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Bioassay evaluation of toxicity reduction in common effluent treatment plant

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Abstract : This paper investigates the utility and validity of bioassay toxicity testing using *Daphnia magna straus* as test organism for monitoring the common effluent treatment plant (CETP) receiving both industrial as well as domestic effluent. The average daphnia toxicity (Gd) at inlet, after primary settling tank (PST), secondary settling tank (SST) and tertiary treatment unit were reported as Gd-16, Gd-12, Gd-4 and Gd-1 respectively. However, a cumulative percentage removal in toxicity after PST, SST and tertiary treatment units was observed as 25%, 75% and 100%, respectively, during entire study period. It showed that a complete removal in daphnia toxicity (Gd) i.e.100% had been achieved only after tertiary treatment unit involving Dual Media Filters followed by activated carbon filters. Further attempts have been made to establish the relationship between key wastewater constituents i.e. Chemical Oxygen Demand (COD) and Suspended Solids (SS) with respects to daphnia toxicity (Gd). It was observed that COD and SS show a statistically significant correlation (r^2) with daphnia toxicity (Gd) i.e. 0.89 and 0.81, respectively. Thus COD and SS can serve as a regulatory tool in lieu of an explicit toxicity standard (to check and improve the operational status of wastewater treatment plants in time).

Keywords: Toxicity, CETP, *Daphnia magna straus*

INTRODUCTION

Water pollution has become a major threat to the existence of living organisms in aquatic environment. A huge quantity of pollutants in the form of domestic and industrial effluents is discharged directly or indirectly into the water bodies, which has severe impacts on its biotic and abiotic environment (Turk and Turk, 1984).

The standard quality of waste effluents has traditionally been based on the control of global parameters such as biochemical oxygen demand (BOD), Chemical Oxygen Demand (COD) or Total Suspended Solids (TSS) according to the waste water treatment directive 91/271/EEC. The detection of these parameters alone is not sufficient, as the waste water generated from small scale industries may contains large amount of chemicals, many of which may be present in such a low concentrations that these may be beyond detection limit and for many of them even the analytical techniques are inadequate. Secondly, the physico-chemical analysis is not only quite complicated, expensive and time consuming process, but also lacks the information about the additive, antagonistic or synergistic effects of various chemicals on biotic community in aquatic ecosystem (Villegas -Navarro *et al.*, 1999). Therefore, effective tools for the evaluation of negative effects of waste effluent on living organisms are needed. The toxicity test is one of such parameters, which covers all above shortcomings and can be used as summary parameters (easier, cheaper, effective and less time consuming). The measure of toxicity is an integral view of the sum of all interacting components in the sample. The purpose of regulatory toxicity testing is to produce baseline data for environmental hazard and risk assessment of chemicals, to be used in regulating the discharge of wastewater treatment systems. Bioassay has been extensively used to document toxicity of surface water and evaluate the potential toxicity of discharges into these waters (Blinova, 2000). Numerous studies have been made to understand the toxic effects of waste effluents on fish, but relatively little attention has been paid to their adverse effects on plankton. In view of the importance of cladocerons as an important link in the food chain in aquatic ecosystem, the present work was carried out upon the toxic effects of waste effluents on *Daphnia magna straus*. Which is highly sensitive to toxic substances, has short generation time, multiplies very rapidly, easily acclimatizes in laboratory condition, cultured in a small space and can be measured in a relatively short period (APHA, 1998 and German Standard Method, 1989). Earlier studies revealed that *Daphnia magna* could be used as a sensitive indicator for effluent

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toxicity measurement (Verma *et al.*, 2003).

The acceptance of bioassay toxicity test as an effective analytical tool requires guarantees of standardization and validation of the experimental procedure to evaluate its sensitivity, accuracy or precision. In this sense, the main objectives of this work was to assess the utility of toxicity tests and to apply the bioassay toxicity testing for monitoring of wastewater treatment plants.

MATERIALS AND METHODS

Site study : Toxicity evaluation study was carried out at the CETP, located at Mangolpuri industrial area, Phase I, West Delhi Segment. The designed treatment capacity of the plant is 2400 m^3/d (2.4 MLD) and it receives the waste effluent from 570 industrial units includes rubber, food processing, dying, plastic, and electroplating industries.

The treatment system consist of primary treatment with screens, grit chamber, equalization tank, primary clarifier followed by secondary treatment with complete mix activated sludge process, secondary clarifier and tertiary treatment units involving dual media filter (sand filter) and activated carbon filters. Sludge handling facilities consists of gravity thickener, sludge digester and sludge drying beds.

Sampling : Wastewater samples were drawn from receiving chamber (raw wastewater), after primary and secondary clarifier and clean water sump (finally treated effluent). Composite sampling was adopted as per standard method (APHA, 1998), to avoid possibly daily variation in the composition of flow. Wastewater samples for toxicological analysis were collected in non- reactive sterilized borosilicate glass bottles of 500 ml capacity. The sample were transported in an ice box to Bio- Science laboratory of Central Pollution Control Board, Delhi and stored at a temperature of 4 ^oC to avoid deterioration. The toxicity test was carried out within next 24 hr.

Sampling Analysis : Bioassay test using *Daphnia magna* were conducted following the German Standard Method (1989), METHOD DIN 38412- L30. COD was defined as the amount of oxygen consumed in the sample during it's chemically digestion over 2 hrs at 150^0C. (APHA, 1998). Data were expressed in mg O_2/l. Suspended Solids (SS) were estimated as the weight of solids material retained on pre-weighted Whatman filter paper after filtering a known volume of sample and drying the filter / membrane at 105^0C until a constant weight was reached. Data were expressed in mg/l. The wastewater samples collected from the various treatment units of CETP were analyzed for weekly/ monthly variations from December 2001- March 2002.

Daphnia magna used in the experiment were selected from the laboratory stock culture, maintained at 24 ± 1^0C in 2 lit. glass beaker and were fed on green alga *Scendesmus substpicatus*. 24 hours prior to the test adult *Daphnia magna* were stored and the young one (neonates) produced from these adults were used in experiments. Dilution water was prepared by using 25 ml of each stock solution i.e.

KCl (Potassium chloride)
$CaCl_2$. $2H_2O$ (Calcium chloride solution)
$NaHCO_3$ (Sodium bicarbonate solutions) and
$MgSO_4.7H_2O$ (Magnesium sulphate solution)
in per liter of double distilled water.

Procedure : The neonates of 1mm was collected for the toxicity test by separating the old Daphnids with a nylon sieve plate with a mesh of approximately 1mm x 1mm. Daphnids were exposed to different concentration of dilution series (i.e. 1, 2 , 3, 4, 6, 8, 12, 16, 24, 32…..) prepared according to German standard method (1989). Five numbers of Daphnids per 20 ml of test solution was taken in 50 ml glass beaker. Tests were run in duplicate. A control set containing dilution water was also run simultaneously. Test was carried out in two phase i.e. Range Finding Test (RFT) and Standard Test. Observation was made for dead or immobilized Daphnids after 24 hrs to 48 hrs of exposure. On the basis of *Daphnia magna* mortality up to 5 consecutive concentrations have to be selected from RFT dilutions for standard test. The standard test is a confirmatory test, performed to determine Gd value, which is the smallest value of dilution for Test solution in which all *Daphnia magna* remain capable of swimming. The lowest value of G (Gd value) was reported for the Test liquid in which at least 4 *Daphnia magna* have retained their swimming ability and indicate dilution factor (Gd) as test result. In case of more than 1 mortality in check liquid/ control set the experiment was discarded and repeated again.

RESULTS AND DISCUSSION

Toxicity removal efficiency of CETP : Table 1 gives the descriptive data (average) on sampling frequency, *Daphnia magna* toxicity (Gd), COD and SS concentration at different treatment levels and the overall plant efficiency w.r.t. to percentage removal of abovementioned parameters.

The variations in Gd, COD and SS concentrations are also illustrated graphically in fig.1, 2 and 3. The mean influent toxicity was Gd- 16. Toxicity after primary, secondary and tertiary treatment units was found to be Gd8- Gd12, Gd3- Gd4 and Gd1, respectively. The toxicity of wastewater is reduced cumulatively from influent to effluent. The mean percentage removal in toxicity after primary, secondary and tertiary treatment units were found to be 29%, 76% and 100%, respectively. It shows that treatment of wastewater up to secondary level is not

Table 1. Summary of test protocol for *Daphnia magna* bioassay.

S.No.	Test Type	Static
1.	Temperature	24± 1^{0}C
2.	Light	Ambient laboratory illumination
3.	Test vessel	50 ml glass beaker
4.	Test solution volume	20 ml
5.	Test organisms/ vessel	5
6.	Replicate	2
7.	Test concentration	1, 2, 3, 4, 6, 8, 12, 16, 24, 32
8.	Feeding regime	No feeding during exposure
9.	Control water	Standard dilution water
10.	Test duration	24 hrs to 48 hrs
11.	End point	Immobilization / death

satisfactory itself to reduce wastewater toxicity completely or its maximum extent. We found the 100% removal in Daphnia toxicity (Gd) only after tertiary treatment of wastewater. The use of *Daphnia magna* in toxicology is accepted in several countries to monitor wastewater treatment systems, establish quality criteria to determine permissible concentrations of pollutants, limits of impurity in water from natural effluents, as well as to determine the efficacy of a good sanitation method (Villegas- Navarro *et al.*, 1999).

The mean influent COD and SS were found in the ranges of 349 mg/l and 160 mg/l, respectively. After primary, secondary and tertiary treatment both COD and SS were observed within a mean range of 254 mg/l, 150 mg/l, 123 mg/l and 146 mg/l, 110 mg/l and 26 mg/l, respectively. The findings revealed that a systematic removal in COD and SS concentration founds from influent to effluent. The mean percentage removal in COD and SS after primary, secondary and tertiary treatment level had been found to be 27%, 57%, 65% and 10%, 31% and 84% respectively. Thus the observations discloses that COD, SS and Daphnia toxicity (Gd) reduce slightly at primary treatment level and significantly at secondary treatment level, while as the optimal and maximum removal in Gd, COD and SS concentration were found at tertiary treatment level. Figure 4 shows the cumulative percentage reduction of performance parameters at different treatment levels of CETP.

Interrelationship between Daphnia toxicity (Gd), COD and SS : On the basis of data obtained we tried to establish relationship between two key wastewater constituents i.e. COD and SS with Daphnia toxicity (Gd). From the plots of fig. 4 and 5 the following relationships were obtained between key parameters,

$$Gd = 0.0631COD - 5.9436 \ (r^2 = 0.899) \ldots\ldots\ldots\ldots\ldots(i)$$

Table 2. Descriptive data and removal efficiency of performance parameters at CETP.

Sampling Frequency Months/ week		Daphnia Toxicity (Gd) I	P	S	E	%	COD (mg/l) I	P	S	E	%	Suspended Solids (mg/l) I	P	S	E	%
Dec	I	16	12	4	1	**100**	335	296	180	120	**64.2**	134	132	104	24	**82.1**
	II	16	12	4	1	**100**	368	256	136	128	**65.2**	142	134	110	24	**83.1**
	III	16	12	4	1	**100**	396	292	188	140	**64.7**	160	142	98	30	**81.3**
Jan	I	16	8	3	1	**100**	366	280	168	130	**64.5**	145	136	104	26	**82.1**
	II	16	12	4	1	**100**	350	220	140	124	**64.5**	154	145	110	18	**88.3**
	III	16	12	4	1	**100**	325	236	144	110	**66.2**	148	129	88	12	**91.9**
Feb	I	16	12	4	1	**100**	342	215	130	110	**67.8**	160	138	106	22	**86.3**
	II	16	12	4	1	**100**	330	260	172	128	**61.2**	188	172	135	32	**83.0**
	III	16	12	4	1	**100**	385	294	190	130	**66.2**	176	166	122	48	**72.7**
March	I	16	8	4	1	**100**	360	244	164	138	**61.7**	187	166	114	25	**86.6**
	II	16	12	3	1	**100**	310	234	158	120	**61.3**	150	134	92	18	**88.0**
	III	16	12	4	1	**100**	315	220	124	108	**65.7**	177	162	134	28	**84.2**

I: Influent or Raw wastewater; P: After primary settling tank; S: After secondary settling tank; E: Effluent or After tertiary Treatment; %: Percentage removal

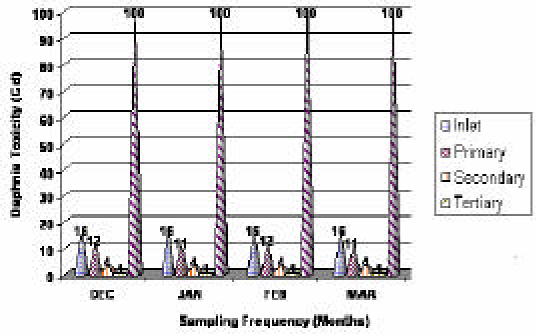

Fig.1. Status of Daphnia toxicity (Gd) at different treatment level of CETP.

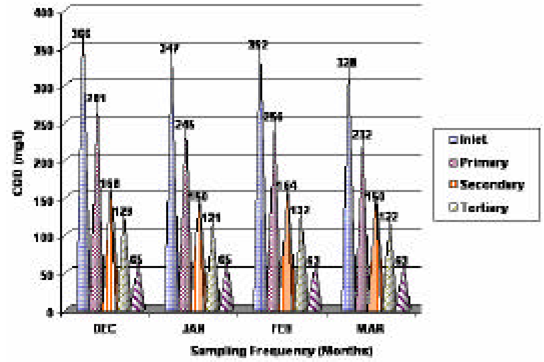

Fig.2. Status of Chemical Oxygen Demand (COD) at different treatment level of CETP.

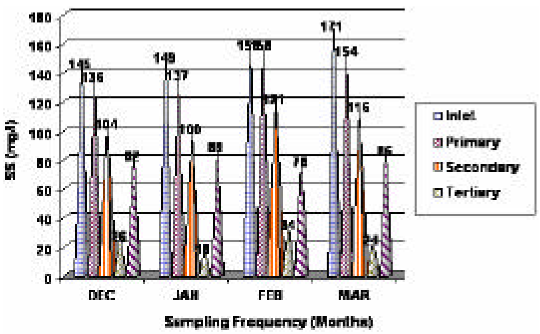

Fig. 3. Status of Suspended Solids (SS) at different treatment level of CETP.

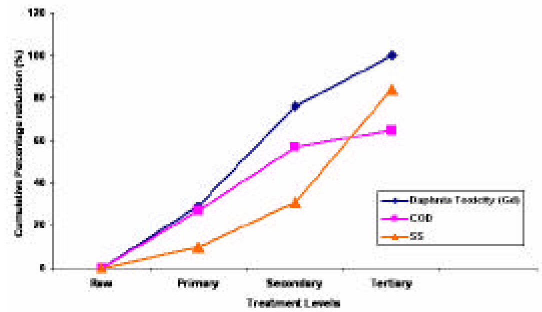

Fig. 4. Cumulative reduction of performance parameters at different treatment levels of CETP.

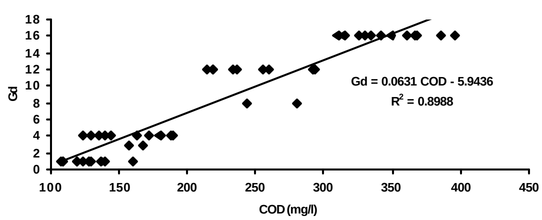

Fig. 5. Interrelationship between Daphnia toxicity (Gd) and chemical oxygen demand.

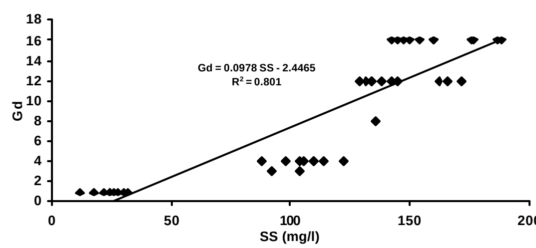

Fig. 6. Interrelationship between Daphnia toxicity (Gd) and suspended solids.

Gd = 0.0978 SS – 2.4465 (r^2 = 0.801)……......…....…….(ii)
The statistical analysis of data revealed that Daphnia toxicity (Gd) exhibit a good coefficient of correlation with COD (r^2 = 0.899) and SS (r^2 = 0.801) (fig.5 & 6). It shows that Gd value could be more strongly correlated to COD than SS.

The observations revealed that an efficient final clarification step, which eliminates COD and SS in wastewater, plays a central role in toxicity reduction in conventional wastewater treatment system. Therefore, for up gradation of sewage treatment plant in terms of toxicological quality, the effluent suspended solids and chemical oxygen demand should be as low as possible by providing optimal treatment and settling conditions to the aeration and sedimentation tanks. Moreover our results indicate that toxicological pollution to receiving natural waters was not strongly dependent on influent water quality or seasonal variations instead, it was related to the efficiency of treatment processes in removing organic loads i.e. COD and SS. Our observations are in concomitant with the findings of Hernando *et al.,* (2004). According to Hernando *et al.,* (2004) the use of biological assay can provide a direct and appropriate measure of toxicity to complement the physico- chemical measures of the quality of wastewater.

Finally the authors believe that more discussion should be undertaken regarding the efficacy of process requirements versus toxicological quality goal of secondary or tertiary treated water.

Conclusion

The following conclusion can be put forward from this study: (i) This test can be considered as useful analytical tools for screening of chemical analysis and early warning system to monitor the different operational units of wastewater treatment plants, (ii) Interrelationship between COD, SS with respect to Daphnia toxicity (Gd) suggests that improvements of the toxicological quality of wastewater could be linked to the removal of both COD and suspended solids. Both the parameters (COD & SS) can serve as a regulatory tool in lieu of an explicit toxicological standard, (iii) The toxicity testing could be help to reduce influent toxicity and thereby avoid impacting microorganism's population in activated sludge systems and (iv) The bioassay method is indispensable and complimentary and support the earlier view that Daphnia magna can serve as a valuable model for bio- monitoring of water pollution and for evaluation of the toxicity of an effluent and risk assessment in an aquatic body, as it is highly sensitive to pollutants.

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