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ScienceDirect
Journal of Hazardous Materials 155 (2008) 403–406
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Industrial effluents and surface waters genotoxicity and mutagenicity evaluation of a river of Tucuman, Argentina

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Received 7 December 2006; received in revised form 23 August 2007; accepted 22 November 2007
Available online 21 February 2008

Abstract

Assessment of water pollution and its effect upon river biotic communities and human health is indispensable to develop control and management strategies. The aim of this work was to ascertain the biotoxicity of water pollution in samples from industrial effluent discharge areas of Tucumán, Argentina by means of biological tests. Chemical characterization of the water pollution was verified by measuring dissolved oxygen concentration or levels of suspended matter and salts. Genotoxic/mutagenic potential was determined using *Allium* anaphase–telophase and Ames/*Salmonella* tests. All samples were phytotoxic and genotoxic for *Allium* roots. Micronucleus and anaphase aberrations were observed, but they did not show mutagenic effects on *Salmonella typhimurium*, TA98 and TA100 strains with and without metabolic activation (S9). Our results show the importance of testing industrial effluents by chemical methods and complementary biological tests to optimize the control policy on these environmental samples.

Keywords: Mutagenicity; Genotoxicity; Argentine industrial effluents; Ames test; *Allium* test

1. Introduction

Environmental mutagens may be a major risk factor for human health. Screening for mutagens in environmental complex mixtures, such as drinking water or industrial wastewater, is gradually being accepted as a routine methodology in monitoring processes [1]. The best way to approach the problem is to use biological tests, especially short-term bioassays on bacteria [2–9] or higher plants [10,11]. Biological assessment (acute toxicity and even genotoxicity testing) is compulsory for effluent discharges in some countries, whereas in others, effluents are evaluated only by their chemical and physical properties. Low toxicity is detected in effluents discharged from efficient secondary treatment plants. However, such effluents can still carry harmful substances with long-term genotoxic/mutagenic or phytotoxic effects. Biological tests to ascertain the quality of industrial effluents in rivers of Argentina were performed in Buenos Aires and Córdoba city areas [12–14]. Different factories from Tucumán, a northwestern state of Argentina, discharge their effluents into the Salí River basin, contaminating it with organic matter, nitrogen, phosphate, salts, chlorophenols and other substances. More than 80% of the residual organic matter derives from the sugar industry and causes serious environmental damage. The Salí River and its tributaries receive 78% of the polluting load spilled, which produces an anaerobic behaviour during the period of industry manufacturing. Dissolved oxygen levels are lower than 4 mg/L from March to October. In fact, this lack of dissolved oxygen is responsible for deterioration and death of a wide range of aquatic organisms. On the other hand, the presence of potential genotoxic chemical in river water use on agricultural fields can harm organisms in the ecosystems as well as human.

To the present, only physical and chemical tests have been used to evaluate the impact of industrial effluents on water systems of Tucumán. These tests satisfy criteria set by State

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doi:10.1016/j.jhazmat.2007.11.080

agencies for environmental control, but several authors have recommended the use of organisms to evaluate toxicity of compounds resulting from anthropogenic activity [15].

The aim of this study was to screen surface water samples from the Salí River in Tucumán in industrial effluents discharge areas to identify water quality problems using biological indicators (short-term bioassays). Whether they contain chemicals that may interact with the DNA is not known yet.

2. Materials and methods

2.1. Sample collection

Water samples were collected for 6 months (from May to October 2004) in the Salí River basin, Tucumán, Argentina, at four different effluent discharge places at the same time. A large volume of industrial waste was dumped in this area during this period. Four different places were selected as sampling sites where a paper mill, a lemon juice factory, a mining company and sugar factories discharge its effluents. Sampling was performed according to the recommended standard method, SM 1060 A [16]. At each sampling point discrete water samples were taken manually each month at 1 m below the surface by a horizontal water bottle. Samples (5 L) were placed in colored plastic containers and kept at low temperature in Styrofoam boxes before transport to the laboratory according to Methods for Aquatic Toxicity Identification Evaluations [17]. The time between sample collection and analysis did not exceed 10 h. Conductivity (μS/cm, 25 °C), pH and temperature (T, °C) were measured *in situ*. The samples were maintained at 4 °C until bioassays were carried out.

Chemical analysis such as dissolved oxygen (DO, mg/L), suspended solids (SS, g/L) and chemical oxygen demand (COD, mg of O_2/L), were frequently measured according to standard methods [16].

2.2. Biotest

2.2.1. Plant genotoxicity test

Allium cepa bulbs, purchased in the local market, were placed in a container with 30 mL of mineral water for 48 h in the dark. Then, they were exposed to different sample dilutions for 24 h. A fraction of root sample was immediately fixed in ethanol–acetic acid, while the other fraction was left in mineral water for another 24 h (recovery time). Next, roots were fixed in 1:3 acetic acid–ethanol solutions for 24 h, and finally stored in 70% ethanol. Mineral water was used as a negative control. Maleic hydrazine (MH) was used as positive control [18,19].

Root tips were hydrolyzed in 1 mol/L HCl at 60 °C for 10 min before staining in Schiff's reagent for 15 min. The meristematic or mitotic zones were immersed in a drop of 45% acetic acid on a clean slide and squashed into single cells. Chromosome staining was carried out with 2% carmine in 45% acetic acid.

Microscopic parameters were mitotic index (MI) to evaluate cellular division rate, and anaphasic aberrations and micronuclei formation, as indicators of DNA damage [20]. Moreover, macroscopic parameters like root length and root modifications in consistency and form (tumor formation, hook roots, and twisted roots) were recorded as a toxicity index [21–25].

Variance analysis and Dunnett's test were performed for data analysis.

2.2.2. Ames test

To examine toxic effects on *Salmonella typhimurium* strains TA98 and TA100, the samples (effluents and different dilutions of surface water) were added to overnight-cultured *S. typhimurium* strains TA98 or TA100 (0.1 mL) and S9 mix (0.5 mL) or 0.1 mol/L phosphate buffer, pH 7 (0.5 mL) instead of S9 mix. The mixture was preincubated at 37 °C for 5 min before being diluted with phosphate buffer and poured onto nutrient agar plates. The plates were incubated at 37 °C for 48 h and the number of colonies was counted. Next, the samples were tested for mutagenic potency in the non-toxic concentration range.

Mutagenic effects were assayed according to the Ames test using *S. typhimurium* strains TA98 and TA100 with and without metabolic activation (S9 mix fraction) [26]. The tested strains were cultured overnight in Oxoid Nutrient Broth for 12 h. Different sample dilutions were added to 2 mL of top agar and 0.1 mL of bacterial culture and poured onto a plate containing minimum agar. The plates were incubated at 37 °C for 48 h and the His^+ revertant colonies were manually counted. The influence of metabolic activation was tested by adding 500 μL of S9 mixture prepared with S9 fraction obtained from liver of Sprague-Dawley rats pretreated with a polychlorinated biphenyl mixture (Araclor 1254).

All experiments were performed in triplicate with at least two replicates. The criterion of positive results was that defined by Maron and Ames [26], twofold or greater increase in the number of revertants exposed to the test material over spontaneous reversion rates. Negative and positive controls were included in each assay. The mutagens used as positive controls were 4-nitro-*o*-phenylenediamine (NPD, 5 μg/plate) which is a direct acting mutagen, and isoquinoline (IQ, 0.1 μg/plate for TA98 and 0.5 μg/plate for TA100), which required S9 mix for metabolic activation. The mutagenicity relation was calculated.

3. Results

The main contamination sources of Tucumán, Argentina, originate in the sugar, lemon juice, paper and mining activities. Hence, the samples used in this work were collected at the discharge sites of these industries in the Salí River basin. Lemon juice and sugar factory wastewaters have primary treatment consisting in solid soluble remove. Mining effluents has been treated for the biodegradable organic matter remove. Physicochemical characterization of water samples was carried out for 6 months. The results are congruent with the industrial processes and kind of effluents treatment. Data are shown in Table 1. Sugar, lemon juice and paper industrial effluents had DO values lower than 2 mg/L, producing anaerobic conditions and affecting the parameters of river water quality. COD values of water samples affected by lemon juice and sugar factory wastewater are higher than the other samples, as a result of organic matter discharged. On the other hand, SS values were lower than in the

Table 1
Physicochemical parameter determination

Parameter	Samples			
	Lemon juice factory	Mining industry	Sugar factory	Paper factory
pH	4.46 ± 0.1	6.12 ± 0.2	4.92 ± 0.1	6.12 ± 0.1
Conductivity (μS/cm)	1261 ± 10	2967 ± 20	627 ± 5	4483 ± 25
DO (mg/L)	0.00	5.25 ± 0.5	0.00	0.00
Temperature (°C)	22.4 ± 0.5	23 ± 0.8	42 ± 1	29 ± 0.7
SS (g/L)	0.70 ± 0.01	1.80 ± 0.05	0.40 ± 0.09	1.80 ± 0.03
COD (mg of O_2/L)	4223 ± 20	23.57 ± 5	5566 ± 30	627 ± 15

The results are the mean of four values of each effluent or surface water sample collected for 6 months at the time of highest industrial production.

Table 2
Root length, anaphase aberrations and mitotic index in *Allium cepa* exposed to different effluent samples collected at a discharge site in the Salí River, Tucumán, Argentina

Samples	Root length (cm)	MI ± S.D. (%)	Anaphase aberrations	% of micronucleus
Negative control	2.6	59 ± 9	–	–
Positive control	2.0	18 ± 6	4 ± 1	1.8
Lemon juice factory	1.9	Toxic	ND	ND
Sugar factory	2.25	Toxic	ND	ND
Paper factory	2.2	40 ± 5	3 ± 1	1.0
Mining industry	2.25	32 ± 6	10 ± 2	1.6

ND, not determined.

rest water samples affected by the industrial effluents analyzed, probably as a consequence of the primary treatment. The highest conductivity values were detected in the mining industry and paper mill effluents. This is a typical characteristic of the kind of processes and used materials, such as high content of dissolved salts and sodium hypochlorite in mining and paper industries, respectively.

Allium root growth inhibition was 20–40% for all assayed effluent samples (Table 2). Macroscopic modification was not observed in treated roots. Paper effluents were less phytotoxic than other samples with a mitotic index of 40 ± 5 against values of 59 ± 9 observed in negative control. Micronucleus formation and aberrant anaphase were observed in all samples (Table 2).

The mutagenicity relation values obtained on *S. typhimurium* TA98 and TA100 (with and without metabolic activation, S9) indicated absence of mutagenicity in all effluent samples at the tested doses (Table 3).

4. Discussion

Mutagenicity/genotoxicity test of surface waters or industrial effluents using a variety of bioassays demonstrates that these mixtures contain many unidentified and unregulated toxicants that may pose risks and carcinogenicity of unknown magnitude [13,14,27–29]. These contamination sources are partially treated or untreated discharges from chemical industries. In general, a number of analytical methods have been extensively used to identify organic contamination in environmental mixtures [30].

High organic matter content and low dissolved oxygen in the Salí River samples analyzed indicated the need for a biological assessment since the genotoxic potential would remain undetected if only chemical analyses were used.

Ecotoxicity bioassays were used to evaluate toxic effects in environmental contamination [6,8,31–35]. There are three kinds of genotoxicity bioassays, according to Ref. [34]: general indicators, biomarkers of exposition to genotoxic agents and integrators of genotoxic agents that include a bacterial biotest. In this work we analyzed the toxicity using the *Allium* test by measuring root length and the genotoxicity using two standardized methods: in a prokaryotic organism, *S. typhimurium* and in an eukaryotic system, *A. cepa* (mitotic index and anaphase aberrations), keeping in mind that there are carcinogens that in a metabolically activated system attack the DNA, in contrast with other agents that act by promoting and enhancing processes through totally distinct mechanisms. Few reports have proved that some rivers of South America show extreme potency (more than 5000 revertants per liter) against *S. typhimurium* TA98 and TA100 [35]. We did not detect

Table 3
Evaluation of mutagenic effect of effluent samples (without dilution) on *S. typhimurium* TA98 and TA100 (with and without metabolic activation, S9 mix)

Effluent samples	Mutagenicity relation (RM) [His^+ revertant per plate/His^+ spontaneous plate]			
	TA98		TA100	
	−S9	+S9	−S9	+S9
Lemon juice factory	0.98	1.45	0.84	1.15
Sugar factory	0.70	0.89	0.74	0.98
Paper factory	0.92	0.98	1.02	0.99
Mining industry	1.23	0.97	0.92	1.01

Data are means ± S.D. of three plates. The number of spontaneous revertants was determined in assays without a sample. The number of spontaneous revertants obtained was 41 ± 5. NPD and IQ were used as positive control. Revertants induced by IQ (0.1 μg/plate) and NPD (5 μg/plate) were 2540 ± 30 and 2343 ± 20, respectively.

mutagenic activity by Ames test with and without metabolic activation, while the samples showed genotoxic effects and a moderate toxicity by *Allium* test. Other authors have indicated that both methods gave complementary results, the plant test can be sensible to both volatile and non-volatile mutagens [9].

According to these results, we suggest that additional assays should be carried out in all water quality monitoring programs to efficiently assess the presence of genotoxic compounds in environmental samples. The use of two or more bioassays, i.e. biological responses in organisms, would be a good complement to chemical analysis.

Acknowledgements

This research was partially supported by the Consejo de Investigación de la Universidad Nacional de Tucumán (CIUNT), Argentina and by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

The authors acknowledge Prof. Silvano Monarca (Dept. of Medical-Surgical Specialities an Public Health, University of Perugia, Perugia, Italy) and Donatella Feretti and Ilaria Zerbini (Dept. of Experimental and Applied Medicine, Hygiene Section, University of Brescia, Brescia, Italy) for the assistance in this research.

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