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Evaluation of Genotoxicity and Toxicity of Buenos Aires City Hospital Wastewater Samples

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ABSTRACT

The untrated wastewaters from health centers present a potential risk aquatic ecosystems because of the content of toxic and genotoxic chemicals. The composition of these wastewaters discharged to the urban sewer system present fluctuations generated by spatial and temporal variations in the discharges of citostatics, heavy metals (Pb, Cr, Hg), antibiotics, etc. In Buenos Aires city the Hospital effluents are discharged to the sewer system with no previous treatment in turn the wastes of the municipal sewage system are released to the Río de la Plata, at a rate of 1,900 m^3/day, with no treatment at all. This river is also the source of water for the plants that provide drinking water to the city. The objective of this paper was the study of the genotoxicity and toxicity from Hospital San Martín wastewaters. This General Hospital releases approximately 560 m^3 daily of effluents to the municipal sewer system, from. Sampling this effluent was performed seasonally, in this paper we report the results obtained in summer 2003, autumn 2004 samples. The determination of toxicity was performed with the *Pseudomonas fluorescens* growth inhibition test and with the determination of mitotic index in *Allium* root tips. On the other hand the genotoxicity was studied with *Saccharomyces cerevisiae D7* assay and with the induction of chromosomal aberrations in *Allium cepa* test. Each sample was assayed either after sterilization by filtration or as an XAD-2 resins extract. The samples of summer 2003 were toxic and genotoxic in the *Allium* test when were assayed at 100% and 50% v/v dilutions, genotoxicity in the *Saccharomyces cerevisiae* test was also detected at 100% and 10% v/v dilutions. The samples of autumn 2004 were no genotoxic, only toxicity for *Saccaromyces* strain was detected when raw effluents and 100X extracts were tested. None of the wastewaters samples assayed demonstrated toxicity in the *Pseudomonas fluorescens* test.

Key words: toxicity, genotoxicity, hospital effluents.

RESUMO

Avaliação de genotoxicidade e toxicidade em efluentes de um Serviço de Saúde de Buenos Aires, Argentina

Os efluentes líquidos não tratados gerados em serviços de saúde representam um perigo potencial pela veiculação de substâncias químicas com efeitos tóxicos e genotóxicos sobre os organismos presentes nos ecossistemas aquáticos. Estes líquidos apresentam grande variação em sua constituição química, pois podem conter compostos químicos como metais pesados (Pb, Hg e Cr), antibióticos não biodegradáveis, entre outros. Na cidade de Buenos Aires, os hospitais lançam seus resíduos líquidos sem tratamento na rede coletora de esgotos, tendo como destino final, com um mínimo de tratamento, o rio de La Plata, principal fonte de abastecimento de água para uma população de 10 milhões de habitantes. Por isso, é importante estudar os riscos que os poluentes presentes nos efluentes podem representar para o meio ambiente. Este trabalho teve por objetivo analisar a toxicidade e genotoxicidade em resíduos líquidos do Hospital de Clínicas da Universidad de Buenos Aires, serviço de saúde geral de grande porte que lança um volume total diário de 564 m^3 de esgoto. As coletas do efluente foram feitas no verão e no outono. As análises de toxicidade e genotoxicidade foram realizadas no efluente utilizando diferentes sistemas biológicos normatizados: ensaio de inibição do crescimento de *Pseudomonas fluorescens* e os testes de genotoxicidade com *Saccharomyces cerevisiae* e *Allium cepa*. Os ensaios foram realizados com o efluente *in natura* e concentrado (100X) em resina trocadora de íons XAD-2. As amostras do efluente *in natura* coletadas no verão apresentaram toxicidade e genotoxicidade para *Allium cepa* (100% e 50% v/v) e genotoxicidade para *Saccharomyces*

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cerevisiae (100% e 10% v/v). Com relação às amostras coletadas no outono, foram consideradas tóxicas para o teste de *Saccharomyces cerevisiae* (100% v/v). As amostras concentradas 100X foram tóxicas para leveduras. Nenhuma das quatro amostras apresentou inibição do crescimento de *Pseudomonas fluorescens*.

Palavras-chave: toxicidade, genotoxicidade, efluente hospitalar.

INTRODUCTION

Hospitals consume daily an important volume of water. Indeed domestic water use in developed countries is, on average, 100 liters/person/day (Gadelle, 1995), while the value generally admitted for hospitals varies from 400 to 1,200 liters/bed/day (Deloffre-Bonnamour, 1995). This important amount of water produces in turn a significant volume of wastewater loaded with micro-organisms, heavy metals, toxic chemicals, and radioactive elements (Kümmerer, 2001). The hospital effluents are discharged, usually, in the urban sewer system were they mix with other effluents and finally reach the sewage treatment plant for purification. The last step of this process is the release of purified wastewaters to a river, a lake, to groundwater or to seawaters. Some of these water bodies are used also as a source of drinking water (Figure 1).

In hospitals a variety of substances are in use for medical purposes. Pharmaceuticals are composed by active substances, formulation adjudants and in some instances pigments and dyes. All these components, and its human metabolites, can reach the wastewaters (Halling-Sorensen, 1998; Stumpf *et al*., 1999; Ternes, 1998). On the other hand unused pharmaceuticals and diagnostic agents are sometimes disposed of in drains. Some drugs like cytostatic agents are genotoxic (Bassi & Moretton, 2003; Ortolan, 1999).

The presence of heavy metals such as mercury and silver and chlorinated compounds were reported in hospital wastewater. Some disinfectants and preservatives contain mercury and chromium, platinum is the main component of a group of cytotstatic agents (Kümmerer, 2001).

Organic matter can reach high concentrations in these effluents. COD values of 1,900 mg/L, with BOD_5 of 700 mg/L have been detected (Emmanuel *et al*., 2001). In previous determinations in Buenos Aires city area hospital samples, lower values of COD and BOD were found (Paz *et al*., 2004).

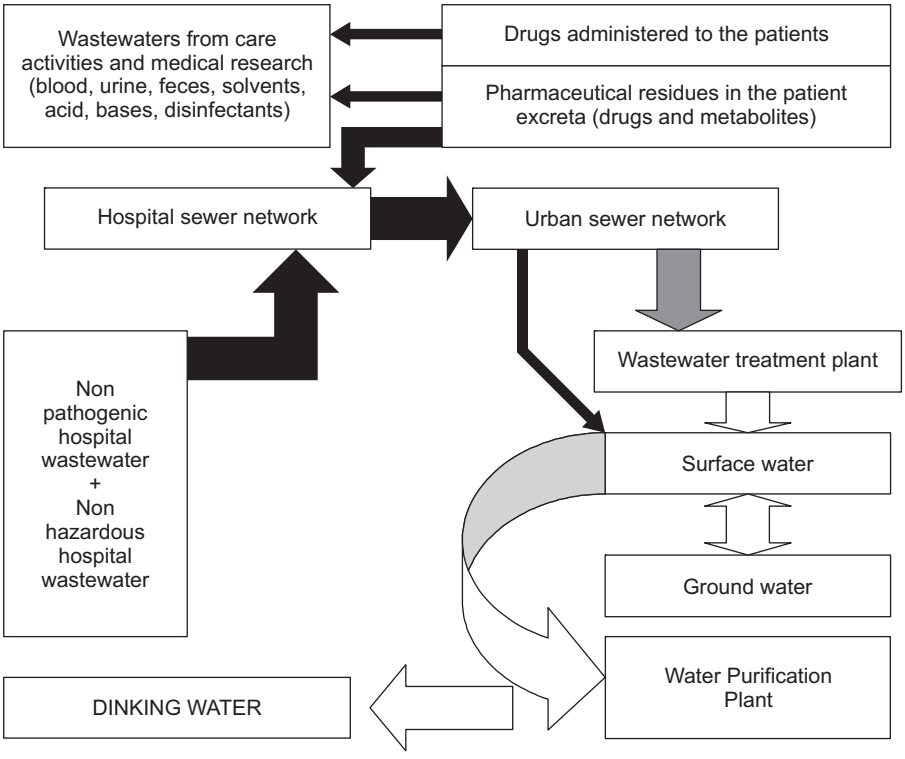

Figure 1 — Cycle of hospital wastewaters.

Iodated X-ray contrast media, solvents, disinfectants, cleaners and drugs containing chlorine are the major mass carriers for the AOX (adsorbable organic halogens) in hospital effluents (Kümmerer *et al*., 1998). Concentrations of nearly 10 mg/L of AOX were detected in the effluents of a university hospital center (Gartiser *et al*., 1996). The AOX are not easily biodegradable.

A mutagenic hazard can be manifested as a heritable change resulting from germ-line mutations and or somatic mutations leading to cancer or other chronic degenerative processes such as aging and coronary hearth diseases. Although there are species differences in metabolism, DNA repair, and other physiological processes affecting chemical mutagenesis, the universality of DNA and genetic code provides rationale for using various non-human test systems to predict the intrinsic mutagenicity of test chemicals (Moreno-Abril & Carrillo-Gallego, 2002). Even when the genotoxicity of compounds like silver, cytostatic drugs, heavy metals, etc were widely studied, no data of the genotoxicity of the complex mixture generated in the hospital wastewater were reported in Argentina.

The aim of this paper is the toxicological and genotoxicological characterization of San Martín General Hospital wastewater samples obtained before their discharge in the urban sewage system. The San Martín General Hospital is a public health center that provides hospital care in a broad category of illness and injuries.

This Hospital operates with 400 licensed beds (maximum number of beds that the facility can operate) and approximately 300 set-up beds (number of actual beds that are in operation and available for patient use). The Hospital's Laboratory conducts test in the following areas: hematology, chemistry, urinalysis, microbiology, blood bank, pathology, and cytology, for both inpatient services as well as out patient services. The San Martín Hospital releases daily approximately 560 cubic meters of effluents to the urban sewer system (Anuario Estadístico 2001). In Argentina few, if any, characterization of hospital effluents were carried on. One of the main problems of the Buenos Aires city area is the lack of sewer treatment plant. The wastes of the urban sewage system are released to the Río de la Plata, at a rate of 1,900 millions of m^3/day, with no treatment at all. This river is also the source of water for the plants that provide drinking water to the city.

MATERIAL AND METHODS

The source of test samples was wastewater obtained from the sewer test chamber, in a representative point located just before the release of the San Martín Hospital effluents to the urban sewer system. The composite samples were taken seasonally during summer and autumn 2003-2004. The sampling was performed during a period of 12 h taking a sample every two hours. The same volume of each partial sample was mixed at the end of the day to obtain the composite sample submitted to biological test. The results reported in this paper correspond to two representative samples of December and April.

Each composite sample was assayed either after sterilization by filtration through a 0.22 μm pore-size cellulose nitrate filter (Millipore), or as an extract obtained as follows: 500 mL of water were filtered (Whatman microfibre paper) and then passed through a column (10 cm high × 1 cm diameter) of XAD-2 resins with a flow rate of 10 mL/min. Ethyl ether was used for the resins elution. Then the ether extracts were collected in beakers and brought to dryness by rotary evaporation at 37ºC. Finally, the dried extracts were dissolved in 5 mL of dimethyl sulfoxide (DMSO), and assayed for genotoxicity (Moretton *et al*., 1990; Moretton *et al*., 1991).The final volumetric concentration factor was 100.

The *Pseudomonas fluorescens* growth inhibition test is based on evaluation of the effect of the test substances on the growth rate of an actively growing bacterial culture in a nutrient broth under defined conditions of temperature and time. The strain *Pseudomonas fluorescens* ATCC 13525 was used as a test organism. The test was conducted in duplicates according to Kümmerer (2001). The wastewater samples were incubated with the bacteria suspension at 26ºC during 18 h. After incubation the optical density at 650 nm was determined for the samples and controls. The dilution of the raw wastewater sample was plotted against optical density for the calculation of IC50, the effective dilution causing 50% reduction of OD or bacterial growth.

The diploid D7 strain in *Saccharomyces cerevisiae (MATa/MATα, ade2-40/ade2-119, trp5-12/trp5-2, ilv1-92/ilv1-92)* was obtained from Dr Giorgio Bronzetti (Laboratorio di Mutagenesi e Differenziamento, Pisa, Italy), and the assay was performed as previously described Moretton *et al*. (1990). Prior to each experiment the *S. cerevisiae* D7 strain was tested for the frequency of spontaneous revertants at the tryptophan (trp) locus. Cells of a stationary phase culture were treated with the samples and incubated 2 and 24 h at 28ºC. After treatment washed cells suspensions were plated on appropriate media. For all the assays the data were analyzed using the modified 2-fold rule in which a response is considered positive if the average response for at least two consecutive dose levels was more than twice the spontaneous frequencies (Moretton *et al*., 1990; Moretton *et al*., 1991). All the samples were assayed, at least, over 3 log concentrations range up to the limit imposed by toxicity of the sample for the tester strains. The data obtained were subjected to an analysis of variance (Sokal & Rohlf, 1994) with computer assistance (STATISTICA 5.0).

For the *Allium* test equal-sized bulbs were chosen from a population of the common onion *Allium cepa*, and series of onions were grown in each test liquid. Prior to the test start,

the outer scales of the bulbs and the brownish bottom plate were removed. The onions were placed directly in the test liquid, that were changed regularly every day. Slides for microscopical studies were prepared after change of liquid in day 2, independent of root length. The roots were cut, suspended in Carnoy solution for 24 h and then conserved in ethanol 70%. The standard procedure for orcein staining of squash material was performed according the description of Fiskejö (1985). From each slide 100 mitosis were scored for detect genotoxic effects and the mitotic index were scored from 1,000 cells. The following days, macroscopic observations like root form and root length, were performed. As the distribution of chromosome aberrations is binomial, the χ^2-test was used for statistical calculations (Sokal & Rohlf, 1994).

RESULTS AND DISCUSSION

The effects of hospital effluents on the growth inhibition of *P. fluorescens* are shown in Figure 2. In the broad rank of wastewater dilution assayed no significant variation in the optical density (OD) was detected, consequently it was not possible the calculation of EC_{50} values for the effluents samples. These results indicated that the effluents were no toxic in the *P. fluorescens* growth inhibition test. The dilution with tap water of heavy metals, phenols, disinfectants, etc. join to the presence of organic matter in the hospital sewer system, would generate an extremely low (below the sensitivity level of the *Pseudomonas* growth inhibition test) concentration of toxic constituents in the wastewater sampled (Muzio *et al.*, 2005).

The results obtained with the *Allium* test were shown in Table 1. This test combines two test targets, toxicity and genotoxicity. Toxicity is easily measured by observation of root growth inhibition and mitotic index, and genotoxicity is detected by frequency of chromosome aberrations. Concentrations of the San Martín Hospital effluent samples submitted to the *Allium* test were selected considering the growth inhibiting effects of the samples in onion roots growth (data not shown). Growth inhibition was detected when more than 50% v/v of the sample in buffer solution, were submitted to the test. Methyl methanesulfonate (MMS) was used as a positive control. The mitotic index did not shown significant differences between the tested concentrations. These results indicate a low toxicity of the effluent samples and are in accordance with the data obtained with *Pseudomonas fluorescens* (Figure 2). Nevertheless the determinations of chromosome aberrations indicated a clear genotoxic response for the sample corresponding to summer 2003. This effect was not detected in the sample of autumn.

The potential of hospital effluent samples and their XAD concentrates to induce genome rearrangements was investigated by the use of the eukaryotic yeast *S. cerevisiae* D7 strain (Figure 3). The results showed only the induction of gene revertion with summer 2003 wastewater samples. On the other hand the XAD concentrates were toxic in all the samples tested.

The hospital wastewater disposed into the urban sewage system, seems not to pose an obvious toxic pollution hazard. Nevertheless the genotoxicity was detected in samples of summer. The hospital effluent is diluted in the sewage system to a degree that its genotoxic activity probably will be no longer detectable in our battery test, unless the samples were submitted to an XAD concentration. However it does not mean that the genotoxicity is lost. It may still be accumulated in one of the environmental compartments and there create long term ecological effects. Therefore it seems necessary to clarify whether a group of organic compounds contribute to the genotoxic potential of the hospital wastewater. Biodegradability and persistence of the main identified compounds will have to be also analyzed to judge the impact on the environment.

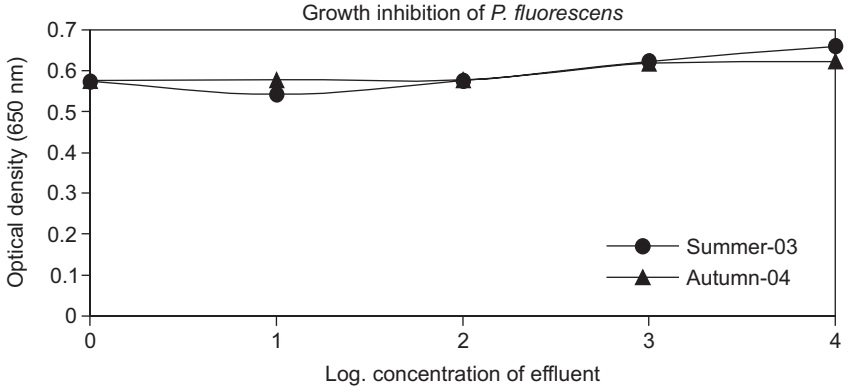

Figure 2 — Results from toxicants assessment of Hospital San Martín waste waters with *P. fluorescens* growth inhibition test. The EC_{50} of the $K_2Cr_2O_7$ was 4.46 ppm.

Table 1 — Results from genotoxicity tests of wastewater samples from San Martín Hospital in the *Allium cepa* chromosome aberration assay.

Effluent %	Mitotic index ± SD	N. of cells examined	Total aberrations %
	Sample 1		
0	80.4 ± 7.8	409	25.67
10	76.9 ± 15.0	597	15.08
50	89.6 ± 9.5	467	31.69*
MMS, 10 mg/L	45.0 ± 8.3	398	59.05**
	Sample 2		
0	74.7 ± 9.4	501	16.57
50	79.2 ± 5.0	529	11.91
MMS, 10 mg/L	57.1 ± 11.6	538	27.51**

*p < 0.05, **p < 0.01 in χ^2-test. Sample 1: summer 2003; sample 2: autumn 2004. MMS: Methyl Methanesulfonate.

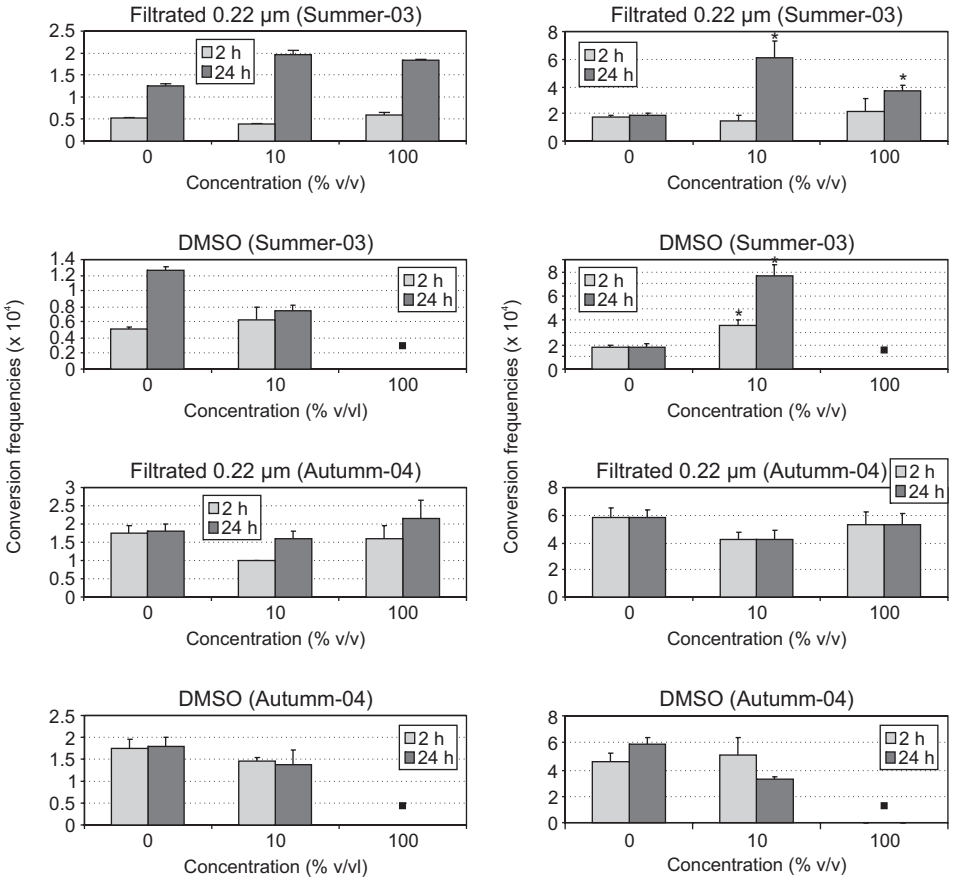

Figure 3 — Conversion and revertion frequences in *S. cerevisiae* D7. *Significant differences between the samples and control. ■ Toxic effect.

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