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RESEARCH ARTICLE

AN IMPERATIVE STUDY ON TOXICOLOGICAL EVALUATION OF HOSPITAL WASTEWATERS

***[1]Pratibha Sharma, [1]Mathur, N., [1]Singh, A. and [2]Bhatnagar, P.**

[1]Department of Zoology, University of Rajasthan, Jaipur 302004, Rajasthan, India
[2]International College for Girls, Mansarovar, Jaipur 302020, Rajasthan, India

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ABSTRACT

Hospital effluents are heavily loaded with pathogenic microorganisms, pharmaceuticals partially metabolized, radioactive elements and other toxic chemical substances such as antitumor agents, antibiotics and organohalogen compounds. The exposure to hazardous health-care wastewater can result in infection, genotoxicity and cytotoxicity, chemical toxicity, radioactivity hazards, physical injuries and public sensitivity. Humans are particularly exposed to such wastes by the drinking water produced from contaminated surface waters. Screening of wastewaters produced from hospitals is, therefore, necessary to predict the actual health hazards that may be caused due to their improper discharge. Thus, concerning the possible threat generated due to untreated hospital wastewater, this paper attempts toxicological characterization of hospital wastewaters in order to investigate their probable toxic effects on microbial population. A bacterial short term bioassay *viz. Pseudomonas fluorescens* growth inhibition assay was used to determine the toxicological effects of effluents from three selected hospitals located in Jaipur (Rajasthan), India.

INTRODUCTION

As one of the crucial and utmost important community services provided, the healthcare sector alone involves the usage of massive amounts of water throughout its operation (Kümmerer, 2001). Viewing from the perspective of wastewater management, a logical assumption can be established to determine that along with the hospitals' use of clean water comes the similar amount of wastewater which requires sewage treatment. Sewers of hospitals are often not watertight, and a significant part of the wastewater in many places may leak into the groundwater. In many developing countries, the major part of hospital wastewater is discharged in surface watercourses or in public sewers or percolates into underlying groundwater aquifers with no or only partial treatment. Hospital effluents are heavily loaded with pathogenic microorganisms, pharmaceuticals partially metabolized, radioactive elements and other toxic chemical substances such as antitumor agents, antibiotics and organohalogen compounds (Emmanuel *et al*., 2002). Wastewater discharged in an uncontrolled manner into the environment may lead to several waterborne diseases that are a threat to human life, especially in developing countries. The exposure to hazardous health-care wastewater can result in infection, genotoxicity and cytotoxicity, chemical toxicity, radioactivity hazards, physical injuries and public sensitivity.

****Corresponding author: Pratibha Sharma***
Department of Zoology, University of Rajasthan, Jaipur 302004, Rajasthan, India

Humans are particularly exposed to such wastes by the drinking water produced from contaminated surface waters. Screening of wastewaters produced from hospitals is, therefore, necessary to predict the actual health hazards that may be caused due to their improper discharge. The analysis of a recognized set of physico -chemical parameters is the most commonly used method of assessing wastewater quality by pollution control boards / regulatory authorities.

However, these parameters alone cannot give a quantitative measure of the impact of pollution. Moreover, study of these parameters of effluents does not throw any light on their synergistic or antagonistic effects on biological systems. Toxicity evaluation is, therefore, an important and cost effective tool in wastewater quality monitoring as it provides the complete response of test organisms to all the compounds present in wastewaters in a cumulative way (Roopadevi and Somashekar, 2012). Hence, the use of biological assays is justified to facilitate a direct and appropriate measure of toxicity to complement the physico - chemical measures of quality of wastewaters (Hernando *et al*., 2005). Thus, concerning the possible threat generated due to untreated hospital wastewater, this paper attempts toxicological characterization of hospital wastewaters in order to investigate their probable toxic effects on microbial population. A bacterial short term bioassay *viz. Pseudomonas fluorescens* growth inhibition assay was used to determine the toxicological effects of effluents from three selected hospitals located in Jaipur (Rajasthan), India.

MATERIALS AND METHODS

Sampling of waste waters

For this work, the samples were collected on January, 2012 and July, 2012 and the sampling was repeated at two consecutive days in a month. Samples were collected from main discharge points (just before the hospitals wastewaters are discharged into the municipal sewerage) of three hospital sites situated in different areas of Jaipur (Rajasthan), India. Sampling sites:

Sawai Man Singh Hospital (SMS)

Sawai Man Singh Hospital is the biggest government hospital in Rajasthan. There are 43 wards with a total of 1563 beds. Samples from this hospital were collected from the main sewer as the hospital doesn't have any wastewater treatment plant to treat its waste water before releasing it into municipal sewerage system.

S.K. Soni Hospital

Soni Hospital is the most frequented private hospital for Critical Care in Rajasthan, with over 300 ICU beds to handle all kinds of trauma and emergency cases. S.K. Soni hospital does not have an effluent treatment plant, but all the liquid waste is disinfected by sodium hypochlorite solution before being released into the municipal sewer. Different concentrations of hyposolution are used for disinfection of different wastes. For instance, the microbiology laboratory uses 1% sodium hypochlorite solution while for cytotoxic waste a 5% hyposolution is preferred. For cleaning of floor and wash rooms also, chlorination is done. From this hospital, samples were taken from the main sewer.

Santokba Durlabhji Memorial Hospital (SDMH) cum Medical Research Institute

SDMH is the most modern hospital of its kind in the state of Rajasthan. It has a bed capacity of 310 including 31 ICC/CCU beds and 161 beds for special care of neo-natals. This hospital has an effluent treatment plant encompassing provision for filtration and chlorination of effluent. Therefore, the samples from this hospital were collected both before and after treatment. Untreated sample was taken from the sewer of the hospital, where the entire water from the hospital is collected before being treated. Treated wastewater was collected from the outlet from where it comes out of treatment plant and is then used for irrigating the gardens of hospital.

Sample Collection

Samples from hospitals were collected in precleaned, sterilized glass bottles. Samples were taken during maximal activity periods, usually 8:00 am-6:00pm in hospitals. All samples were stored at 4°C until testing. These samples were further tested for physico-chemical parameters and cytotoxic potential in their crude state without being concentrated.

***Pseudomonas fluorescens* growth inhibition bioassay**

P. fluorescens growth inhibition assay facilitates the determination of the effect of toxic substances on the growth of a pure culture of this bacterium. The test was performed as described by Dutka and Kwan (1981). The tester strain *Pseudomonas fluorescens* MTCC103 equivalent to ATCC13525 was obtained from Microbial Type Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology (IMTech), Chandigarh, India. 15 ml of logarithmic growth phase culture of *P. fluorescens* were aseptically inoculated into 1 L of sterile nutrient broth; this constituted the test inoculum which was immediately dispensed in 25 ml volumes into 125 ml test flasks. To each flask, a 25 ml of sample was added and then the flasks were placed on a rotator shaker at 25-30°C for 10h, the inhibition of P. fluorescens growth was evaluated by absorbance determination on a spectrophotometer at 650nm. Five concentration levels (2%, 5%, 10%, 50% and 100%) of samples were tested and each set of experiment was repeated twice. Results of this toxicity test were expressed as inhibition percentage compared with negative controls. Inhibition of an increase in turbidity or the % growth inhibition in the samples was compared with that of the control using the following equation:

$$\%I = OD_c - OD_t \ / \ OD_c \ X \ 100$$

Where %I is the growth inhibition, expressed as a percentage, OD_t is the optical density (at 650nm) of a culture incubated with t^h concentration of test sample and OD_c is the measured turbidity i.e. optical density of biomass at the end of the test period in the control,. The inhibition values (%I) for each concentration were then plotted against the corresponding concentration (%). MS-excel 2007 was used for drawing dose-response plots. Statistical analysis of dose-response curves to calculate EC_{20} and EC_{50} values were performed by logistic regression using a MS excel software XLSTAT 2012; Addinosoft.

RESULTS AND DISCUSSION

Little or negligible is known about the health hazard caused by improper discharge of hospital waste waters in Jaipur. This study is amongst the few initial studies in India attempting to measure and compare the cytotoxic potential of untreated and treated hospital wastewaters.

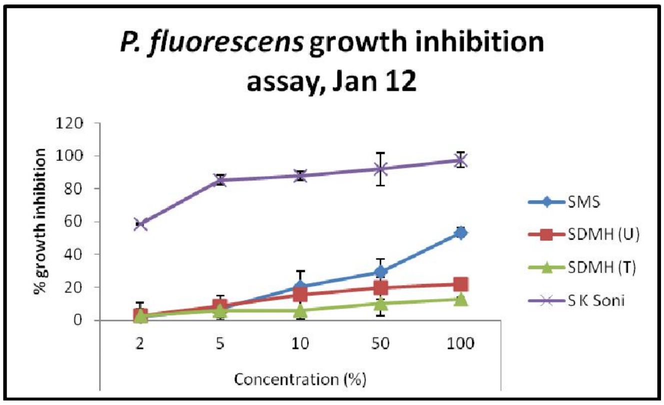
***P. fluorescens* growth inhibition assay**

When the samples were analysed with the *P. fluorescens* growth inhibition assay, responses observed were more or less similar for all facilities in terms of their concentration-response profiles during the months of January and July representing little or no seasonal influence (Fig 1.a & b). The observations revealed that the wastewater samples from S. K. Soni hospital were most cytotoxic with EC_{20} and EC_{50} values of 0.08% (for January) & 0.41% (for July) and 0.7% (for January) & 3.41% (for July), respectively (Table 1). These EC_{20} and EC_{50} values were obtained using logistic regression of killed cells by the log of sample concentration (%). Lower values of EC_{20} and EC_{50} for Soni samples show that at very low concentrations these samples were able to cause 20% and 50% death of bacterial cells thus indicating highly cytotoxic nature of these samples. Among the untreated samples, samples from SMS hospital were found to be significantly less cytotoxic (EC20 = 16.54% for January & 13.66% for July and

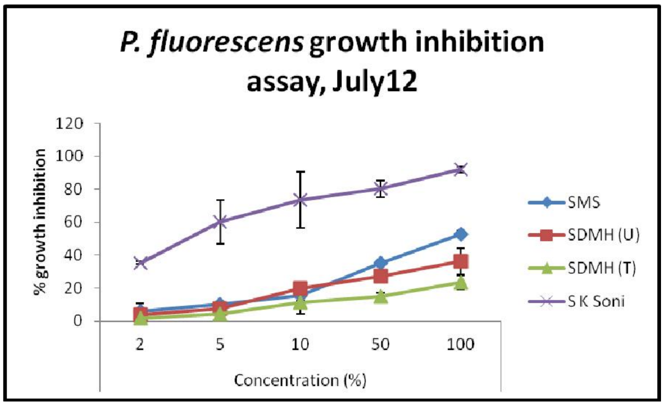
EC50 almost reached 100% for both January and July) while that obtained from SDMH was not proven to be toxic to P. fluorescens at all (EC20 = 54.21% and 21.36% for January and July respectively; EC50 not observed). Much higher EC_{20} and EC_{50} values for treated waste water sample from SDMH (EC20 = 71.01% for July; EC50 not observed) indicated further reduced cytotoxicity after treatment (Table 1). Concentration response curves clearly show that at lower concentrations of all waste water samples, except for those collected at the Soni hospital, had similar response, although at higher concentrations their cytotoxic potential differed accordingly.

Table 1. EC20 and EC50 Values for hospital wastewaters in *Pseudomonas fluorescens* growth inhibition bioassay

S. No.	Sample site	EC20 (%)		EC50 (%)	
		Jan, 12	Jul, 12	Jan, 12	Jul, 12
1	SMS	16.54	13.66	101.93	100.84
2	S.K. Soni	0.08	0.41	0.7	3.41
3	SDMH (Untreated)	54.21	21.36	-	-
4	SDMH (Treated)	-	71.01	-	-



(a)



(b)

Figure 1. Concentration-response curve for hospital wastewaters with *P. fluorescens* in growth inhibition assay

Analysing the results obtained with *P. fluorescens growth inhibition assay*, it could be seen that untreated samples only from S K Soni hospital were showing significant cytotoxic nature towards bacterial cells. SMS hospital samples and untreated SDMH samples were showing little cytotoxicity whereas treated samples were having further reduced cytotoxicity level that could even not be estimated. These findings are in agreement with the results of study executed by Paz *et al*. (2006) which indicated that the effluents were not toxic in the P. fluorescens growth inhibition test. The probable explanation for such results can be given as, the dilution with tap water of heavy metals, phenols, disinfectants, etc. joined with 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). However, highly cytotoxic character of Soni samples could be related to chlorinated by-products in its wastewaters as the hospital uses hypochlorite for cleaning and disinfection purposes. Previously, Emmanuel *et al*. (2004) evaluated the toxicity of hospital wastewater from services using NaOCl in pre-chlorination to aquatic organisms and reported considerable acute toxicity in *Daphnia magna* and *Vibrio fischeri* (bioluminescent marine bacterium); these findings were in support of results obtained with present study. Further, the ETP of SDMH was found competent enough in reducing the cytotoxicity of effluents.

Conclusion

Toxicity testing has grown steadily in recent years and is a useful tool in environmental risk assessment. Recent studies deal with the use of rapid, reproducible and cost effective bacterial assays for screening and assessment as the bacterial assay do not require a priori knowledge of toxicant identity and/or physico – chemical properties. There is a growing interest in short term microbial tests due to the fact that despite the existence of different toxicity for various organisms of different species, a substance that is toxic for an organism often demonstrates similar toxic effects on the other organisms (Kaiser, 1998). Further they can be easily performed at any microbiological laboratory within the hospital. Therefore, evaluation of biological effects using a quick, simple and sensitive method could indicate specific information on toxicity and ecotoxicity and allow incorporation of toxicity parameters in the regulatory framework (Parvez *et al*., 2006). Thus a short term microbial bioassay like *P. fluorescens* growth inhibition assay seems to be relevant for assessing the toxic potential and health hazard caused by untreated hospital effluents to human beings and other higher aquatic and terrestrial organisms, upto a considerable extent. Furthermore, based on the observations with short term *in vitro* tests, it is recommended to also test these effluents in *in vivo* animal studies should also be performed in order to the potential relevance of the toxic effects of health care wastewaters regarding potential health effects in humans and wildlife.

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