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SAFETY ASPECTS OF EDUCATIONAL INSTITUTE'S DRAINAGE WATER

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ABSTRACT

Effluent discharges of educational institutions as a possible source of environmental pollution has not been reported yet. Therefore, preliminary analysis of these effluents was carried out to assess the genotoxic potential along with the physicochemical parameters. In the present study, effluent samples were collected during pollution loading period and non-loading period from different educational institute's drainage. Tap water was also collected as influent water source to compare the effluent water quality with it. Collected effleunts were analyzed for pH, temperature; BOD, COD and genotoxicity by Ames test using *S.typhimurium* TA 98 and TA 100 at 5 doses level i.e. 2 µl, 5µl, 10 µl, 50 µl and 100 µl. Compared to tap water, Educational Institute's drainage water was found to be polluted and genotoxic with either one strain or both strain of *S.typhimurium* which reveals the presence of frameshift and basepair mutagens in it. These genotoxicants may arise in effluent due to use of various chemicals, dyes etc. in experiments or may be produced due to the interaction of various chemicals in the effluent. Level of genotoxicants was found to be higher in experimental timings as compared to non-experimental timings. Further, the genotoxic effect reduced with the addition of S9mix. Hence, our study clearly indicates the presence of genotoxicants in the Educational Institute's drainage water and it can be a new source of environmental pollution. It is unsafe for discharge in untreated form.

Key words: institutional drainage, effluent, genotoxicity, mutagenicity.

1. INTRODUCTION

Educational institute is one of the biggest growing industries where wide range of chemicals at different concentrations used in experimentation. Some of these, if not all, to some extent are ending up in institutional effluents which is generally a mixture of various wastes and chemicals. The volumetric proportion of the daily discharge of these effluents rarely exceeds industrial discharges but still it is of great concern from an environment and biosafety point of view. As institutional effluent may be mutagenic and have an impact on the environment and human health, study of this is utmost importance to check the pollution on its source. In the recent years, attention has drawn towards assessing the potential health hazards of effluents. Many reports have been published on physico-chemical, toxicological and genotoxicological analysis of industrial discharges, sewage discharges and various polluted water bodies (Claxton *et. al.*, 1998; Haddad et *al.*, 1998; Prakash and Somashekhar, 2006; Khurana and Bansal, 2008; Kulshreshtha *et al.*, 2008; Garg et *al.*, 2009 and Mulani et *al.*, 2009). However, the knowledge about the institute's wastewater toxicity is scarce.

Identifying compounds present in water on a routine basis is not feasible in any case because of

time and expense required. Besides, it is also extremely difficult to quantify the risk associated with these chemical pollutants because they usually occur in concentrations too low to allow analytical determination.

A growing concern for continuing deterioration of environment and public health enforced us to assess the quality of effluent and genotoxicity hazard posed to environment and public health. Quality of effluents can be assessed by short-term genotoxicity assays which have proved to be an important tool in studying of effluents because of their simplicity, sensitivity to genetic damage, speed, low cost of experimentation and small amount of sample required (Ames et *al.*, 1975; Ames, 1979) Ames assay is valid and sensitive assay which is mentioned in an OECD guideline 471 (July, 1997) and is the part of battery of tests conducted for genotoxicity, accepted by USFDA and ICH. Thus, the present study was planned to assess the genotoxic potential of institutional drainage effluent along with physico-chemical parameters which is helpful in deciding its safety perspectives.

2. MATERIALS AND METHODS

2.1 Collection of samples

Tap water is a source of influent water for all activities going in an institute which comes out from these institutes as effluent. Tap water becomes polluted with many chemical compounds during use in several processes. In the present study, effluent characteristics were compared with tap water to find out that whether influent water source is previously contaminated or it becomes contaminated after use. Therefore, drainage water samples of institutes(A, B, C, D and E) were collected for 15 days during college hours and after college hours which are considered as pollution loading period (PLP) and non-loading period (NLP) respectively. Tap water was also collected as influent water source. All samples were collected in pre-sterilized containers and stored at 4ºC until analyzed.

2.2 Physicochemical parameters

Collected effluent samples were analyzed for pH, temperature by using probes of water analyzer kit as given in instruction manual. Initially, probes were calibrated according to instruction given in manual and then used for measuring parameters. COD and BOD were analyzed by open reflux method and membrane electrode method respectively as given in APHA (1995).

2.3 Ames mutagenicity/ genotoxicity test

The Salmonella/microsome reversion assay was conducted using the plate incorporation procedure described by Ames *et al.* (1973) and revised by Maron and Ames (1983). Effluents and influents were tested with TA 98 (frameshift mutagens detector) and TA 100 (basepair mutagens detector) strain of *S. typhimurium*, which was obtained from Microbial Type Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology (IMTech), Chandigarh (INDIA). The tester strain genotypes (Histidine requirement, *rfa* mutation, *uvr* B and R-factor) were confirmed immediately after receiving the cultures.

Each effluent was tested on duplicate plates at five dose levels (2µl, 5µl, 10 µl, 50µl and 100µl). Positive control used for TA 98 and TA 100 was 2-Nitrofluorine (1 µg / plate: 104 revertants) and Sodium azide (1 µg / plate: 594 revertants) respectively. On adding metabolic activation system i.e., S9 mix (prepared from uninduced liver of mouse as explained by Prival et *al.*, 1984) revertants per plate in positive control increased as TA 98 (1 µg / plate: 481) and TA 100 (1 µg / plate: 897). Sterile distilled water was used as negative control. Fresh solutions of the reference mutagen were prepared immediately in Dimethylsulfoxide before the beginning of each experiment. The revertant colonies were clearly visible in a uniform background lawn of auxotrophic bacteria.

2.4 Data analysis

The pH, temperature, COD and BOD of each sample was analyzed twice a day. The data obtained for each sample was pooled. Mean and Standard deviation was calculated for each sample.

The most common method of evaluation of data from the Salmonella 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 compounds doubles or more than doubles, the mean spontaneous mutation frequency obtained on the day of testing, and 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 *Salmonella typhimurium*.

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

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). The following values of spontaneous revertants were obtained for the two strains: Revertant/plate: without metabolic activation TA 98 (42), TA 100 (165); with metabolic activation, slightly higher values were obtained: TA 98 (44), TA 100 (168). Mutagenicity ratio of 2.0 or more is regarded as a significant indication of mutagenicity (Maron and Ames, 1983).

3. RESULT AND DISCUSSION

In the present investigation, temperature of the effluent collected during effluent loading time was not found to be significantly differing from tap water. Besides this, there was no significant difference in pollution loading time and non-loading time (Table 1). Similarly, pH of almost all institutional water samples was slightly basic in effluent loading time and non-loading time. The pH of effluent was not found to be significantly differing from tap water (Table 1). Hence, no significant change in pH and temperature in institutional effluent were measured due to the discharge of chemicals.

Table 1*: Physicochemical properties of water sample collected from drainage of five institutes (A, B, C, D, and E)*

S no	Sample	Temperature (^{o}C)			pH			COD (mg/l)			BOD (mg/l)		
		TW	DCH	ACH	TW	DCH	ACH	TW	DCH	ACH	TW	DCH	ACH
1	A	27.4± 0.0	30.5± 0.4	28.3± 0.2	7.0± 0.0	7.7±1.0	6.9± 0.8	0.0	9200 ± 2205	733± 393	0.0	80.6± 8.0	82.6± 6.41
2	B	28.2± 0.0	29.0± 0.7	27.2± 0.6	7.1± 0.0	7.1±0.8	7.2± 0.5	0.0	13266 ± 2458	733± 467	0.0	75.6± 7.0	51.6± 13.3
3	C	28.2± 0.0	29.7± 0.4	28.4± 0.2	7.5± 0.0	7.9±1.0	8.1± 1.0	0.0	10533 ± 3304	466± 163	0.0	57.1± 9.2	42.8± 15.3
4	D	33.8± 0.0	32.3± 0.2	35.7± 0.7	7.8 0.0	7.6±1.2	8.3± 0.7	0.0	13133 ± 2296	666± 326	0.0	66.3± 12.4	95.8± 20.7
5	E	31.2± 0.0	31.6± 2.0	31.0 ±0.7	7.8± 0.0	7.8±0.9	7.8± 0.9	0.0	12400 ± 2039	866± 467	0.0	75.3± 11.6	116.3± 30.2

DCH: During college Hours, ACH: After college Hours, TW: Tap Water

Effluents of all institutes were found to possess very high COD i.e. ranging 9000-13,000mg/l and 466-866mg/l during PLP and NLP respectively. Similar, findings were obtained with BOD i.e. 57-80mg/l and 47-116mg/l during PLP and NLP respectively. Secondly, both COD and BOD were found to be increased significantly during the PLP compared to NLP (Table 1). This reflects that effluents of institutes must be treated before discharge as their BOD and COD was found to be beyond the discharging limits i.e., 30mg/l and 350 mg/l respectively (CPCB, 2000).

In genotoxicity analysis, influent water i.e., tap water was not found to be genotoxic with both strains of *Salmonella typhimurium* in the presence and absence of metabolic activation system of mouse liver revealing the absence of frameshift and basepair mutagens (mutagenicity ratio<2) in it. In contrast to this, educational institute's effluents collected during pollution loading time were found to have both frameshift and basepair type of genotoxicity as detected by strains of *Salmonella typhimurium* TA 98 and TA 100 respectively at all dose level. Out of the 5 samples, Sample A did not show any genotoxicity both during and after experimental timings. While the other 4 samples, B, C, D and E, showed genotoxicity, which increased with increase in the dose level (Table 2). Moreover, on adding S9 mix, genotoxicity was not found to be decreased as revealed by the number of revertants per plate. This revealed that liver enzymes of eukaryotic system are not able to detoxify genotoxic compounds present in the institutional effluent. Furthermore, during pollution loading time mutagenicity was found to be higher compared to non-pollution loading time which is possibly due to the chemicals in the effluent.

The mutagenicity of institutional effluents has not been reported so far. The study on genotoxicity in educational institute's drainage effluent has revealed the presence of genotoxic compounds. These genotoxic compounds may be used in the laboratories or may be formed due to the reaction of various chemicals. It is possible that the higher experimental activity in the educational institutes leads to effluent discharge in larger quantity, and thus a higher effluent genotoxicity than tap water. Besides this, the range of experimental BOD and COD was not matching with the effluent discharge standards as prescribed by CPCB (2000). Therefore, this problem must be taken seriously that release of these compounds in to the environment not only pollutes the environment but may causes health hazards due to mutagenicity.

New institutes are setting up in the outskirts of cities and releasing water to the nearby area. Sometimes this waste water is used for irrigation purpose due to scarcity of water resources and ignorance of the institutional drainage water may lead to the spreading of mutagenicity and associated diseases like cancer, and therefore, cannot considered as safe for discharge. The results of these studies must, however, be interpreted with caution because the exposure to genotoxic water was only estimated and not really measured. However, these results emphasized the importance of the determination of water genotoxicity with an aim at controlling the population exposure and need of establishing suitable treatment plant for the treatment of effluents coming out from institutes.

4. CONCLUSION

Educational institutes represent an incontestable release source of many chemicals compounds in the surrounding environment due to laboratory activity into wastewater. However, the knowledge about the educational institute's effluent toxicity is scarce and must be studied. Keeping this in mind, the educational institute drainage effluent was analyzed for physicochemical parameters and genotoxicity and the data was compared with influent (tap water). Institute's effluents were found to possess high BOD and COD, therefore considered as severely polluted during the effluent loading period. Besides this effluents were found to be mutagenic with both strains of *S.typhimurium*. Moreover, mutagenicity was found to present in pollution loading and non-loading period. It is hypothesized that the possible reason of this mutagenicity is either mutagenic chemicals or conversion of non-mutagenic compound into mutagenic compounds through chemical reactions. From public health standpoint, our

preliminary investigation suggests that institutional effluents are not safe to be released in environment. Since these are one of the sources of discharge of genotoxic compounds in wastewater, efforts must be undertaken by institute in order to integrate the knowledge and the control of their wastewaters, and thus the environment management, in the infection and environmental control programs.

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