Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

Vol. 9	No. 1	pp. 57-61	2010

Original Research Paper

Genotoxicity Assay of Three Different Surface Water Systems of Madurai District, Tamil Nadu Using Ames Test

N. Arun Nagendran
Department of Zoology, Thiagarajar College, Madurai-625 009, Tamil Nadu, India

Nat. Env. Poll. Tech.
ISSN: 0972-6268
www.neptjournal.com

Key Words:
Genotoxicity assay
Ames test
Surface water
Mutagenicity

ABSTRACT

Three water samples, viz., Vaigai river (near PTR Bridge), Vilangudi pond water and Tadagainachiamman stream water, Sirumalai hill were subjected to mutagenicity assay by Ames test using the strains *Salmonella typhimurium* TA 98 and *E. coli* to detect the presence of mutagenic substances. The mutagenic index in strain *Salmonella typhimurium* was 6.12 and 4.02 and in *E. coli*, the mutagenic index was 6.2 and 5.7 for Vilangudi pond water and Vaigai river water respectively. From the results it is concluded that the discharge of effluents in River Vaigai and petroleum products and household materials like detergents in Vilangudi pond might be the reason for the presence of xenotoxic compounds in these samples. The Thadagainachiamman stream water, since not subjected to severe anthropogenic stress, is free from these toxic compounds.

INTRODUCTION

Various pollutants, which exhibit genotoxic activity, continue to be deposited into the environment especially in water bodies. Genotoxicological safety of water, particularly drinking water, represents an important issue for safeguarding of health and well being of humans (Lal et al. 2005a). Mutagenic studies are important in safety evaluation of various polluted waters. Significant mutagenic activity has been detected in a variety of industrial wastewater effluents and sludges (Andon et al. 1986, Pancorbo et al. 1987, Sanchez et al. 1988).

The Ames test is an *in vitro* test, well validated and widely applied because of its simplicity, sensitivity and accuracy to detect environmental mutagens and carcinogens (Ames 1979, Ames et al. 1975), is used as an assessment of polluted water. As no study has earlier been carried out to observe the presence of mutagens in Vaigai river basin and adjacent areas, the present study focuses on the mutagenic activity of water polluted by industrial effluents and other discharges in this area using Ames reversion assay and standardize the reproducibility of the test.

MATERIALS AND METHODS

Bacterial tester strains: *Salmonella typhimurium* TA98-MTCC 1251-(detects frame shift mutation) and *E. coli* - MTCC Code: 1591 strains were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), IMTech, Chandigargh. The tester strains were subcultured in ampicillin growth medium No. 114 with following composition, and incubated at 37°C for 48 hours.

50 X VB Salts	-	20.0 mL
40% Glucose	-	50.0 mL
Histidine HCl.H_2O	-	10.0 mL
0.5 mM biotin	-	06.0 mL

Ampicillin solution	-	3.15 mL
Agar	-	15.0 g
Distilled water	-	910.0 mL

Test samples: Test samples were collected from three different places viz., Vagai river near PTR bridge, pond from Vilangudi and Thadagainachiamman stream water, Sirumalai hill. The samples were brought to the laboratory in sterilized vials in ice boxes and stored under refrigeration until use.

Ames mutagenicity test: The *Salmonella* reversion assay test was conducted using the plate incorporation procedure described by Ames et al. (1975) and revised by Maron & Ames (1983) with TA98 *Salmonella typhimurium* and *E. coli* strains. Samples were impregnated in sterile Whatman No 1 filter paper and tested on triplicate plates in two independent experiments. Sterile distilled water was used as negative control. The colonies clearly visible in a uniform background lawn of auxotrophic bacteria were considered as revertant colonies. All the tester strains were maintained and stored according to the standard methods (Mortelamans & Zeiger 2000).

Data analysis: The following criteria were used to interpret results following Mathur et al. (2005).

Positive test: A compound was considered mutagen if it produces a reproducible, dose-related increase in the number of revertant colonies in one or more strains of *Salmonella typhimurium*. A compound was considered weak mutagen if it produces a reproducible dose-related increase in the number of revertant colonies in or more strains but the number of revertants was not double the background number of colonies.

Negative test: A compound was considered a non-mutagen if no dose-related increase in the number of revertant colonies was observed in at least two independent experiments.

Inconclusive test: If a compound cannot be identified clearly as a mutagen or a non-mutagen, the results were classified as inconclusive.

Mutagenicity index: Mutagenicity index was calculated by the following formula.

$$\text{Mutagenicity index} = \frac{\text{No. of revertant colonies in test}}{\text{No. of revertant colonies in negative control}}$$

RESULTS AND DISCUSSION

Among the three samples tested against *Salmonella typhimurium* TA 98, pond water collected at Vilangudi showed more mutant colonies (75.33 ± 6.79) followed by Vaigai river water collected near PTR bridge (50.33 ± 6.01). The water collected from Thadagainachiamman stream showed low number (12.33 ± 2.05) of revertant mutants. The mutagenic index calculated from the observed data indicates that Vagai river water and Vilangudi pond water are positive in inducing mutation (Table 1).

Table 2 depicts the results of Ames test conducted with the three samples of water using *E. coli* strain. A maximum count of 56.0 ± 6.48 revertant colonies were counted in the plates inoculated with Vilangudi pond water. This is followed by Vaigai water sample with 51.33 ± 4.92 mutant colonies. The mutagenic index shows positive results for Vaigai river water and Vilangudi pond water. Similar to *Salmonella typhimurium* TA 98 strain, *E. coli* also does not show mutagenic induction with Thadagainachiamman stream water.

Genetic toxicology is an area of science in which interaction of DNA damaging agents with the cell's genetic material is studied in relation to subsequent effects on the health of organisms. Structural changes to the integrity of DNA caused by DNA damaging agents are useful end points for

assessing exposure to hazardous environmental pollutants on human health (Kohn 1983). The use of bioassay is an essential part of the hazard assessment and control procedures of toxic chemicals (Derelanko & Hollinger 1995). The Ames *Salmonella* assay is based on the promise that bacterial assay systems provide an efficient way to detect agents, which would interact with DNA and cause mutations (Mathur et al. 2005).

This is the most widely used test for assessing the mutagenic properties of chemicals. The *Salmonella typhimurium* histidine (his) reversion system is a microbial assay that uses a set of histidine requiring strains of bacteria to detect frame shifts and base pair substitution mutations (Maron & Ames 1983). Treatment with mutagens can induce the mutations in the histidine operon and shift growth of the strains from a histidine requiring to a histidine independent patterns. The change in the growth phenotype represents an indicator of mutagenic response (Rao et al. 2004).

In the present experiment, the potentiality of Ames test was applied to assess the mutagenicity of water samples collected from various places using two tester strains, viz., *Salmonella typhimurium* and *E. coli.* The test was earlier carried out by Sivaselvi et al. (2005) to assess the mutagenicity of Noyyal river basin. Malik & Ahmed (1995) have evaluated the genotoxicity of water samples contaminated with electroplating industries of Aligarh, India. The genotoxicity of industrial wastewater samples from Aligarh and Ghaziabad cities was compared using Ames plate test by Fatima & Ahmad (2006). According to GenPharm Tox guidelines, a mutagenic potential of the test item, tested with Ames test is assumed to be positive if the mutagenic index is above 2. If the index is below 2, then no mutagenic potential is assumed. The mutagenicity index values prescribed by GenPharm Tox have been applied by Lal et al. (2005) while monitoring genotoxicity in drinking water in Ljubljana.

The results indicate presence of 50 and 75 revertant colonies in Vagai river water and Vilangudi pond water with the strain *Salmonella typhimurium* TA 98 respectively. The mutagenic index value generated by Vaigai river water is 5.7, and pond water is 6.2. Similar results were obtained by Sivselvi et al. (2005) in Noyyal river basin with tester strain TA 102.

The Thadagainachiamman stream water does not show any mutagenic ability with the tester stain *Salmonella typhimurium* TA 100. Though a few colonies (15.66 ± 2.63) were observed in the plates incubated with this sample, the index value is only 1.74. The Ames test did not prove genotoxic potential in the stream water and the results are similar to the mutagenicity studies in drinking water by Lal et al. (2005b).

Table 1: Number of revertant colonies induced by the tested water samples in *Salmonella typhimurium* TA 98 strain.

S.No.	Water Sample	No. of revertant colonies	Mutagenic index	Interpretation
1	Vaigai river	50.3 ± 6.01	4.08	+
2	Vilangudi pond	75.33 ± 6.79	6.12	+
3	Thadagainachiamman stream	17.33 ± 2.49	1.40	-
4	Control (Negative)	12.3 ± 2.05	-	-

Table 2: Number of revertant colonies induced by the tested water samples in *E. coli.*

S.No.	Water Sample	No. of revertant colonies	Mutagenic index	Interpretation
1	Vaigai river	51.3 ± 4.92	5.7	+
2	Vilangudi pond	56.0 ± 6.48	6.2	+
3	Thadagainachiamman stream	15.66 ± 2.63	1.74	-
4	Control (Negative)	12.3 ± 2.05	-	-

The same three samples were tested for their mutagenic potential with *E. coli*. Reversion of the TrpE65 mutation can occur via a number of genetic pathways (Ohta et al. 2002). The *E. coli* Trp assay is accepted as a valid short term genotoxicity test by International regulatory agencies (Mortelmans & Riccio 2000).

It was noted that Vaigai river water has induced 50.3 ± 6.01 colonies, and Vilangudi pond water produced 75.33 ± 6.79 colonies. The mutagenic index values are 4.08 and 6.12 respectively. Sanchez et al. (1988) have analysed metallurgical wastes and found that about 60% of them are mutagenic in *E. coli* WP_2 test. *E. coli* mutants were used to assess the genotoxicity in some wastewaters in India (Malik & Ahmad 1995).

Similar to the results with *Salmonella typhimurium*, with *E. coli* also the Thadagainachiamman stream water has produced low revertant colonies with the index of 1.4. The *E. coli* reverse mutation assay was used to evaluate the capacity of 2-dodecyclobutanone to induce mutations and the results are in agreement with the negative results obtained from other short term and long term genotoxic tests (Sommers 2003).

The results reveal that among the three samples tested, Vaigai river water and Vilangudi pond water samples are contaminated with mutagenic agents whereas, Thadagainachiamman stream water is not polluted with such contaminants. The mutagenicity of river water may be either due deposition of industrial wastes or organic deposition. Velema (1987) reported that organic substances in river water may naturally result from plant decay process or from industrial effluents and upon subsequent chlorination, which might be one of the reasons for the presence of genotoxic chemicals in river water.

The discharge from large number of workshops present in the Vilangudi region might be the cause for high mutagenicity in Vilangudi pond water. The studies of Amer et al. (1990) support the mutagenicity of water due petroleum products. Also domestic discharge containing detergents might also contribute to the presence of mutagenic compounds in Vilangudi pond water.

Thadagainachiamman stream, though intervened by humans for recreational purpose, is not severely subjected to anthropogenic stress. The human disturbance to the stream is also confined to down stream at the foot hills, and this might be the reason for the absence of xenotoxic compounds in this sample.

Since correlations between genotoxicity of drinking water and increased cancer risk are proven, detection of low levels of genotoxicity in water is very important. As chemical analysis alone cannot predict the actual risks humans are exposed by consuming drinking water, because they are limited by sensitivity and determination of single compounds and their derivatives (Lal et al. 2005b), it is necessary to use sensitive test methods. The approach proves to be helpful in environmental monitoring, since these biological assays appear more sensitive and relevant. Nevertheless, further investigations are necessary for more complete detection of genotoxic compounds in environmental samples, like use of bioassays with organisms of different trophic levels.

REFERENCES

Amer, M.H., El Yazigi, A., Mohamed, H.A. and Mohamed, M.E. 1990. Water contamination and oesophagus cancer at Gassim region, Saudi Arabia. Gastroenterology, 98: 1141-1147.

Ames, B.N. 1979. Identifying environmental chemicals causing mutations and cancer. Science, 204: 587-593.

Ames, B.N., Mecann, J. and Yamasaki, E. 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/ mammalian microsome mutagenicity test. Mutat. Res., 31: 347-364.

Andon, B., Jackson, M., Houk, V. S. and Claxton, L. 1986. Evaluation of chemical and biological methods for the identification of mutagenic and cytotoxic hazardous waste samples. In: Petros, J.K., Lacy, W.J. and Conway, R.A. (eds). Hazardous and Solid Waste Testing, Fourth Symposium, ASTM STP 886, Philadelphia, PA.

Derelanko, M.J. and Hollinger, M.A. 1995. CRC Handbook of Toxicology. CRC Press, Boca Raton, New York.

Fatima, R.A. and Ahmad, M. 2006. Genotoxicity of industrial wastewaters obtained from two different pollution sources in northern India: A comparison of three bioassays. Mutat. Res., 609(1): 81-91.

GenPharmTox, www.genpharmtox.com

Kohn, H.W. 1983. The significance of DNA-damaging assays in toxicity and carcinogenicity assessment. Ann. Ny. Acad. Sci., 407: 106-118.

Lal, B., Zinko, B., Narat, M. and Marinsek-Logar, R. 2005a. Monitoring of genotoxicity in drinking water using *in vitro* Commet assay and Ames test. Food Technol. Biotechnol., 43(2): 139-146.

Lal, B., Zinko, B., Tatjana, T. and Marinsek-Logar, R. 2005b. Genotoxicity detection in drinking water by Ames test, Zimmermann test and Commet assay. Acta. Chim. Slov., 52: 341-146.

Malik, A. and Ahmad. M. 1995. Genotoxicity of some wastewaters in India. Environmental Toxicology and Water Quality, 10: 287-293.

Maron, D.M. and Ames, B.N. 1983. Revised method for *Salmonella* mutagenicity test. Mutat. Res., 101: 173-215.

Mathur, N., Bhatnagar, P. and Bakre, P. 2005. Assessing mutagenicity of textile dyes from Pali (Rajasthan) using Ames bioassy. Applied Ecology and Environmental Research, 4(1): 111-118.

Mortelmans, K. and Zeiger, E. 2000. The Ames *Salmonella*/microsome mutagenicity assay. Mutat. Res., 455: 29-60.

Mortelmans, K. and Riccio, E.S. 2000. The bacterial tryptophan reverse mutation assay with *Escherichia coli* WP2. Mutat. Res., 455: 61-69.

Ohta, T., Tokishita, S., Tsunoi, R., Ohmal, S. and Yamagata., H. 2002. Characterization of Trp + reversions in *Escherichia coli* strain WP2 uvr A. Mutagenesis, 17(4): 313-316.

Pancorbo, O.C.P., Lenin, P.J. and Belvins, R.D. 1987. Mutagenic activity of surface waters adjacent to a nuclear fuel processing facility. Arch. Environ. Contam. Toxicol., 16: 631-637.

Rao, K.S., Yongxy, M.D., Ellenshaw, M.S. and Parton, J.W. 2004. Mutagenicity testing applied for regulation of developing products. Current Separation, 20(4): 141-144.

Sanchez, P.S., Sato, M.I.Z., Passhoal, C.M.R.B., Aives, M.N., Furlan, E.V. and Martins, M.T. 1988. Toxicity assessment of industrial effluents from Sao Paulo, Brazil using short term microbial assay. Toxic Asses., 3: 55-80.

Sivaselvi, R., Santhy, K.S., Sukumar, S. and Arulraj, P. 2005. Screening and evaluation of mutagenicity of *Salmonella typhimurium* in the river and well water samples. J. Ecotoxicol. Environ. Monit., 15(1): 79-84.

Sommers, C.H. 2003. 2-dodecyclobutanone does not induce mutations in the *Escherichia coli* tryptophan reverse mutation assay. J. Agric Food Chem., 51: 6367-6370.

Velema, J.P. 1987. Contaminated drinking water as potential cause of cancer in human. J. Environ. Sci. Health, 5: 1-28.