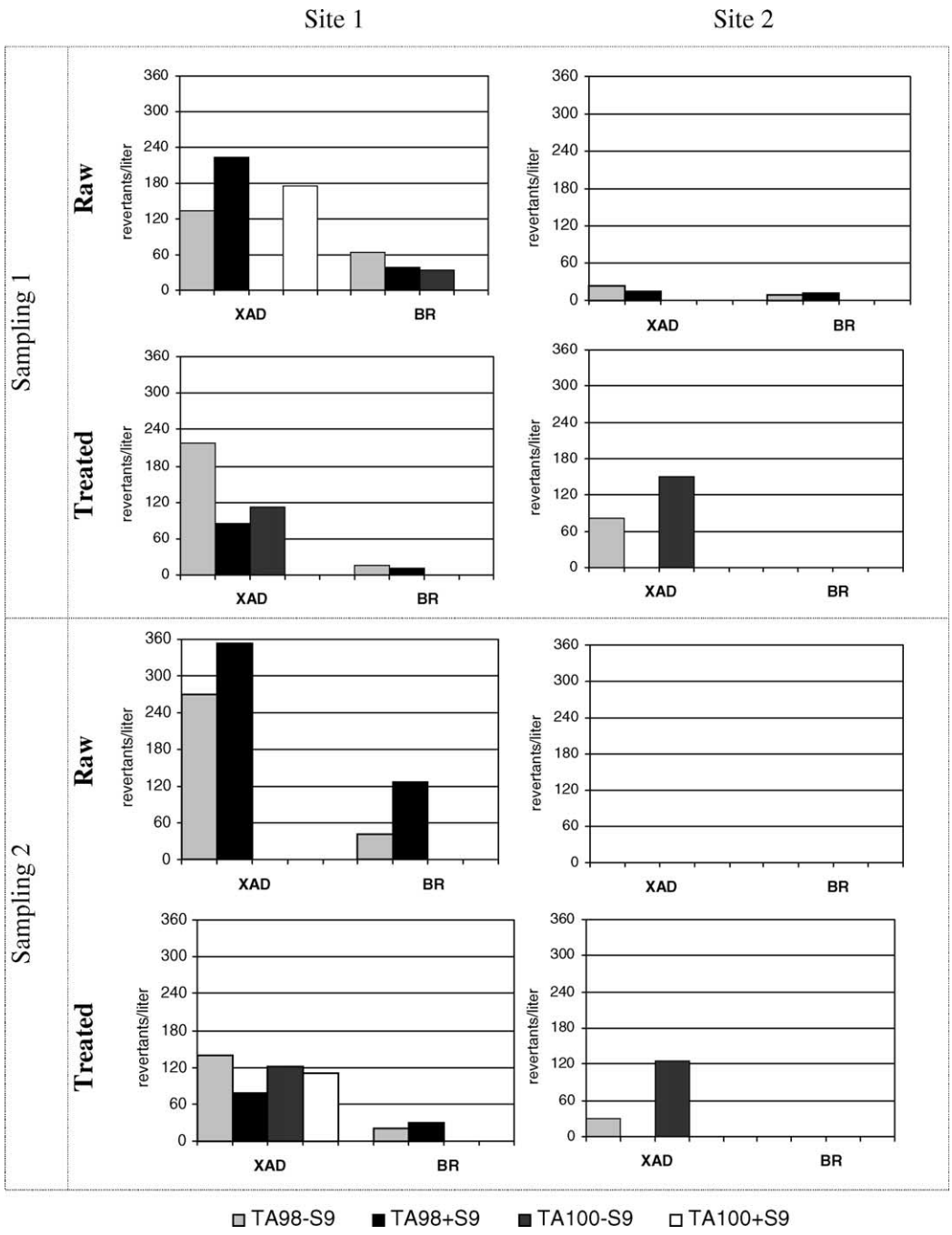

Fig. 2. Comparison of the total mutagenicity in number of revertants per liter of raw and treated water obtained using both blue rayon and XAD4 methods using TA 98 and TA100 with and without S9, from the two sites analyzed. Site 1 is a river under influence of an azo dye-processing plant and site 2 is a reservoir that receives untreated domestic sewage, not directly impacted with industrial discharges.

According to Table 2, comparing the potencies of the samples collected at site 1, the mutagenic activity of raw and treated water samples were always higher for the XAD4 extracts than for the blue rayon extracts, indicating the presence of other genotoxic compounds other than the polycyclic ones. In that case, mono-aromatic amines or mono-aromatic nitro amines could be responsible for this activity because those compounds are expected to be present in surface waters contaminated with dye-related compounds [45].

4. Conclusions

Using the blue rayon method in columns described in the present work, we were able to compare the results with the ones obtained with XAD resin method. The efficiency of the blue rayon to recover the mutagenicity of 2-aminoantracene was similar to the XAD resin. This was also observed for the recovery of PAHs extracted by XAD resin and blue rayon [15,34]. Although blue rayon fibers can be reused, it is important to test them for mutagenicity before each use in order to avoid false-positive responses. When blue rayon is used to extract drinking water samples, its lifetime can be reduced.

The XAD4 extraction procedure seems to be more adequate to access the total river and drinking water mutagenicity in agreement with several authors [13,14], although the complementary extraction with blue rayon indicated the presence of mutagenic polycyclic planar compounds in river and drinking water samples collected in the site under the influence of the discharges azo-type dye-processing plant. Compounds such as azo dyes, polycyclic aromatic amines, and PBTAs could account for the observed mutagenicity. In order to better characterize the classes of genotoxic compounds present in those samples, we suggest further investigation with the YG strains of *Salmonella*, which are more sensitive to arylamines [46,47], combined with the *Salmonella* assay in micropreincubation [48], which is more sensitive than the standard plate incorporation protocol used in this work. Chemical identification of those extracts could confirm the results obtained in this work.

References

[1] M. Koivusalo, J.J.K. Jaakkola, T. Vartiainen, T. Hakulinen, S. Karjalainen, E. Pukkala, J. Tuomisto, Drinking water mutagenicity and gastrointestinal and urinary tract cancers: an ecological study in Finland, Am. J. Public Health 84 (1994) 1223–1228.
[2] S. Monarca, A. Zanardini, D. Feretti, A. Dalmiglio, E. Falistocco, P. Manica, G. Nardi, Mutagenicity of extracts of lake drinking water treated with different disinfectants in bacterial and plant tests, Water Res. 32 (1998) 2689–2695.
[3] J.R. Meier, R.B. Knohl, W.E. Coleman, H.P. Ringhand, J.W. Munch, W.H. Kaylor, R.P. Streicher, F.C. Kopfler, Studies on the potent bacterial mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2 (5*H*)-furanone: aqueous stability, XAD recovery and analytical determination in drinking water and in chlorinated humic acid solutions, Mutat. Res. 189 (1987) 363–373.
[4] US Environmental Protection Agency, Office of Water (1997). Manual for the Certification of Laboratories Analyzing Drinking Water. Criteria and Procedures, 45268. EPA 815 B-97-001, March 1997, Cincinnati, OH.
[5] United Nations University 2003. http://www.lnweh.uni.edu/biology447/modules/modules1/1g-t3.htm. Accessed on 01/04/2003. United Nations.
[6] A. Oguri, T. Shiozawa, Y. Terao, H. Nukaya, J. Yamashita, T. Ohe, H. Sawanishi, T. Katsuhara, T. Sugimura, K. Wakabayashi, Identification of a 2-phenylbenzotriazole (PBTA)-type mutagen, PTBA-2, in water from the Nishitakase river in Kyoto, Chem. Res. Toxicol. 11 (1998) 1195–1200.
[7] M. Grifoll, A.M. Solanas, J.M. Bayona, Bioassay-directed chemical characteristics of genotoxic agents in the dissolved particulate water phases of the Besos and Llobregat rivers (Barcelona, Spain), Arch. Environ. Contam. Toxicol. 23 (1992) 19–25.
[8] M. Grifoll, A.M. Solanas, J.M. Bayona, Characterization of genotoxic components in sediments by mass spectrometric techniques combined with *Salmonella*/microsome test, Arch. Environ. Contam. Toxicol. 19 (1990) 175–184.
[9] I. Tolosa, J.W. Readmam, L.D. Mee, Comparison of the performance of solid-phase extraction techniques in recovering organophosphorus and organochlorine compounds from water, J. Chromatgr. 725 (1996) 93–106.
[10] J.C. Loper, Mutagenic effects of organic compounds in drinking water, Mutat. Res. 78 (1980) 241–246.
[11] T. Vartiainen, A. Liimatainen, High levels of mutagenic activity of chlorinated drinking water in Finland, Mutat. Res. 169 (1986) 29–34.
[12] W. Sloof, D. De Zwart, J.F.J. Van de Kerkhoff, Monitoring the rivers Rhine and Meuse in the Netherlands for toxicity, Aquat. Toxicol. 4 (1983) 189–198.
[13] L. Guzzella, S. Sora, Mutagenic activity of lake water samples used as drinking water resources in northern Italy, Water. Res. 32 (1998) 1733–1742.
[14] H. Sakamoto, T. Ohe, T. Hayatsu, H. Hayatsu, Evaluation of blue-chitin column, blue-rayon hanging, and XAD–resin column techniques for concentrating mutagens from two Japanese rivers, Mutat. Res. 371 (1996) 79–85.

[15] H. Hayatsu, Cellulose bearing covalently linked copper phthalocyanine trisulphonate as an adsorbent selective for polycyclic compounds and its use in studies of environmental mutagens and carcinogens, J. Chromatgr. 597 (1992) 37–56.
[16] H. Sakamoto, H. Hayatsu, A simple method for monitoring mutagenicity of river water. Mutagens in Yodo river system, Kyoto, Osaka, Bull. Environ. Contam. Toxicol. 44 (1990) 521–528.
[17] Z. Rehana, A.A.M. Malik, Mutagenic activity of the Ganges water with special reference to the pesticides pollution in the river between Kachla to Kannauj (U.P.), India, Mutat. Res. 343 (1995) 137–144.
[18] N. Kinae, J. Tanaka, N. Kamio, C. Sugiyama, M. Shimoi, K. Tanji, Detection and origin of 3-chloro-4-dichloromethyl-5-hydroxy-2(5*H*)furanone (MX) in river water, Water Sci. Technol. 30 (2000) 117–123.
[19] S. Galassi, L. Guzzella, M. Mingazzini, L. Vigano, S. Capri, S. Sora, Toxicological and chemical characterization of organic micropollutants in river Po water (Italy), Water. Res. 26 (1992) 19–27.
[20] P.A. White, K.T. Ho, T. Ohe, D.M. Demarini, C. Blaise, A mass balance of surface water genotoxicity in the providence river (Rhode Island, USA), Environ Mol. Mutagen. 33 (1999) 68.
[21] G.U. Valent, M.I.Z. Sato, M.C.L.S. Coelho, C.A. Coimbrão, P.S. Sanchez, M.T. Martins, R. Bonatelli Jr., Monitoring São Paulo state rivers in Brazil for mutagenic activity using Ames test, Environ. Toxicol. Water Qual. 8 (1993) 371–381.
[22] G.A. Umbuzeiro, D.A. Roubicek, M.I.Z. Sato, P.S. Sanchez, The *Salmonella* mutagenicity assay in a surface water quality monitoring program based on a 20-year survey, Mutat. Res. 491 (2001) 119–126.
[23] S. Kira, K. Taketa, Y. Nogami, H. Hayatsu, A simple technique for monitoring mutagenicity and Benzo(a)pireno content in mussels and their ambient water, Environ. Toxicol. Water Qual. 10 (1995) 167–172.
[24] H. Kataoka, T. Hayatsu, G. Hietsch, H. Steinkellner, S. Nishioka, S. Narimatsu, H. Hayatsu, Identification of heterocyclic amines (IQ, Trp-P1 and A alfa C) in the water of Danube river, Mutat. Res. 466 (2000) 27–35.
[25] H. Nukaya, J. Yamashita, K. Tsuji, Y. Terao, T. Ohe, T. Sawanishi, T. Katsuhara, T. Kiyokawa, M. Tezuca, A. Oguri, T. Watanabe, T. Sugimura, K. Wakabayashi, Isolation and chemical-structural determination of a novel aromatic amine mutagen in water for the Nishitakase river in Kioto, Chem. Res. Toxicol. 11 (1997) 1061–1066.
[26] H. Nukaya, T. Shiozawa, A. Tada, Y. Terao, T. Ohe, T. Watanabe, M. Asanoma, H. Sawanishi, T. Katsuhara, T. Sugimura, K. Wakabayashi, Identification of 2-[2-(acetyl-amino)-4-amino-5-methoxifenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole (PBTA-4) as a potent mutagen in river water in Kyoto and Aichi prefectures, Mutat. Res. 492 (2001) 73–80.
[27] T. Watanabe, Y. Takahashi, T. Takahashi, H. Nukaya, Y. Terao, T. Hirayama, K. Wakabayashi, Seasonal fluctuation of the mutagenicity of river water in Fukui, Japan, and the contribution of 2-phenylbenzotriazole-type mutagens, Mutat. Res. 519 (2002) 187–197.
[28] T. Ohe, P.A. White, D.M. DeMarini, Mutagenic characteristics of river waters flowing though large metropolitan areas in North America, Mutat. Res. 547 (2002) 1–12.
[29] J.R. Meier, Genotoxic activity of organic chemicals in drinking water, Mutat. Res. 196 (1988) 211–245.
[30] E.R. Nestmann, G.L. leBel, D.T. Williams, D.J. Kowbel, Mutagenicity of organic extracts from Canadian drinking water in the *Salmonella*/mammalian-microsome assay, Environ. Mutagen. 1 (1979) 337–345.
[31] H.P. Ringhand, J.R. Meier, F.C. Kopler, K.M. Schenk, W.H. Taylor, D.E. Mitchell, Importance of sample pH on recovery of mutagenicity from drinking water by XAD resins, Environ. Sci. Technol. 21 (1987) 382–387.
[32] T. Vartiainen, A. Liimatainen, S. Jääskelainen, P. Kauranen, Comparison of solvent extractions and resin adsorption for isolation of mutagenic compounds from chlorinated drinking water with high humus content, Water Res. 21 (1987) 773–779.
[33] APHA Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, DC, 1998.
[34] G.A. Junk, J.J. Richard, M.D. Grieser, D. Witiak, J.L. Witiak, M.D. Arguello, R. Vick, H.J. Svec, J.S. Fritz, G.V. Calder, Use of macroreticular resin in the analysis of water for trace organic contaminants, J. Chromatogr. 99 (1974) 745–762.
[35] T. Ohe, N. Takeuchi, T. Watanabe, A. Tada, H. Nukaia, Y. Terao, H. Sawanishi, T. Hirayama, T. Sugimura, K. Wakabayashi, Quantification of two aromatic amine mutagens, PBTA-1 and PBTA-2, in the Yodo river system, Environ. Health Perspect. 107 (1999) 701–704.
[36] D.N. Maron, B.N. Ames, Revised methods for the *Salmonella* mutagenicity test, Mutat. Res. 113 (1983) 173–215.
[37] L. Bernstein, J. Kaldor, j. McCann, M.C. Pike, Na empirical approach to the statistical analysis of mutagenesis data from the *Salmonella* test, Mutat. Res. 97 (1982) 97–267.
[38] G. Krishna, J. Xu, J. Nath, Comparative mutagenicity studies of azo dyes and their reduction products in *Salmonella typhimurium*, J. Toxicol. Environ. Health 18 (1986) 111–119.
[39] T.M. Reid, K. C Morton, C.Y. Wang, C.M. King, Mutagenicity of azo dyes following metabolism of different reductive and oxidative systems, Environ. Mutagen. 6 (1984) 705–717.
[40] F. Joachim, A. Burrell, J. Andersen, Mutagenicity of azo dyes in the *Salmonella*/microsome assay using in vitro and in vivo activation, Mutat. Res. 156 (1985) 131–138.
[41] S. Venturini, M. Tamaro, Mutagenicity of anthraquinone and azo dyes in Ames' *Salmonella typhimurium* test, Mutat. Res. 68 (1979) 307–312.
[42] J.R. Meier, W.F. Blazak, R.B. Knohl, Mutagenic and clastogenic properties of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone: a potent bacterial mutagen in drinking water, Environ. Mol. Mutagen. 10 (1987) 411–424.
[43] K.M. Shenck, L.J. Wyner, B.W. Lykins Jr., R.M. Clark, Aplication of a Finnish mutagenicity model to drinking water in the U.S., Chemosphere 37 (1998) 451–464.
[44] N.C. Stoppe, D.A. Roubicek, C.A. Coimbrão, M.I.Z. Sato, G.U. Valent, Mutagenicity of chlorinated drinking water treatment plants (WTP) of São Paulo metropolitan area, Genet. Mol. Biol. 21 (1998) 125.

[45] E.J. Weber, R.L. Adams, Chemical and sediment-mediated reduction of the azo dye disperse blue 79, Environ. Sci. Technol. 29 (1995) 1163–1170.
[46] Y. Hagiwara, M. Watanabe, Y. Oda, T. Sofuni, T. Nohmi, Specificity and sensitivity of *Salmonella typhimurium* YG1041 and YG1042 strains processing elevated levels of both nitroreductase and acetyltransferase activity, Mutat. Res. 291 (1993) 171–180.
[47] M. Watanabe, M. Ishidate Jr., T. Nohmi, Sensitive method for detection of *Salmonella typhimurium* tester strains possessing elevated *o*-acetyltransferase levels, Mutat. Res. 234 (1990) 337–348.
[48] N.Y. Kado, D. Langley, E. Eisenstadt, A simple modification of the *Salmonella* liquid incubation assay, Mutat. Res. 121 (1983) 25–32.