Int. J. Chem. Sci.: 7(1), 2009, 125-137

USE OF MICROBIAL BIOASSAY IN MONITORING SEWAGE TREATMENT PLANT

NUPUR MATHUR*, PRADEEP BHATNAGAR and TEENA YADAV

Environmental Toxicology Unit, Department of Zoology, University of Rajasthan, JAIPUR – 302004 (Raj.) INDIA

ABSTRACT

Wastewaters are treated through different processes at municipal wastewater treatment plants. Nevertheless they often contain mutagens especially when the proportion of industrial wastewater in comparison to municipal wastewater is high. Properly designed waste treatment systems can remove or destroy many of the harmful contaminants in industrial wastewaters. Such effluents can then be safely discharged to receiving waters. Monitoring parameters that are used to assess the efficiency of these municipal waste water plants are only physico-chemical such as BOD, COD, pH, TDS and appearance in terms of color. No biological parameters are used to check the efficiency of these plants. From the results obtained in this study, it can be concluded that the treatment procedure carried out at sewage treatment plant, Amer Road is successful in treating the wastewater in terms of physico-chemical parameters but are inefficient in removing genotoxicants. The mutagenic compounds present in the sewage can have a negative effect on aquatic life of natural water bodies, where the treated wastewater is finally discharged.

Key words: Municipal wastewater, Physico-chemical parameters, Genotoxicants, Mutagenicity, Ames test

INTRODUCTION

Pollution and water quality degradation interfere with vital and legitimate water uses at any scale, i. e. local, regional or international. Pollution may result from point sources or diffuse sources (non-point sources). The major point sources of pollution to freshwaters originate from the collection and discharge of domestic wastewaters, industrial wastes or certain agricultural activities. Untreated, or inadequately treated, sewage disposal is probably still the major point source of pollution to the world's waters. Multi-dimensional approaches to water quality assessment have, therefore, become an inevitable necessity.

* Author for correspondence; Tel.: +91-141-2707927; E-mail: nupurmathur123@rediffmail.com

Sewage treatment is a multi-stage process to renovate wastewater before it reenters a body of water. The goal of treatment is to reduce or remove organic matter, solids, nutrients and disease causing organisms and other pollutants from wastewater. Monitoring different physico-chemical parameters is necessary to verify proper operation of treatment plant. Thus, usually monitoring parameters that are used to asses the efficiency of most of the municipal waste water treatment plants are only physico-chemical such as BOD, COD, pH and TDS. However, such analytical studies are not enough regarding the potential effects of wastewaters on human health. Consideration of only physico-chemical analyses has been thought to be inadequate in protecting the aquatic environment against hazardous discharges[1].

Tests to establish the sub-lethal carcinogenic, teratogenic or mutagenic capacity of chemicals in the aquatic environment are at present poorly developed. However, with the production and use of thousands of new chemical substances each year (many of which may eventually be discharged to fresh-waters) such tests are becoming of increasing importance, particularly with respect to human health. Routine biological monitoring programmes should therefore include biological testing. An example of a biological test, which has been standardized, is the reverse mutation assay with *Salmonella typhimurium* or the "AMES" test[2, 3].

The present study was planned to evaluate the physico-chemical and genotoxicity parameters of industrial effluents and different levels of municipal wastewater treatment plant of Jaipur.

EXPERIMENTAL

Materials and methods

Sampling site -The wastewater samples were collected from sewage treatment plant Brahampuri, Amer road, Jaipur. It has capacity to treat 27 Megalitres per day.

Sample collection - Samples were obtained from the sewage treatment plant at different levels of treatment to measure different physico-chemical parameters and mutagenicity. Sampling was done in months of June and July from year 2005 to 2007. Samples were collected from five different stages of treatment:

(i) **S1-Inlet chamber :** The inlet chamber receives wastewater from sever lines. There are two in the sewage treatment plant. First one is 50 mm screen and next is a fine screen of 5 mm. Both these screen removes solids from wastewater.

(ii) **S2-Grit chamber :** After screening the wastewater passes through a grit chamber where the grit particles (size < 5 mm) settle down at the bottom. This grit is collected and disposed off in the nearby land.

(iii) **S3-Aeration chamber**: After the removal of grit, the wastewater goes into the aeration tank. This tank was 25-30 feet deep and divided into eight zones. Water from grit chamber enters from one end and moves through these zones and finally to clarifier tank. In each zone, there were pipes with perforated ending dipped into water, to aerate the water. The bacteria degrade the organic content of wastewater which forms sludge. This sludge also contains bacteria and other aerobic organism.

(iv) **S4-Return sludge:** This sludge is collected and some part of it is mixed with the wastewater in the aeration tank. This is known as return sludge. Return sludge is mixed with water from grit chamber and then aerated in aeration chamber. Remaining sludge is disposed off in the nearby land.

(v) **S5-Effluent (Clarifier)**: This wastewater moves to clarifier and is finally send to Jalmahal lake. In the clarifier, no chemicals are mixed to further clear the water.

Physico-chemical analysis

Systronics water analyser 371, a microcontroller based instrument was used for measuring pH, mV, dissolved oxygen (D. O.), salinity, conductivity, TDS, temperature and turbidity in water samples. BOD and COD were analyzed according to protocols given in APHA manual[4].

Genotoxicity analysis : Microbial mutagenicity assay (Ames test)

The *Salmonella*/microsome reversion assay was conducted using the plate incorporation procedure described by Ames et al.[2] and revised by Maron and Ames[3]. TA 98 and TA 100 strains of *Salmonella typhimurium* were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (IMTech), Chandigarh (India). Samples were tested on duplicate plates in two independent experiments. Five dose levels of individual samples were tested. Positive controls used were 2-nitrofluorene for TA 98 (2.5 µg/plate, 208 revertants) and sodium azide for TA 100 (5 µg/plate, 2969 revertants). Sterile distilled water was used as the negative control (TA 98 : 42 revertants; and TA 100 : 142 revertants). Fresh solutions of the reference mutagens were prepared immediately before the beginning of each experiment. All tester strains were maintained and stored according to standard methods[5]. The strains were regularly checked for genetic markers. All reagents used were of analytical grade, supplied by Himedia Laboratories Limited (India) and Sigma-Aldrich.

RESULTS AND DISCUSSION

A complete assessment of water quality is based on appropriate monitoring of physico-chemical and biology components. Summary characteristics, such as total dissolved solids, conductivity and redox potential provide a general classification of water bodies of a similar nature. Mineral content, determined by the total dissolved solids present, is an essential feature of the quality of any water body resulting from the balance between dissolution and precipitation. Oxygen content is another vital feature of any water body because it greatly influences the solubility of metals and is essential for all forms of biological life.

In the present study, pH of samples obtained in June and July were 7.43 and 7.30, respectively. According to Indian standards for sewage effluents discharge, pH value should be 5.5 to 9. Thus, pH of all the samples was well in the permitted limit. The temperature of effluent did not exceed 45 ^{o}C and the temperature measured was between 30.8 ^{o}C to 31.6 ^{o}C. The TDS (Total Dissolved Solids) of influent was 0.94 ppt and that of effluent 0.92 ppt in June. In the next sampling (in July), the values were 1.64 ppt and 0.61 ppt respectively. Conductivity is related to the concentration of ionized substances in the water. Mostly dissolved inorganic substances in water are in the ionized form and hence, contribute to conductance. Its value varied from 1.92 ms – 1.97 ms in the effluent water. The conductivity of distilled water was less than 29 μs and therefore, values of effluent water showed presence of ions in them (Tables 1 and 3).

The analysis for DO is a key test in water pollution control activities and wastewater treatment process control. The DO test provides information about the condition of the wastewater for the operator to make process control decisions. In all samples, at different levels of treatment, the D. O. was found to be lowest in return sludge i. e. 1.5 ppm. The return sludge is the residue from aeration chamber, which is again mixed with wastewater. Natural water has a BOD varying from 110 to 440 mg/L and conventional sewage treatment reduces this by 95%. The water entering in the studied treatment plant had a BOD value of 8.7 mg/L and 13.8 mg/L. After the entire treatment procedure, it was reduced to 5.6 mg/L and 6.7 mg/L, respectively. COD is also a value, which can be related empirically to BOD, organic carbon or organic matter for samples for specific sources. COD values shows organic load of the samples but cannot distinguish between biologically inert and biologically oxidizable substances. The values of Inlet Chamber (S1) and Effluent sample COD were 48.35 and 35.40 mg/L for sampling in June. In second sample these values were 88.15 and 41.20, respectively. Thus, the reduction of BOD and COD values again shows proper operation of treatment plant (Tables 1 and 3).

Table 1 : Physico–chemical parameters of sample obtained in June

Parameters	Distilled water	Inlet chamber S1	Grit chamber S2	Aeration chamber S3	Return sludge S4	Effluent (clarifier) S5
pH	7.74	7.79	7.71	7.56	7.50	7.43
mV	-35 mV	-41mV	-40 mV	-39 mV	-39 mV	-49 mV
Temperature	32.6ºC	30.5ºC	31ºC	30.4ºC	30.4 ºC	30.8ºC
Salinity	0.01 ppt	1.04 ppt	1.05 ppt	1.02 ppt	1.07 ppt	1.01 ppt
TDS	12.1 ppm	0.04 ppt	0.96 ppt	0.92 ppt	0.95 ppt	0.92 ppt
Conductivity	28.8µS	2.02mS	2.04 mS	1.96 mS	2.11 mS	1.97 mS
Turbidity	0.29 NTU	111 NTU	97 NTU	100 NTU	106 NTU	76 NTU
Dissolved oxygen (D. O.)	3.5 ppm	2.8 ppm	2.5 ppm	2.6 ppm	1.5 ppm	3.1 ppm
BOD (mg/L)	0.6	8.7	8.4	6.7	6.8	5.6
COD (mg/L)	2.25	48.35	46.00	40.52	41.81	35.40

Table 2 : Mutagenicity ratio of sample in June with strain TA 98 and TA100 of *S. typhimurium*

Salmonella strain	Doses (µL)	Sample (June)				
		S1	S2	S3	S4	S5
TA 98	2	+	+	+	+	+
	5	+	+	+	+	+
	10	+	+	+	+	+
	50	+	+	+	+	+
	100	+	+	+	+	+

Cont…

Salmonella strain	Doses (µL)	Sample (June)				
		S1	S2	S3	S4	S5
TA 100	2	+	+	-	+	+
	5	+	+	+	+	+
	10	+	+	+	+	+
	50	+	+	+	+	+
	100	+	+	+	+	+

+ : Ratio greater than 2.0 indicating possible mutagenicity.
– : Ratio less than 2.0 indicating non- mutagenicity.

Table 3 : Physico–chemical parameters of sample obtained in July

Parameters	Distilled water	Inlet chamber S1	Grit chamber S2	Aeration chamber S3	Return sludge S4	Effluent (clarifier) S5
pH	6.64	7.88	7.73	7.76	6.82	7.30
mV	-42 mV	-51 mV	-47 mV	-43 mV	-43 mV	-41 mV
Temperature	32ºC	31.9ºC	31.6ºC	31ºC	31.3ºC	31.6ºC
Salinity	0.001 ppt	0.57 ppt	0.58 ppt	0.58 ppt	0.58 ppt	0.57 ppt
TDS	9.52ppm	1.64 ppt	0.62 ppt	0.61 ppt	0.74 ppt	0.61 ppt
Conductivity	27.4 µS	1.89 mS	1.92 mS	1.93 mS	1.90 mS	1.92 mS
Turbidity	0.01NTU	136 NTU	120 NTU	118 NTU	95 NTU	58 NTU
Dissolved oxygen (D. O.)	6.8 ppm	5.5 ppm	4.7 ppm	2.9 ppm	1.5 ppm	4.8 ppm
BOD (mg/L)	0.6	13.8	7.5	6.2	6.0	6.7
COD (mg/L)	2.33	88.15	45.00	38.24	36.39	41.20

Table 4 : Mutagenicity ratio of sample in July with strain TA 98 and TA100 of *S. typhimurium*

Salmonella strain	Doses (µL)	Sample (June)				
		S1	**S2**	**S3**	**S4**	**S5**
TA 98	2	-	+	+	-	+
	5	+	+	+	+	+
	10	+	+	+	+	+
	50	+	+	+	+	+
	100	+	+	+	+	+
TA 100	2	-	-	-	-	-
	5	-	+	-	-	+
	10	+	+	+	+	+
	50	+	+	+	+	+
	100	+	+	+	+	+

+ : Ratio greater than 2.0 indicating possible mutagenicity.
- : Ratio less than 2.0 indicating non- mutagenicity.

In contrast to the chemical quality of water bodies, which can be measured by suitable analytical methods, the description of the biological quality of a water body is a combination of qualitative and quantitative characterization ways. Biological assessment is often able to indicate whether there is an effect upon an ecosystem arising from a particular use of the water body. It can also help to determine the extent of ecological damage. Biological methods are cheap and can be easily integrated into other studies. Compared with physico-chemical analysis, much less equipment is necessary and a large area can be surveyed very intensively in a short time, resulting in a large amount of information suitable for later assessment. Further no special equipment or facilities are needed for basic methods. Therefore, to predict the additive, synergistic or antagonistic effects of various chemicals on biological systems, use of bioassays is very essential. In this respect, use of biological assays to evaluate the toxicity of wastewater effluents, will be of prime importance.

The response of the Ames tester strain is expressed as the mutagenicity ratio, which is the ratio of average and induced revertants on test plates (spontaneous revertants +

induced revertants). Mutagenicity ratio of 2.0 or more is regarded as a significant indication of mutagenicity provided all controls confirm to specifications[5].

The mutagenicity ratio calculated for all samples indicates significant mutagenicity in samples. For June sample, inlet chamber and return sludge have negative response for TA 98 at 2 µL doses. Thus, they were negatively mutagenic towards TA 98 at low dilution of sample. At higher dilutions; however, all were positively mutagenic. With TA 100 all samples have a mutagenicity ratio less than 2.0 for 2 µL doses. But at higher doses, they were again positively mutagenic. During July, only one sample that is Aeration chamber (S3) shows negative response for TA 100 at lowest dose level i. e. 2 µL. Apart from that, all other samples were positively mutagenic.

More detailed observations were made with the dose-response curves of the water treatment plant samples (Fig. 1 to 4).

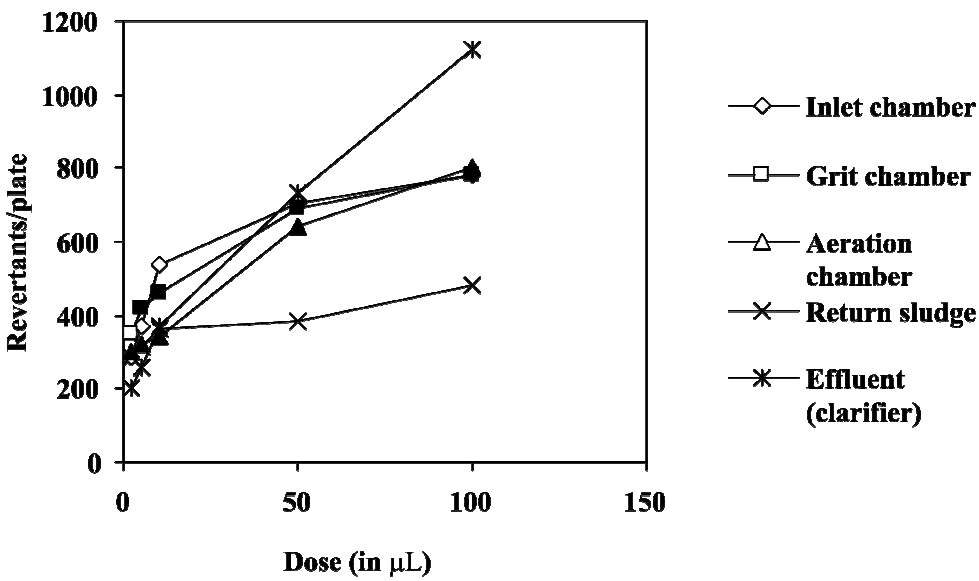

Fig. 1 : Dose response curve of sample in June with strain TA 98 of *S. typhimurium*

A clear dose dependent increase in the number of revertants was seen in all the concentrations tested. The water samples from different stages of treatment showed high mutagenicity as seen from the number of induced revertants obtained, over the control value, with strain TA 98 and TA 100. Significant difference was observed in the number of induced revertants obtained with strain TA 98 during the months of June (779 induced

revertants, per 100 μL of inlet chamber sample) (Fig. 1) and July (554 induced revertants, per 100 μL of inlet chamber sample) (Fig. 3).

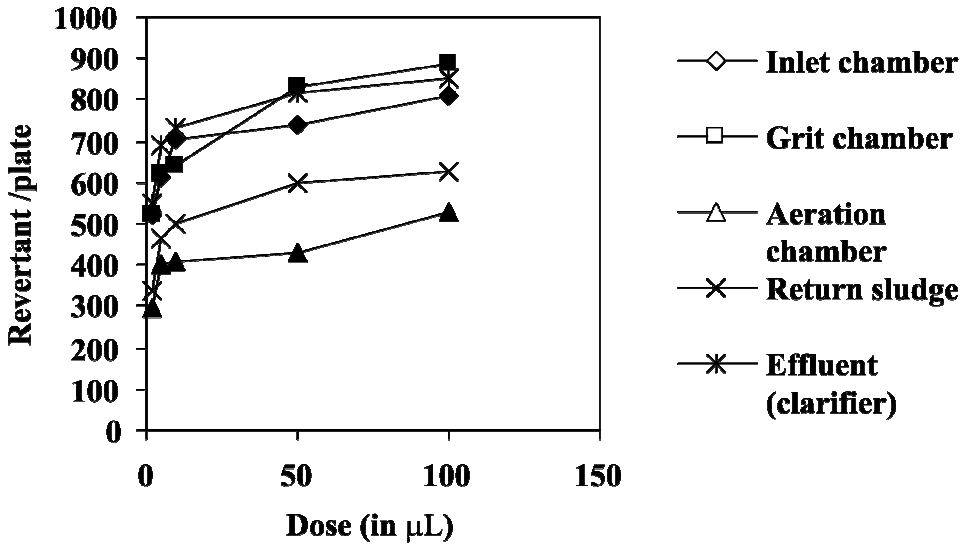

Fig. 2 : Dose response curve of sample in June with strain TA 100 of *S. typhimurium*

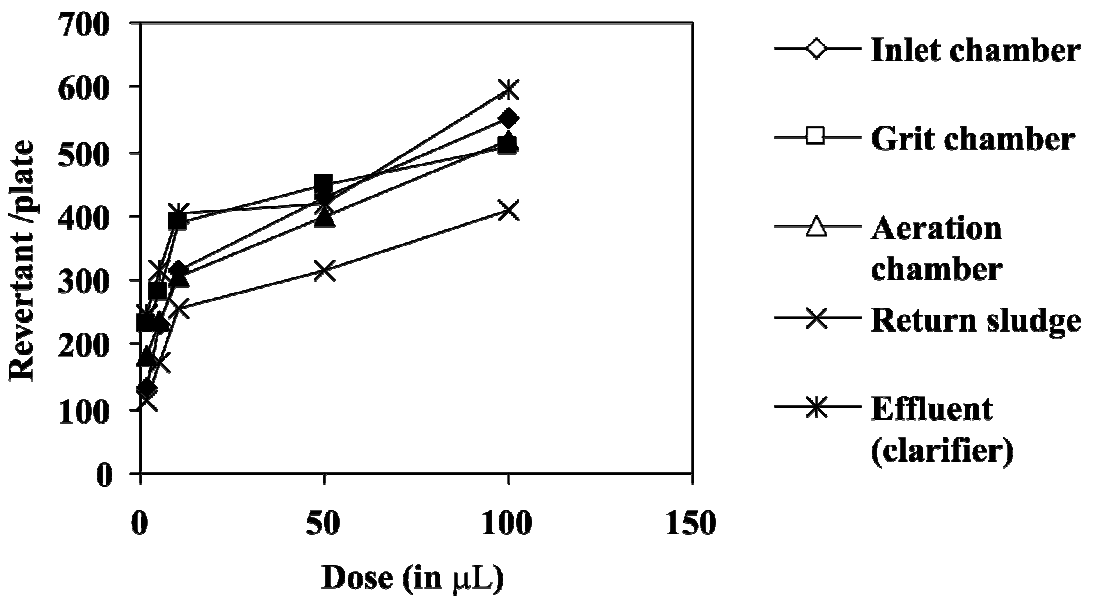

Fig. 3 : Dose response curve of sample in July with strain TA 98 of *S. typhimurium*

Similarly, the effluent waters, which are finally discharged to Jalmahal lake showed difference in number of induced revertants during the months of June (1120 induced revertants, per 100 µL sample) (Fig. 1) and July (596 induced revertants, per 100 µL of sample) (Fig. 3). Thus in all the five samples viz. Inlet chamber, Grit chamber, Aeration chamber, Return sludge and effluent (clarifier) higher mutagenicity was observed during the month of June. With strain TA 100 also, the numbers of induced revertants observed in June (807 induced revertants, per 100 µL of inlet chamber sample) (Fig. 2) and July (540 induced revertants, per 100 µl of inlet chamber sample) (Fig. 4) were comparable to those seen in the month of June (852 induced revertants, per 100 µL sample) (Fig. 2) and July (623 induced revertants, per 100 µL of sample) (Fig. 4). This clearly indicates that mutagenicity shows variations on monthly basis.

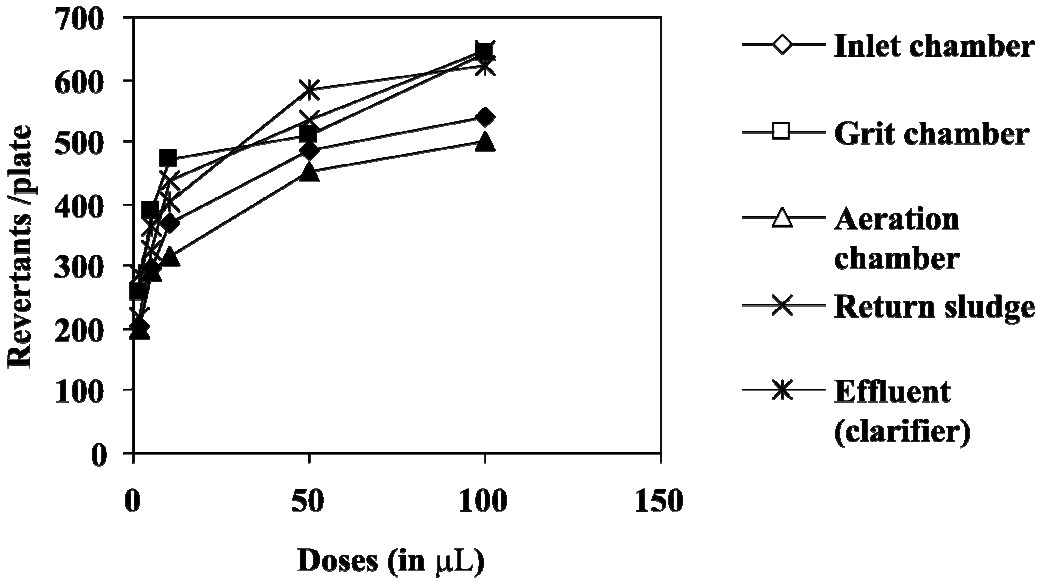

Fig. 4 : Dose Response Curve of Sample in July with strain TA 100 of *S. typhimurium*

Comparisons, between influent and effluent waters of treatment plant revealed that the number of revertants obtained were almost similar for both the samples. Number of induced revertants with strain TA 98 during the months of June (779 induced revertants, per 100 µL of inlet chamber sample) (Fig. 1) was lower than those of effluent waters, which are finally discharged (1120 induced revertants, per 100 µl sample) (Fig. 1). Similarly, number of induced revertants with strain TA 100 during the months of June (807

induced revertants, per 100 μL of inlet chamber sample) (Fig. 1) was lower than those of effluent waters, which are finally discharged (857 induced revertants, per 100 μL sample) (Fig. 1). During July month also, same trend was observed. This is an unexpected finding as the overall mutagen concentrations in the final effluents were found to be no less than those present in the influent wastewaters. Comparison of the mutagenic response of CETP influent and effluent waters; thus revealed that the treatment method employed at this plant has failed to remove mutagenic substances, which are present in Jaipur wastewaters.

These results are in agreement with several previous studies, which have shown that many of the conventional and advanced wastewater treatment plants have been unsuccessful in adequately removing potentially hazardous chemical mutagens from the wastewaters[6-8]. Many scientists have also shown that treatment plants are in fact capable of introducing mutagens[8,9].

If the treated effluent generated by the municipal wastewater treatment plants are not monitored, it would be like transferring the waste from one form to another[10]. As not much work has been done regarding the biological characteristics of waste generated from these treatment plants, with this project, we wish to incorporate the biological toxicity testing of waste characterization in monitoring programme of all treatment plants. This would ensure that the effluent being released from treatment plants are completely safe for the disposal in environment. Due to the discharge of treated sewage water into rivers and lakes; there is concern that genotoxic chemicals may pose risk to organisms in the ecosystems as well as humans, by accumulation in the food chain.

CONCLUSION

From the results of this study, it can be concluded that the treatment procedure carried out at 27 MLD sewage treatment plant, Amer Road is successful in treating the wastewater in terms of physico-chemical parameters but is inefficient in removing genotoxicants. The mutagenic compounds present in the sewage can have a negative effect on aquatic life of Jalmahal, where the treated wastewater is finally discharged. In order to efficiently assess the presence of mutagens in the water, in addition to the chemical analysis, mutagenicity/genotoxicity assays should be included as additional parameters in water quality monitoring programs. The present study also emphasizes the importance of the Ames *Salmonella* mutagenicity assay as a short-term test. It can be used as complement

to other ecological, toxicological and conventional chemical tests and for establishing priorities of pollution control. 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 in one species have the potential to produce similar effects in other species. This microbiological test could thus be of use in toxicity identification and evaluation (TIE) and toxicity reduction and terrestrial (TRE) schemes applied in aquatic and terrestrial environments.

REFERENCES

1. L. Lambolez, P. Vasseur, J. F. Ferard and T. Gisbert, the Environmental Risks of Industrial Waste Disposal. An Experimental Approach Including Acute and Chronic Toxicity Tests. Ecotoxicol. Environ. Saf., **28**, 317-328 (1994).

2. B. N. Ames, J. Mccann and E. Yamasaki, Methods of Detecting Carcinogens and Mutagens With the Salmonella/Mammalian Microsome Mutagenicity Test, Mut. Res., **31**, 347-364 (1975).

3. D. M. Maron and B. N. Ames, Revised Methods for the Salmonella Mutagenicity Test, Mut Res., **113**, 173-215 (1983).

4. APHA Standard Methods for the Examination of Water and Wastewater, (19^{th}) Edition), American Water Works Association, 6666 Quincy Ave., Denver CO, 80235, 1-800-926-7337 (1995).

5. K. Mortelmans and E. Zeiger, The Ames Salmonella / Microsome Mutagenicity Assay, Mut. Res., **455**, 29-60 (2000).

6. S. M. Rappaport, M. G. Richard, M. C. Hollstein and R. E. Talcott Mutagenic Activity in Organic Wastewater Concentrates, Environ. Sci. Technol., **13**, 957-961 (1979).

7. J. Saxena and D. J. Schwartz, Mutagens in Wastewaters Renovated by Advanced Wastewater Treatment, Bull. Environ. Contam. Toxicol., **22**, 319-326 (1979).

8. J. R. Meier and D. F. Bishop, Evaluation of Conventional Treatment Processes for Removal of Mutagenic Activity from Municipal Wastewaters, Journal WPCF, **57(10)**, 999-1005 (1985).

9. W. O. K. Grabow, P. G. Van Rossum, N. A. Grabow and R. Denkhaus, Relationship of the Raw Water Quality to Mutagens Detectable by the Ames Salmonella / Microsome Assay in a Drinking Water Supply, Water Research, **15**, 1037-1043 (1981)

10. N. Mathur, P. Bhatnagar, K Mohan and P. Bakre, Mutagenicity Evaluation of Chemical and Biological Sludge from Common Effluent Treatment Plant, Chemosphere, **67**, 1229–1235 (2006)

Accepted : 06.10.2008