



## Comparative Study of Usage of Microbial Strains for Monitoring Waste Water Treatment Plants

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### Abstract:

Domestic sewage treated in municipal sewage treatment plants is only analyzed for its physicochemical properties correction prior to its discharge but as domestic sewage is proved to contain genotoxicity also, it is suggested that the discharge should also be checked for genotoxicity. Many microbial genotoxicity tests are employed for genotoxicity testing. These tests are simple, quick and employ a variety of auxotrophic bacterial strain battery detecting frame shift, point mutation, base pair substitution causing mutagens presence in the sample. Among them various *Salmonella typhimurium* strains TA98, TA100, TA97a, TA97b, Ta102, *E.Coli*. WP2 etc. are very commonly used which requires repetition of experimentation and makes it tedious. Actual speed of experimentation in detection of mutagens is also lost. In the present study four most commonly used strains of auxotrophic bacterium viz. *Salmonella typhimurium* TA98, TA100, Ta102 and *E.Coli*. WP2 strain was taken into consideration and were analyzed that in domestic sewage which types of mutagens are present and which strain among these four most commonly used strains is most sensitive and quick in detection of these mutagens. The results of the study showed that domestic sewage and municipal waste water consists of mutagens causing frame shift mutations and base pair substitutions and the auxotrophic bacterial strain which is most effective among the four strain is *Salmonella typhimurium* strain TA 102 which along with the frame shift mutation detecting strain TA98 can be used for quick assessment of genotoxicity of domestic waste prior to its discharge.

**Keywords:** *Salmonella typhimurium*, *E. coli*. WP2 assay, genotoxicity, physico chemical parameters, auxotrophic

### 1.0 Introduction:

Short-term genetic bioassays have proved to be an important tool in genotoxicity evaluation studies because of their simplicity, sensitivity to genetic damage, speed, low cost of experimentation and small amount of sample required, Results from genetic bioassays are relevant to human health because the toxicological target is DNA, which exists in all cellular life forms. Thus, it can be extrapolated that compounds shown to be reactive with DNA of one species have the potential to produce similar effects in other species. In perturbations of genetic material are deleterious to the organism and can lead to severe and irreversible health consequences. The whole idea behind the conduct of bioassays is that if a compound is found to be mutagenic for bacterial strain is likely to be a carcinogen in laboratory animals, and thus, by extension, present a risk of cancer to humans (Mathur et al., 2006, Gupta et al., 2009, Thewes et al., 2011).

Many types of genotoxicity and mutagenicity assays employing microorganisms and mammalian cells have used for monitoring of complex environmental samples (Verschaeve, 2002; Isidori *et al.*, 2004; Reinecke and Reinecke, 2004; Russo *et al.*, 2004, Mielli *et al.*, 2009, Thewes M. et. al., 2011). Popularity of the bacterial assay is based on the fact that bacteria are an integral part of the ecosystem and the bacterial assays are relatively quick and simple. The growing interest in these tests is due to the fact that despite the existence of different toxicity mechanisms for various organisms of different species, a substance that is toxic for an organism often demonstrates similar toxic effects on other organisms. (Gupta et. al, 2009) Microorganisms have demonstrated several attributes that make them attractive for use in quick screening of effluents and chemicals for toxicity.

In this study, we used the *Ames* test including a tester strain battery of TA98, TA100, TA102 and *E. coli*. WP2 reverse mutation assay using *E.Coli*.WP2 strain to evaluate the toxicity and genotoxicity of domestic waste water at each treatment stage of municipal waste water treatment plant. Comparative studies of all the strains were done to check which strain is the most sensitive strain for the detection of genotoxicity of such water samples. Since decades these tests are done with a tester strain battery of several different strains containing auxotrophic mutations In the present study it was checked whether the battery of different strains or repetition of alike experimentation is required for genotoxicity evaluation or not and which strain is most useful and accurate for detection of possible mutagenicity in such water samples coming from such waste water treatment plants handling domestic sewage. Treated waste water is often utilized for irrigation and other recreational activities. However, safe utilization of waste water for irrigation requires proper treatment and various precautionary measures to use as it may cause environmental and human health hazards (Qadir, *et. al.*, 2005, Butt *et.al*, 2005). If proper pollution management / abatement practices are not in place other uses of water are affected (Persson *et.al.*, 2009).

## 2.0 Material and Methods:

### 2.1 Sampling:

The sewage treatment plant situated at Amer road, Jaipur (Rajasthan, India) is taken under study. This plant has a capacity of 270 lakhs per liters per day. It benefits the entire population of the area and also prevents the pollution of Jalmahal Jheel, (a natural water resource present in this area). This sewage treatment plant was basically commissioned for the treatment of domestic and industrial waste of north zone of city during 1979. Before its commissioning the waste water was directly discharged without any pre treatment. The sewage treatment plant (27MLD) consist of screening, grit chambers, extended aeration tanks, secondary clarifier, sludge recirculation, sludge thickener, aerobic sludge digester and sludge drying beds. Sampling of water was done after each treatment stage to check whether genotoxicity is reducing at every stage and to compare which bacterial strain is most specific and sensitive for the detection of genotoxicity of such water samples. Water samples were taken from four different sites, firstly, the water was taken from the site where the sewage water is discharged for

further treatment in tanks after primary treatment (site 1), Secondly, the sample was taken from the effluent water coming out of clarifier (site2), Thirdly water sample was taken from the discharge of aeration tanks (site 3), and Finally water sample was collected from the treated discharge coming out of the final stage of treatment process (site 4). All the collected samples from different sites were collected in clean sterile bottles and refrigerated at 4°C. No further fractionation or treatment of samples was done. The samples were directly used for the analysis in the test doses 2µl, 5µl, 10µl, 50µl and 100µl. These sample mixtures were treated as a single entity and were tested in the crude form. All the samples are taken twice in a year in two months of June and December to check seasonal variations and also in two subsequent years i.e. 2008, 2009 to check the consistency of results obtained.

### 2.2 Bacterial Strains:

Four different strains were utilized for two different bioassays viz. *Salmonella typhimurium* strain TA98, TA 100, TA 102 and *E.Coli* WP2 strain. Strain TA 98 detects frame shift mutations. Strain TA 100 detects base pair mutations. Strain TA 102 also detects base pair mutations. Strain *E.Coli* WP2 detects base pair substitution mutation. All the strains were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh (India). They were stored as glycerol cultures at -20°C. All tester strains were maintained and stored according to the standard methods (Ames *et al.*, 1975; Maron and Ames, 1983). The tester strain genotypes (histidine requirement, *rfa* mutation, *uvr* B and R- factor) were confirmed immediately after receiving the cultures and every time a new set of frozen permanents were prepared or used.

### 2.3 Salmonella Mutagenicity Test:

The Salmonella/microsome reversion assay was conducted using the plate incorporation procedure described by Ames *et al.*, 1975 and revised by Maron and Ames (1983). The tester strains of *Salmonella typhimurium* viz. TA98, TA100 and TA102 were obtained from Microbial Type Culture Collection & Gene Bank, Institute of Microbial Technology (IMTEC), Chandigarh (India).The samples were analyzed without the hepatic S9 fraction. Five dose levels of individual samples were tested (2, 5, 10, 50 and 100 µl). The positive controls used in this assay were sodium azide used for TA 100, 2-Nitrofluorene,

used for TA98 and TA 102. All the plates were run in duplicate. Each set of experiment was repeated twice. Spontaneous revertant colonies per plate for the strains TA98, TA100 and TA102 were  $50 \pm 2$ ,  $120 \pm 4$  and  $140 \pm 4$ , CFU, respectively as shown in Fig. 1. Sterile distilled water was used as negative control. The *S. typhimurium* strains viz.TA98, TA100 and TA102 were grown at 37°C, with shaking, for 10h to obtain final cell concentration of  $10^9$  bacterial cells. 0.1ml of this fresh culture was mixed with 0.2 ml of his/bio solution, 0.1ml or less of test chemical, 0.5 ml of buffer and total volume was made upto 1.0 ml by autoclaved distilled water. This mixture was then shaken and poured on plates containing about 25 ml of minimal glucose agar medium. The test concentrations were selected from a set of standard test doses for liquids. The plates were immediately

covered with paper to protect photosensitive chemicals present in the test compounds. Plates were then inverted and placed in a dark incubator for 48h at 37 °C. The revertant colonies were clearly visible in a uniform background lawn of auxotrophic bacteria. After 48h the revertant colonies on the test and control plates were counted. All tester strains were maintained and stored according to standard methods (Ames *et al.*, 1975; Maron and Ames, 1983). The tester strain genotypes (Histidine requirement, rfa mutation, uvr B and R-factor) were confirmed immediately after receiving the cultures and every time a new set of frozen permanents was prepared or used. All reagents used were of analytical grade, supplied by Himedia Laboratories Limited (India) and Sigma-Aldrich.

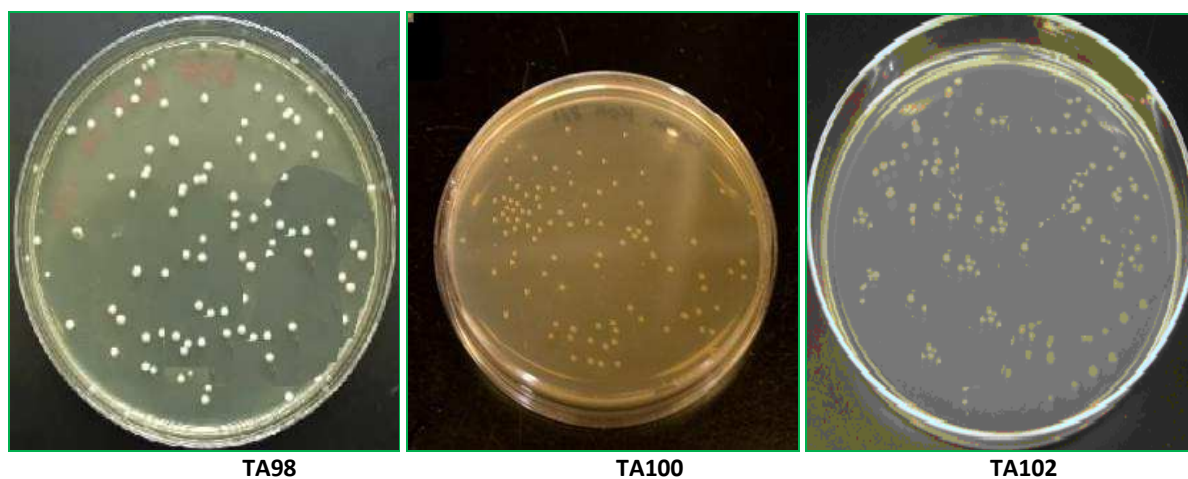


Fig 1: Showing plates with spontaneous revertant colonies obtained with TA 98, TA 100, TA 102 strains in Ames assay.

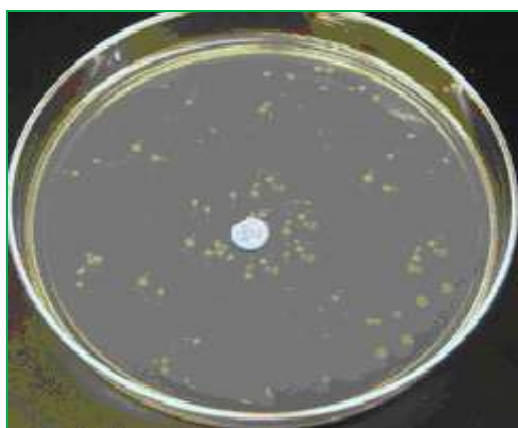


Fig 2: Figure showing number of spontaneous revertant colonies obtained with *E.Coli* WP2 strain.

### 2.4 *E.coli*. WP2 Bioassay:

*Escherichia coli* strain WP2 and its repair- deficient derivatives are suitable strains for mutagen screening. In these strains, agents which cause base substitution mutations can be shown to increase the frequency of *trp*<sup>+</sup> revertants. In addition, agents causing many types of DNA damage can be detected through increased killing of the repaired deficient derivatives. *E.coli*. tryptophan reversion system has been used extensively in microbial studies (including chemical screening, radiation studies and analysis of bacteria DNA-repair pathways and in numerous non-genetic applications). In contrast to the *Salmonella* strains that have different unique target DNA sequences in the Histidine operon, the four most commonly used WP2 strains carry the same tryptophan marker, *trpE* (Wilcox *et al.*, 1990). The assay is currently used by many laboratories in conjunction with the Ames *Salmonella* assay for screening chemicals for mutagenic activity (Mortelmans and Riccio, 2000). The strain was obtained from Microbial Type Culture Collection and gene Bank (MTCC), Institute of Microbial Technology (IMTech), Chandigarh (India). The number of spontaneous revertants obtained by this method was 82± 2 CFU as shown in Fig 2. Autoclaved distilled water was used as a negative control. The positive controls used in this assay were 4-nitroquinoline oxide and methyl methane sulfonate. The cultures were grown overnight in 10 ml of growth medium and re incubated with aeration for an additional 2.5 h at 37°C till the cell density reached 2 x 10<sup>8</sup> cells/ml. Selective agar 2 (SA2) plates were spread in agar with 0.1 ml of a culture. SA2 agar contains a trace of tryptophan. After solidification, 25µL of the test material was added via saturated filter paper disc. The plates were incubated at 37°C for 48 h, permitting diffusion of the chemical into agar. All reagents used were of analytical grade, supplied Himedia Laboratories Limited (India) and Sigma-Aldrich (India). All samples were tested in at least two independent experiments using five doses and three plates per dose.

### 3.0 Data Analysis of Mutagenicity Tests:

#### 3.1 Interpretation of Test Results:

The most common method of evaluation of data from the mutagenicity assay is the “two fold rule” according to which doubling of spontaneous reversion rate at one or two test chemical concentrations constitutes a positive response

(Mortelmans and Zeiger, 2000). This rule specifies that if a test compound doubles or more than doubles mean spontaneous mutation frequency obtained on the day of testing, then the compound is considered significantly mutagenic. Using this procedure the following criteria were used to interpret results:

**Positive**—A compound is considered a mutagen if it produces a reproducible, dose-related increase in the number of revertant colonies in one or more strains of *S. typhimurium* and *E.Coli* WP2. A compound is considered a weak mutagen if it produces a reproducible dose- related increase in the number of revertant colonies in one or more strains but the number of revertants is not double the background number of colonies.

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

**Inconclusive**—If a compound cannot be identified clearly as a mutagen or a non-mutagen, the results are classified as inconclusive (e.g. if there is one elevated count). For this analysis the dose-related increases in the number of revertant colonies were observed for the test compounds and mutagenicity ratios were calculated. Mutagenicity ratio is the ratio of average induced revertants on test plates(spontaneous revertants plus induced revertants)to average spontaneous revertants on negative control plates(spontaneous revertants)(Mathur *et al.*,2005).

#### 3.2 Statistical Analysis:

To observe the effects of dose in 4 sampling sites, during 2 seasons and 3 types of strains for *S. typhimurium* and 1 strain of *E. coli* WP2, the techniques of regression analysis were used. Under this, 3 types of regression models had been used, they were the general univariate linear model (Draper and Smith, 1966; Moore and Felton, 1983), inverse linear model (Draper and Smith, 1966) and quadratic model (Draper and Smith, 1966). The upward trend between dose and number of revertants/plate suggested applying these 3 types of regression models to find out the relationship between dose level and number of revertants/ plate. To observe whether the relation between number of revertants /plate and dose response is constant throughout the study, the linear model was used, and to observe the non-linearity quadratic model,

the inverse exponential model was used. Revertants were taken as the dependent variable and dose as the independent variable ; time and strains (TA98,TA100,TA 102 and *E.coli* WP2) were fixed for the six water samples. Comparison-wise P values of 0.05 were considered to be statistically significant and all tests were two- tailed. The Statistical Package for Social Sciences, Release 10.0 was used for the statistical analysis and graphical representations.

#### 4.0 Results and Discussion:

The results of Salmonella mutagenicity assay for four different treatment stages are summarized in Table1 and Table 2 as the mutagenicity ratio of average induced reversions to spontaneous reversions.

#### 4.1 Stage 1: Inlet Water Coming for Treatment:

Raw sewage coming for treatment in the waste water treatment plant showed positive mutagenicity with all the strains as indicated in Table 1.The mutagenicity ratios of the sample of primary inlet with strain TA98 at each dose level of 2µl to 100µl, is much higher than two indicating positive mutagenicity (Table 1). At each dose level as the dose of sample aliquot increases there is significant increase in the mutagenicity of the sample showing increased response of mutagenicity with increased dose of sample aliquot. Little seasonal variation is also observed in the number of induced revertant colonies, the net number of induced revertants observed for inlet water were higher in the month of June than those obtained in the month of December

2008 the time of second sampling. For comparison purpose highest dose level of each sample was compared. The number of induced revertants obtained for water sample in the month of June 2008 (3283 induced TA98 colonies per 100µl of sample in the absence, fig. 3) were found to be higher than those obtained in the month of December for the same samples of waste water (2885 induced TA98 revertant colonies per 100µl of sample in the absence, fig. 3).With strain TA 100, TA 102, and *E.coli*. WP2 also all the samples showed positive mutagenicity with mutagenicity ratio much higher than 2.0 .During first sampling time of June 2008 the number of induced revertants are slightly more (3058 induced revertant colonies at 100µl sample aliquot fig 4 ,with TA 100 strain), With TA 102 (3450 induced revertant colonies per 100µl of sample, fig 5) and with *E.coli* WP2 (3277 induced revertant colonies per 100µl of sample fig. 6) .While in the month of December 2008 little seasonal variation were obtained and the number of revertant colonies obtained were little less than what was observed in the month of June'08 (3014 induced revertant colonies per 100µl of sample with TA 100, fig 4), (3471 induced TA102 revertant colonies per 100µl of sample, fig. 5 ), (3175 induced revertant colonies per 100µl of sample with *E. coli*. WP2 fig. 6). The number of revertant colonies obtained at this stage was very high which is a strong indication of positive mutagenicity and presence of both frame shift and base pair substituting mutagens in the sample.

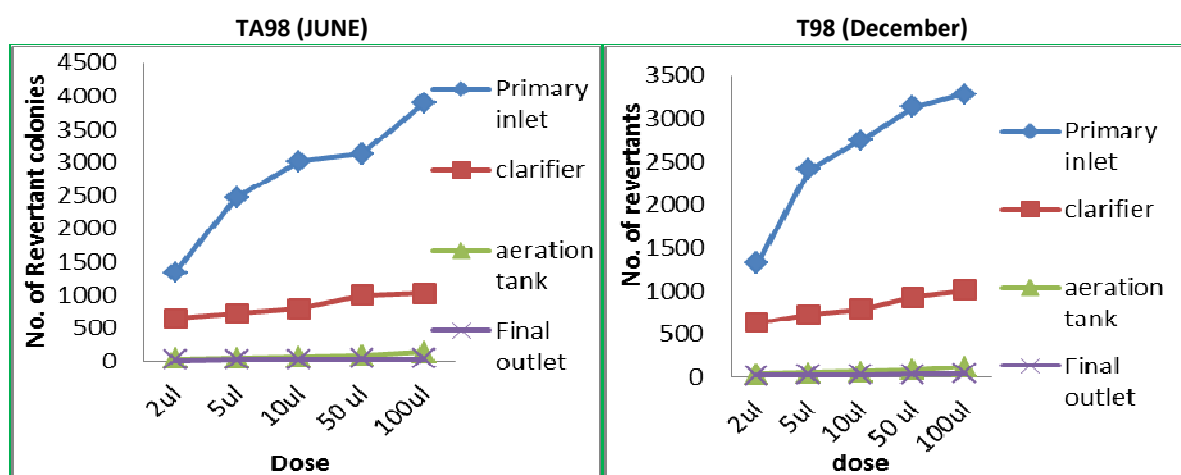


Fig. 3. Dose-response curve of different water samples with strain TA98,

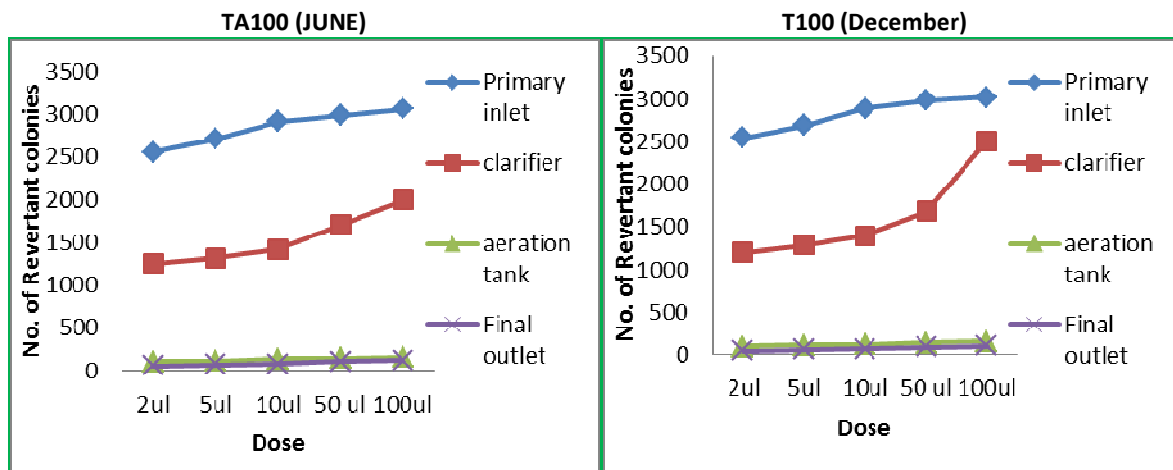


Fig. 4. Dose-response curve of different water samples with strain TA100,

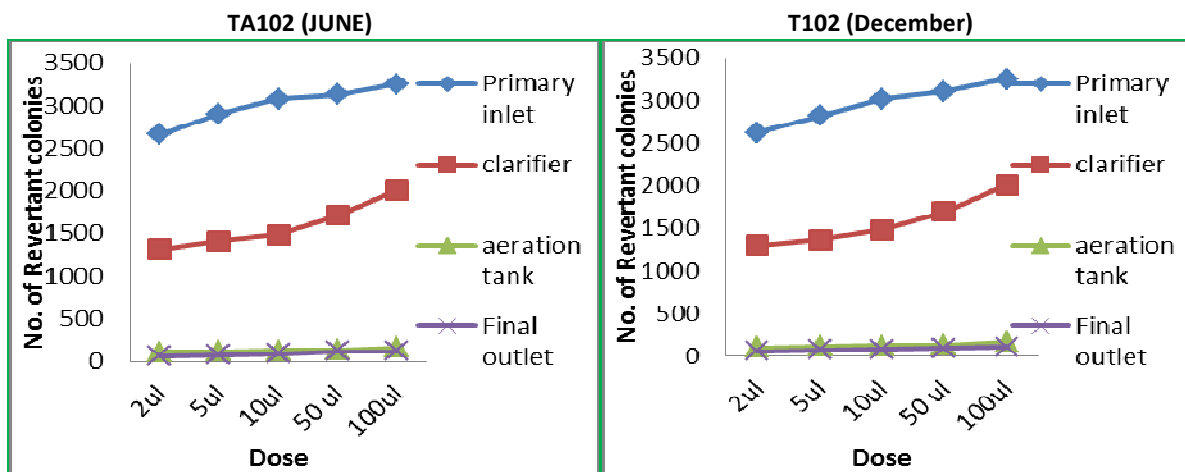


Fig.5. Dose-response curve of different water samples with strain TA100,

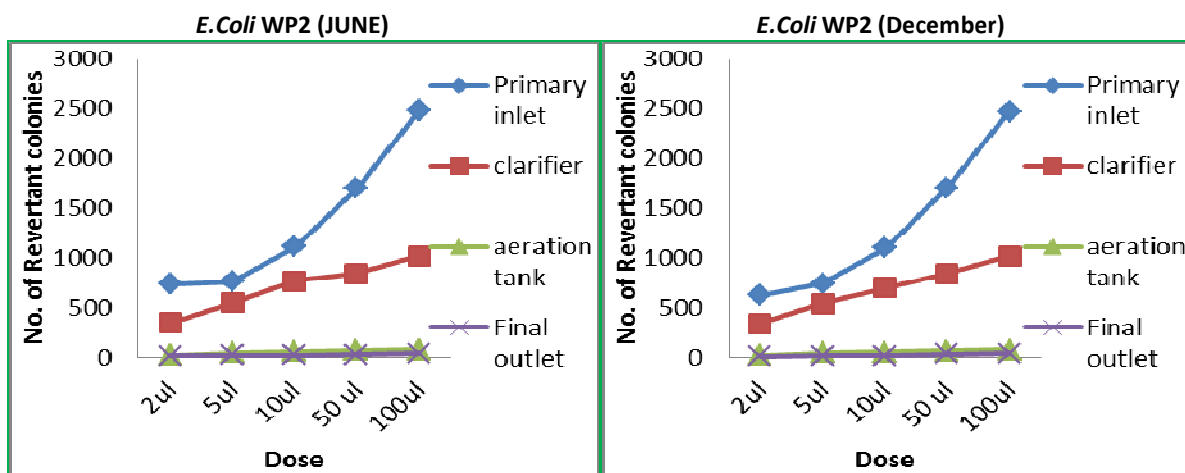


Fig. 6. Dose-response curve of different water samples with strain *E.Coli*. WP2,

**Table 1:** Mutagenicity ratio of *Salmonella* tester strain TA 98, TA 100, TA 102 in Ames test and *E. Coli* WP2 tester strain on water samples from different stages of Amer road Sewage treatment plant, Jaipur (2008)

Site	Dose ( $\mu$ l)	Mutagenicity Ratio							
		June				December			
		TA98	TA100	TA102	<i>E. Coli</i> WP2	TA98	TA100	TA102	<i>E. Coli</i> WP2
Primary inlet	2	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+
	50	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+
Clarifier	2	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+
	50	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+
Aeration tank	2	-	-	-	-	-	-	-	-
	5	-	-	-	-	+	-	-	-
	10	+	-	-	-	+	+	-	-
	50	+	+	-	-	+	+	-	-
	100	+	+	+	+	+	+	+	+
Final outlet	2	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-

\*+, Ratio greater than 2.0 indicating possible mutagenicity. \*- , Ratio less than 2.0 indicating non mutagenicity.

#### 4.2 Stage 2: Clarifier:

Clarifier is the first stage of the treatment procedure, it comprises of filters where raw sewage is filtered to remove solid waste particles present in the sewage collected from different sites of the city. Dose response curves indicate that at each dose level as the dose of sample aliquot increases there is significant increase in the mutagenicity of the sample showing increased response of mutagenicity with increased dose of sample aliquot. The number of induced revertant colonies obtained for sample in the month of June (1025 induced revertant colonies per 100 $\mu$ l of sample with TA 98, fig3) (2508 induced revertant colonies at 100 $\mu$ l sample aliquot with TA 100, fig 4) (2011 induced revertant colonies per

100 $\mu$ l of sample with TA 102, fig 5) (1020 induced revertant colonies per 100 $\mu$ l of sample with *E.coli* WP2, fig 6) are higher than those obtained in the month of December for the same samples of effluent discharged (1010 induced revertant colonies per 100 $\mu$ l of sample with TA 98, fig 3) (1995 induced revertant colonies per 100 $\mu$ l of sample with TA 100, fig 4) (2007 induced revertant colonies per 100 $\mu$ l of sample with TA 102, fig 5) (1021 induced revertant colonies per 100 $\mu$ l of sample with *E.Coli* WP2, fig 6). The numbers of revertant colonies obtained at this stage are also very but are less in comparison to what was obtained with primary inlet stage showing reduction in mutagenicity of samples at this stage of treatment.

**Table 2:** Mutagenicity ratio of *Salmonella* tester strain TA 98, TA 100, TA 102 in Ames test and *E. Coli* WP2 tester strain on water samples from different stages of Amer road Sewage treatment plant, Jaipur (2009)

Site	Dose ( $\mu$ l)	Mutagenicity Ratio							
		June				Dec			
		TA98	TA100	TA102	E.Coli WP2	TA98	TA100	TA102	E.Coli WP2
Primary inlet	2	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+
	50	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+
Clarifier	2	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+
	50	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+
Aeration tank	2	-	-	-	-	-	-	-	-
	5	+	-	-	-	+	-	-	-
	10	+	+	-	-	+	+	-	-
	50	+	+	-	-	+	+	-	-
	100	+	+	+	+	+	+	+	+
Final outlet	2	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-

\*+, Ratio greater than 2.0 indicating possible mutagenicity. \*- , Ratio less than 2.0 indicating non mutagenicity.

#### 4.3 Stage 3: Outlet Coming From Aeration Tank:

Aeration is that stage of sewage water treatment plant at which oxidation of sewage waste is done. It was observed that much of the mutagenicity potential of the sewage water is reduced at this particular stage. Findings reveal that, the samples showed negative mutagenicity at 2 $\mu$ l, 5 $\mu$ l dose with TA 98 strain, with mutagenicity ration less than 2.0 (Table1) but as the dose of sample aliquot is increased it showed positive mutagenicity. In June 2008, the number of revertant colonies obtained was high but are very less in comparison to what was obtained with two previous stages (139 induced revertant colonies per 100 $\mu$ l of sample, fig 3). Similarly in December 2008. the samples at 2 $\mu$ l dose level showed negative mutagenicity but at all other higher dose levels, all the samples showed positive mutagenicity. The number of revertant colonies obtained in December was little less than what was obtained in the month of June (123 induced revertant colonies per 100 $\mu$ l of sample, fig 3). Similar

trends of results were obtained in the subsequent year also. Strain TA 100 during first sampling time of June showed negative mutagenicity at 2 $\mu$ l, 5 $\mu$ l and 10 $\mu$ l dose and at all other high dose levels it showed positive mutagenicity, (155 induced revertant colonies at 100 $\mu$ l sample aliquot fig 4) .While in the month of December little seasonal variation were obtained and the sample showed negative mutagenicity at 2 $\mu$ l and 5 $\mu$ l but positive at higher dose levels (155 induced revertant colonies per 100 $\mu$ l of sample fig 4). Similar results were also obtained in the subsequent year that is 2009 when second sampling was done. And with strain TA 102 at all the four sampling times in two years ,all the sample doses shows negative mutagenicity with mutagenicity ratio lower than 2.0 indicating no mutagenic potential except at highest dose (Table 1) The rise in the number of induced revertant with increasing dose level of sample aliquot is observed with dose response curves. In June 2008 the number of induced revertants obtained (168 induced revertant colonies per 100 $\mu$ l of sample, fig 5) is



higher than what was obtained in the month of December 2008 (167 induced revertant colonies per 100µl of sample fig 5). Similar observations are noted in the subsequent year also (Table 2). All the four samples of water from aeration tank outlet collected in two years showed negative mutagenicity with *E. Coli* WP2 assay also at all doses with mutagenicity ratio lower than 2.0 except at highest dose level (Table 1). The rise in the number of induced

revertant with increasing dose level of sample aliquot was indicated in the fig. 6. In June 2008 the number of induced revertants obtained (86 induced revertant colonies per 100µl of sample in the absence of S9 mix, fig 6) is higher than what was obtained in the month of December 2008 (82 induced revertant colonies per 100µl of sample in the absence of S9 mix, fig 6).

**Table 3:** Analysis of physical parameters of samples from Amer road plant

Sr. no.	Physical parameters	Primary inlet	Clarifier underflow	Clarifier final	Aeration tank	Outlet digest
1	Total dissolved solid (TDS) (mg/l)	$1.4 \pm 0.2 \times 10^4$	$1.2 \pm 0.1 \times 10^4$	$1 \pm 0.3 \times 10^4$	$0.8 \pm 0.2 \times 10^4$	$0.6 \pm 0.1 \times 10^4$
2	Dissolved oxygen (DO) (mg/l)	44.76±2	53.15±3.1	75.53±2	92.32±0.4	106.30±4
3	Chlorine concentration (mg/ml)	298.20±2.0	291.10±3.7	269.80±2.4	184.60±2.7	170.40±3.2
4	CO <sub>2</sub> concentration (mg/ml)	268.40±6.4	257.20±2.2	243.20±2.1	233.20±2.3	213.20±2.4
5	Acidity (mg/ml)	440±10	370±9	365±7	310±10	260±6
6	Alkanity (mg/ml)	90±7	70±7	60±5	40±5	30±4
7	Total hardness (CaCO <sub>3</sub> ) (mg/ml)	158±2	142±7	118±4	104±5	76±4
8	Biological dissolved oxygen (BOD) (mg/ml)	30.77±0.5	27.98±0.7	13.99±0.1	11.18±0.4	8.10±0.8
9	pH	5.9±0.2	6.2±0.8	6.5±0.2	6.7±0.1	6.8±0.1

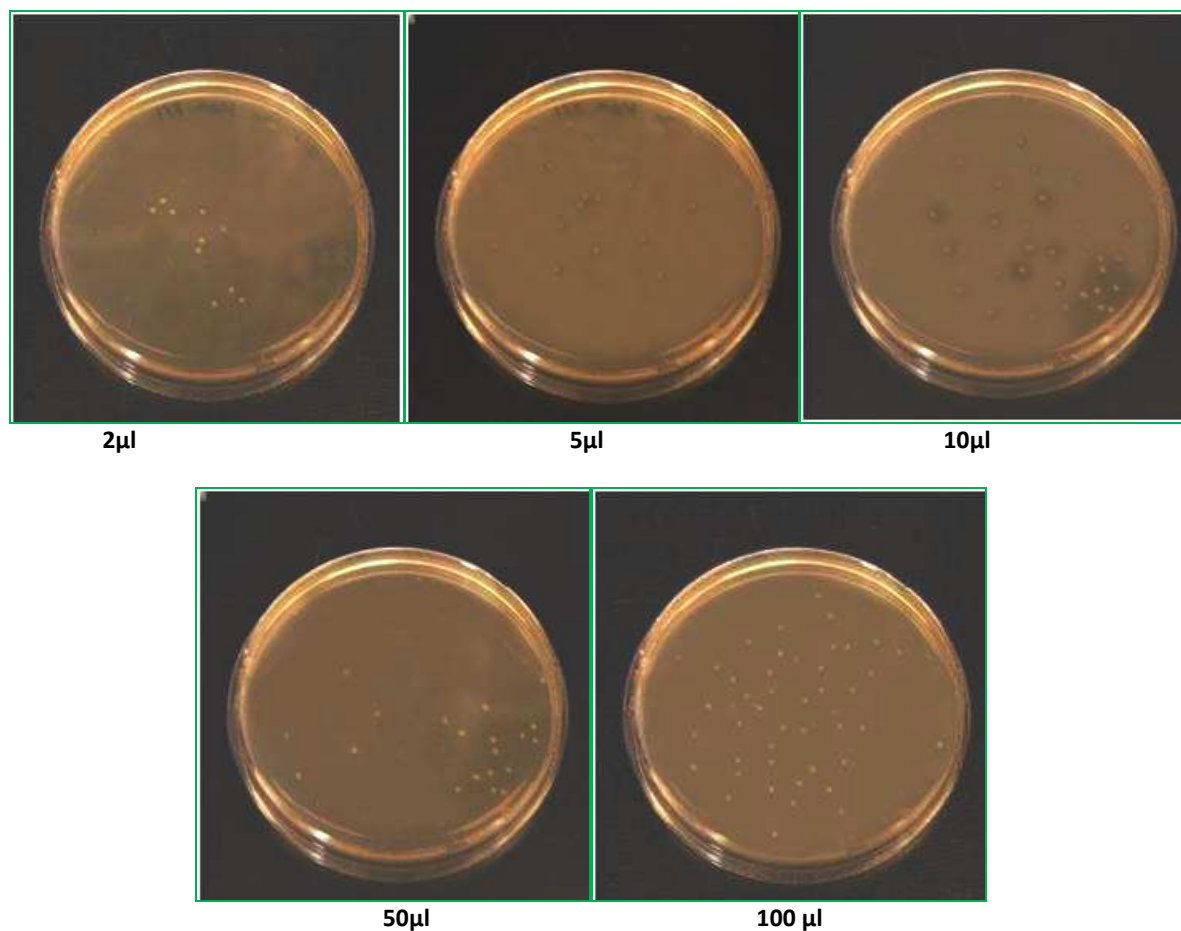
#### 4.4 Stage 4: Digester Outlet:

This sample is the final liquid discharge coming out of the sewage water treatment plant. As this water is used for irrigational and other recreational purposes it is very necessary to check the mutagenic potential of the water coming out of the treatment plant. Note worthy is that, the samples showed negative

mutagenicity at all doses. In June 2008, with strain TA98 the sample showed negative mutagenicity at all doses with mutagenicity ratio less than 2.0 (Table 1), (44 induced revertant colonies per 100µl of sample fig 3) and in Dec 2008 a little lesser number of revertant colonies were observed (41 induced revertant colonies per 100µl of sample fig 1). Similar

trends of results were obtained in the subsequent year also. Strain TA 100, TA 102 and *E.coli*. WP2 all the four samples of water from digester showed negative mutagenicity at all dose levels (Table 1) , (119 induced revertant colonies at 100µl sample with TA 100,fig 4 ) (123 induced revertant colonies per 100µl of sample with strain TA 102, fig 5) (47 induced revertant colonies per 100µl of sample with Strain *E.coli* WP2, fig 6 ).While in the month of December 2008 little seasonal variation were

obtained and the sample showed negative mutagenicity (103 induced revertant colonies per 100µl of sample with strain TA 100, fig 4) (114 induced revertant colonies per 100µl of sample with strain TA 102, fig 5) (44 induced revertant colonies per 100µl of sample with strain *E.Coli* WP2, fig 6). This indicated that with each treatment stage there was reduction in the genotoxicity of the samples which is a clear indication of efficient working of the plant as shown in Fig 7.



**Fig 7: Dose dependent increase in the number of induced revertants of *Salmonella* tester strain TA 98 by treated sewage waters from Final discharge of Common Sewage Treatment Plant Amer road, Jaipur in absence of S9 hepatic fraction in Ames assay (June' 08)**

Evaluation of safety of a Sewage Treatment plant (STP) and Industrial Effluent Treatment Plants (ETP) are till date only done using physico- chemical parameters testing only as shown in Table 3, it was observed that organic matter is efficiently reduced

from the sewage till it becomes the effluent discharged by the STP. The values of BOD, COD, TDS, total nitrogen and total phosphorus showed marked reduction where as the values for dissolved oxygen remarkable up gradation was observed. But, a

complete evaluation of the safety of these plants must include an evaluation of their genotoxicity or mutagenicity also. As the bacterial mutagenicity assays can be carried out in 48 hours, they have been suggested as rapid pre- screens for distinguishing between carcinogenic and non-carcinogenic chemicals, allowing many thousands of components in our environment, not previously tested, to be screened for potential hazards. Histidine requiring R- factor containing frame shift mutant of *Salmonella typhimurium* TA 98, has been shown to be easily mutated by a large number of aromatic compounds (Vincent-Hubert F *et al.* 2012, Aouadene A *et al.*, 2008 ). A good correlation has been obtained by several groups, for a number of carcinogenic aromatic amines in their ability to induce mutation in the above strain and the ability to induce a response in animals (Ames *et al.*, 1983, Gupta *et. al.*, 2009). To check the efficiency of working of this STP sampling was performed at each stage of treatment and the samples were studied using two biological assays. Ames assay with tester strain battery of TA98, TA100 and TA102 and *E.coli* WP2 assay with *E.coli* WP2 strain yielded positive mutagenicity with ratios much higher than 2.0 and the also a clear dose related increase in the number of revertants was seen with all the strains of *Salmonella typhimurium*. Samples taken from aeration tank showed a little lesser genotoxicity with both the assays as at this stage the oxidation of waste was done. At lower dose level the mutagenicity ratio was less than 2.0 indicating no mutagenic potential but as the dose of the sample is increased to 50µl and 100µl the sample showed positive genotoxicity and the number of revertant colonies were also more which is the indication for reduction of genotoxicity of sewage in this particular stage but still the genotoxicity is not properly removed. Samples taken from digester outlet when studied using two bacterial assays, Ames assay and *E.coli* WP2 assays yielded negative mutagenicity both in concern of mutagenicity ratio and number of revertant colonies obtained which is an indication of the proper functioning of the STP and the biosafety of the discharge coming out of the STP.

The strain which is observed as most sensitive among the four strains taken under consideration is TA 102 which is the most recently devised strain and shows maximum sensitivity with maximum number of revertant colonies obtained. This suggest that domestic sewage and municipal waste water consists of mutagens causing base pair substitutions

and the auxotrophic bacterial strain which is most effective among the four strain is TA 102 which along with the frameshift mutation detecting strain TA98 can be used for quick assessment of genotoxicity of domestic waste prior to its discharge.

## 5.0 Conclusions:

Following conclusions can be drawn from the study done:

- With the treatment procedure in STP along with physicochemical parameters bioassays for genotoxicity evaluation should also be included as a part of regular treatment protocol.
- Among all the strains studied it can be concluded that *Salmonella typhimurium* strain Ta 102 was found to be most sensitive with the type of mutagens present in the sample thus along with TA 98 only can satisfactorily detect the presence of mutagens in the sample.
- As the procedure for Ames assay is easy, efficient , economic and less time consuming, it is an efficient tool for the assessment of genotoxicity evaluation of the discharges coming out of waste water treatment plants.

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