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Short Contribution

Assessment of Wastewater Toxicity by *Vibrio fischeri* Bioassay

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ABSTRACT

Increased industrialization and urbanization created ecological and toxicological problems from the release of toxic contaminants into the environment. Conventional toxicity bioassays that use animals are time consuming and expensive. The *V. fischeri* bioassay in which the inhibition of light emission by *V. fischeri* is considered a rapid, sensitive and simple test for toxicity as compared to other biological methods and assays using algae, protozoa and fish which require 7-10 days. In the present study the suitability of *V. fischeri* bioassay for the evaluation of toxicity of municipal and industrial wastewater in Allahabad (India) was conducted. Twenty five municipal wastewater streams in city of Allahabad and industrial wastewater from electronic component manufacturing unit with electroplating were evaluated for toxicity using *V. fischeri* bioassay. The majority (88%) of tested municipal wastewater samples were found to be highly toxic (EC_{50}) in summer as compared to monsoon season. Untreated industrial wastewater showed high toxicity (EC_{50} <10) as compared to treated wastewater (EC_{50} <51-75). The results indicated that the *V. fischeri* bioassay test is sensitive and suitable for evaluating the toxicity of wastewaters and other contaminants.

Key Words: Wastewater; *Vibrio fischeri* Bioassay, Toxicity

INTRODUCTION

With increased industrialization and urbanization nations face increasing ecological and toxicological problems from the release of toxic contaminants into the environment. Municipal wastewater represents the largest source of effluent discharge all over the world. The volume of the wastewater, the pollutants they contain, and potential for impacts to water quality make municipal wastewater a matter of concern. Municipal wastewater is a complex mixture of human waste, suspended solids, debris and a variety of chemicals derived from residential, commercial, and industrial sources (Araujo et al. 2005).

Toxicity measurement of wastewater, and contaminated water bodies is a very important part of environmental pollution monitoring and control strategy. The assessment of biological effects of wastewater discharges in the ecosystems is today considered relevant and ecotoxicological tests identifying the ecological hazard are useful tools for the identification of environmental impacts (Mendonca et al. 2009, Lundström et al. 2010). Direct toxicity assessment, making use of ecotoxicological tests, can play an important role in supporting decision-making, either regulatory driven or on a voluntary basis (Lai 2013).

Traditionally, crustaceans, fish and other animals are used for aquatic toxicity measurement. The tests based on these organisms require large exposure time and sample volume. Therefore, toxicity measurements based on microorganisms which are rapid, cost effective and reproducible are gaining popularity. As a result a wide range of short term bioassays have been developed to meet the demand for rapid, sensitive and inexpensive toxicity screening procedures. One of the bioassay using luminescent bacterium *V. fischeri* was developed by Bulich and co-workers as a principle bioassay organism (Bulich et al. 1981) and several studies have highlighted

the sensitivity of this bioassay (Burman et al. 2001, Ramteke and Kanan 2003, Szczepanska et al. 2016, Rodriguez-Loaiza et al. 2016). In the present study the suitability of *V. fischeri* bioassay for the evaluation of toxicity of municipal and industrial wastewaters in Allahabad (India) was conducted. Twenty five municipal wastewater streams in city of Allahabad and industrial wastewater from electronic component manufacturing unit with electroplating were evaluated for toxicity using *V. fischeri* bioassay.

MATERIALS AND METHODS

Municipal wastewater samples from 25 from different localities of Allahabad city were collected for their toxicity testing. Industrial wastewater from electronic component manufacturing unit with electroplating included raw, treated and effluent from confluence point. Toxicity tests were performed using *V. fischeri* bioassay. The samples were analysed using the Microtox analyser and reagents as described by manufacturer. The test samples were adjusted to contain 2 % sodium chloride using osmotic adjustment soloution and dilutions were made using Microtox diluents. The diluted samples were equilibrated to 15º C. The luminescent bacterial reagent was hydrated with 1 ml of precooled (4ºC) reconstitution solution and 10 ml aliquots were pipetted into cuvette containing 0.5 ml Microtox diluents equilibrated to 15ºC. For every sample, the simultaneous testing of a control with five dilutions of the test sample was conducted. Light measurements were made at 5, 15, 30 minutes after addition of the test sample to bacterial suspension. The supporting computer software model was used to calculate the effective concentration of EC_{50} (50% inhibition) value of the test samples. Toxicity was graded on a scale given by Colman and Qureshi (1985). Statistical analysis was performed by student's 't' test.

RESULTS AND DISCUSSION

Comparative summary of results of toxicity of municipal wastewaters in summer and monsoon seasons as measured by *V. fischeri* bioassay is presented in Figure 1. From the figure it is clear that overall municipal waste waters were found toxic by *V. fischeri* bioassay. Toxicity of municipal wastewaters in summer season was found higher as compared to monsoon season. In summer, majority (88%) of the 25 municipal wastewater streams

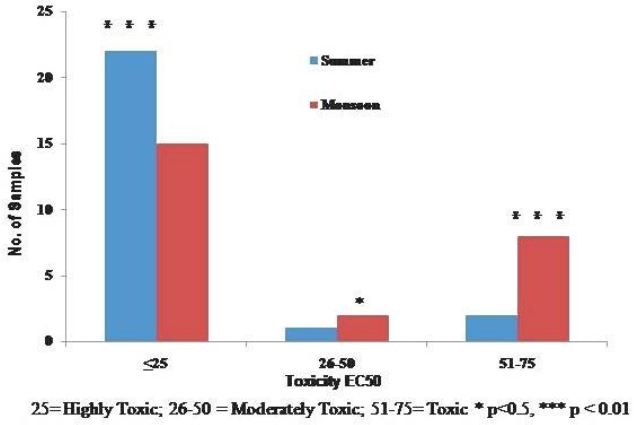

Figure 1. Toxicity of municipal wastewater

were found to be highly toxic i.e.EC_{50} <25 ($p< 0.01$). In summer only one municipal wastewater streams was moderately toxic (EC_{50} < 26-50) whereas two wastewater streams were toxic (EC_{50}<51-75). The observation of reduction in toxicity of municipal wastewaters in monsoon may be due to dilution effect of rain waters.

In case of industrial wastewater, untreated effluent was found highly toxic and the toxicity increased with increase in the incubation period (Table 1). Treated effluent also showed the same pattern of toxicity but the degree of toxicity was comparatively less. Sample from the confluence point was non-toxic after 5minutes incubation but it toxicity increased to the moderate level after the incubation of 30 minutes.

Table 1. Toxicity of industrial wastewaters

Samples	*V. fischeri* bioassay EC_{50}%		
	5 Minutes	10 Minutes	30 Minutes
Untreated effluent	9.2	8.4	6.3
Treated effluent	60.3	57.4	28.2
Confluence point	>100	74.3	33.55

<25 = Highly toxic; 26- 50 = Moderately toxic; 51- 75 = Toxic; >75 = Slightly toxic; >100 = Nontoxic

Population growth and continued urbanization will continue to increase the quantity of wastewater discharged in the environment. Several monitoring studies of water bodies reveal that the main source of pollution is the discharge of raw sewage (Belmont and

Metcalf, 2002, Belmont et al. 2004) Toxicity measurement of wastewater, and contaminated water bodies is an important part of environmental pollution monitoring.

The results of the present study very clearly indicated that the *V. fischeri* bioassay is sensitive and suitable for evaluation of toxicity of municipal and industrial wastewaters. The prior analysis of the effluent samples from electronic component manufacturing unit with electroplating showed elevated levels of toxicants and contaminants such as Zn, Cu, Ni, Cr, Cd and Pb and had detrimental impact on soil and plants (Burman et al. 2001).The sensitivity of *V. fischeri* bioassay and the reliability of this test in monitoring toxicity of treatment plant wastewaters have also been observed by other authors (Weltens et al. 2014, Szczepanska et al. 2016, Rodriguez-Loaiza et al. 2016).

Toxicity testing using *V. fischeri* bioassay has several advantages. The main bio-agent used in *V. fischeri* bioassay have relatively short life cycles and respond rather quickly to changes in environment. It is stable and easily maintained at low cost. Relatively large numbers of cells are exposed to the toxicant, offering statistical advantage. In terms of sensitivity, reproducibility, low sample requirement and rapidity, the bioluminescent bacterial test has an edge over other systems.

The results of our study suggest that the *V. fischeri* bioassay test is sensitive and suitable for evaluating the toxicity of wastewaters.

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REFERENCES

Araujo, C.; Nascimento, R.; Oliviera, C.; Strotmann, U. and Da Siva, E. 2005. The use of Microtox to assess toxicity removal of industrial effluent from the industrial district of Camacari (BA, Brazil). Chemosphere 58: 1277-1281.

Barman, S.C.; Kisku, G.C.; Salve, P.R.; Misra, D.; Sahu, R.K.; Ramteke, P.W. and Bhargava, S.K. 2001. Assessment of industrial effluent and its impact on soil and plants. Journal of Environmental Biology 22: 251-256.

Belmont, M. A. and Metcalfe, C. D. 2002. Contaminacion del agua enla cuencal del rio Texcoco, The first Intercultural Conference on the Integrated Management of Human Settlements Within Watersheds, Otavalo, Ecuador, May 28–31.

Belmont, M.A.; Cantellano, E.; Thompson, S.; Williamson, M..S.; Anchez, A.; Chris, D. and Metcalfe, C.D. 2004. Treatment of domestic wastewater in a pilot scale natural treatment system in central Mexico. Ecological Engineering 23: 299–311.

Bulich, A.A.; Green, M.M. and Isenberg, D.L. 1981. Reliability of the bacterial luminescent assay for determination of the toxicity of the pure compounds and complex effluents. Aquatic Toxicology and Hazard Assessment: Fourth Conference, American Society for Testing and Materials STP 737.

Coleman, R.N. and Qureshi, A.A. 1985. Microtox and Spirillum voluntas tests for assessing toxicity of environmental samples. Bulletin of Environmental Contamination and Toxicology 35: 443-451.

Lai, E.P.C. 2013. Bioassay kits for ecological testing of wastewater with nanoparticles. Biochemistry & Analytical Biochemistry 2: article el139.

Lundström, E.; Adolfsson-Erici, M.; Alsberg, T.; Björlenius, B.; Eklund, B.; Lavén, M. and Breitholtz, M. 2010. Characterization of additional sewage treatment technologies: Ecotoxicological effects and levels of selected pharmaceuticals, hormones and endocrine disruptors. Ecotoxicology and Environmental Safety. 73: 1612-1619.

Mendonca, E.; Picado, A.; Paixao, S.M.; Silva, L.; Cunha, M.A.; Leitão, S.; Moura, I.; Cortez, C. and Brito, F. 2009 2009. Ecotoxicity tests in the environmental analysis of wastewater treatment plants: case study in Portugal. Journal of hazardous Material 163: 665-670.

Ramteke, P.W. and Kannan, A. 2003. Toxicity of nickel (II) in microbial test systems. Asian Journal of Microbiology, Biotechnology and Environmental Science 5: 77-80.

Rodriguez-Loaiza, D. C.; Ramirez-Henao, O. and Ponuda-Mesa, G. A. 2016. Assessment of toxicity in industrial wastewater treated by biological processes using luminescent bacteria. Actualidades Biologicas 38: 211-216.

Szczepanska, N.; Namiesnik, J. and Kudtak, B. 2016. Assessment of toxic and endocrine potential of substances migrating from selected toys and baby products. Environmental Science and Pollution Research 23: 24890-24900.

Weltens, R.; Deprez, K. and Michiels, L. 2014. Validation of Microtox as a first screening tool for waste classification. Waste Management 34: 2427-2433.

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