



Evaluation of *Daphnia magna* as an indicator of Toxicity and Treatment efficacy of Municipal Sewage Treatment Plant

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ABSTRACT: Performance evaluation of wastewater treatment plants (WWTPs) with special reference to toxicity reduction using *Daphnia magna straus* as test organism is very important to study the likely adverse effects of the treated wastewater on the aquatic ecosystem of receiving waters and to detect common environmentally realistic concentrations of pollutants at different concentration levels and toxicity discriminatory ability to distinguish different degree of toxicity and toxic specificity of the compounds on target organisms. This test can be considered as useful analytical tool for screening of chemical analysis and early warning system to monitor the different operational units of wastewater treatment plants. Interrelationship between COD, SS with respect to *Daphnia* toxicity (Gd) suggests that improvement 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. An important feature of this work was to emphasize the significance of toxicity tests. It could help to reduce influent toxicity and thereby avoid impacting microorganisms' population in activated sludge systems. This study shows the difference between using physico-chemical and biological criteria to define the quality or toxicity of wastewater, making it clear that both methods are 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. @JASEM

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 Council Directive (1991) concerning waste water treatment. The detection of these parameters alone is not sufficient, as the waste water generated from small scale industries may contain 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 on living organisms are needed. The use of biological assays can provide a direct and appropriate measure of toxicity to complement the physico- chemical measures of quality of wastewaters (Hernando *et al.*, 2005). 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 cladocerans as an important link in the food chain in aquatic ecosystem, the present work on toxic effects of waste effluents to *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 GSM, 1989). The use of *Daphnia magna* in toxicology is accepted in several countries to monitor wastewater treatment systems, to establish quality criteria to determine permissible concentrations of pollutants, limits of impurity in water from natural effluents, and to determine the efficacy of a good sanitation method (Villegas- Navarro *et al.*, 1999).

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 were to assess the utility and validity of toxicity tests and to apply the bioassay toxicity testing for monitoring of wastewater treatment plants.

Site Study: Toxicity evaluation study was carried out at the Common Effluent Treatment Plant located at Mangolpuri industrial area, Phase I, West Delhi Segment. It was worked from November 2001 with a construction cost of about Rs.5.5 Crore. The plant covers an area of 5472 m². The designed treatment capacity of the plant is 2400 m³/d, received the waste effluent from around 570 different 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.

MATERIAL AND METHODS

Sampling: Wastewater samples were drawn from four different treatment units i.e. receiving chamber (Raw wastewater), after primary and secondary clarifier and clean water sump (finally treated effluent). Composite sampling procedure was adopted as per standard method, 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 ice to Bio- Science laboratory of Central Pollution Control Board, Delhi and stored at a temperature of 4°C to avoid deterioration. The toxicity test was carried out within next 24 hr. (APHA, 1998)

Sampling Analysis: *Daphnia* tests were conducted following the GSM (1989). COD was defined as the amount of oxygen consumed in the sample during it's chemically digestion over 2 hrs at 150°C. Data were expressed in mg O₂ /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°C until a constant weight was reached. Data were expressed in mg/l.

Daphnia magna used in the experiment were selected from the laboratory stock culture, maintained at 24± 1°C in 2 l glass beaker and were fed on green alga *scenedesmus substpicatus*. Twenty-four hours prior to the test adult daphnia were stored and the young one (neonates) produced from these adults were used in experiment. Dilution water was prepared by using 25 ml of each stock solution of KCl (Potassium chloride), CaCl₂. 2H₂O (Calcium chloride solution), NaHCO₃ (Sodium bicarbonate solutions) and, MgSO₄.7H₂O (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 for acute toxicity. On the basis of daphnia 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 remain capable of swimming. The lowest value of G (Gd value) was reported for the Test liquid in which at least 4 daphnia have retained their swimming ability and indicate Dilution factor (Gd) as test result. In case of more than 1 mortality occurred in check liquid/control set the experiment was discarded and repeated again (Summary of Test Protocol cited in Table 1).

Table 1. Summary of Test Protocol for *Daphnia* Bioassay

Sampling Frequency (Months/ week)	<i>Daphnia</i> Toxicity (Gd)					COD (mg/l)					Suspended Solids (mg/l)					
	I	P	S	E	%	I	P	S	E	%	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

RESULTS AND DISCUSSION

Toxicity removal efficiency of CETP: Earlier studies on effluent test using *Daphnia magna* showed that macro-invertebrates could be used as a sensitive indicator for effluent toxicity study (Verma *et al.*, 2003). The wastewater samples collected from the various treatment units of CETP were analyzed for weekly/ monthly variations from December 2001-March 2002. Table 2 gives the descriptive data on sampling frequency, *Daphnia* toxicity (Gd), Chemical Oxygen

Demand (COD) and Suspended Solids at different treatment levels and the overall plant efficiency w.r.t. to percentage removal of above mentioned parameters.

Table 2: Descriptive data and removal efficiency of performance parameters at Common effluent treatment plant, Mangolpuri (Del

S.No.	Test Type	Static
1.	Temperature	24± 1°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

* I: Influent or Raw Wastewater; P: after Primary Settling Tank; S: after Secondary Settling Tank; E: Effluent or after Tertiary Treatment; %: Percentage removal

The variations in Gd, COD and SS concentrations and removal in percentage terms are also illustrated graphically in figs.1, 2 and 3. The mean influent toxicity was Gd- 16. Toxicity after primary, secondary and tertiary treatment units was in the range of 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 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.

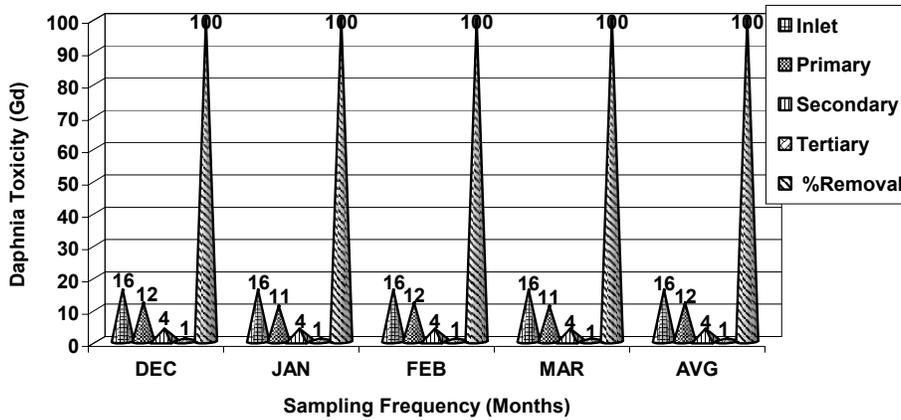


Fig 1: Status of *Daphnia* toxicity (Gd) at different treatment levels of CETP

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 were observed within a mean range of 254 mg/l, 150 mg/l, 123 mg/l and 146 mg/l, 110 mg/l & 26 mg/l respectively. The findings revealed that a systematic

removal in COD and SS concentration found from influent to effluent. The mean percentage removal in COD and SS after primary, secondary and tertiary treatment level were found to be 27%, 57%, 65% and 10%, 31% and 84% respectively.

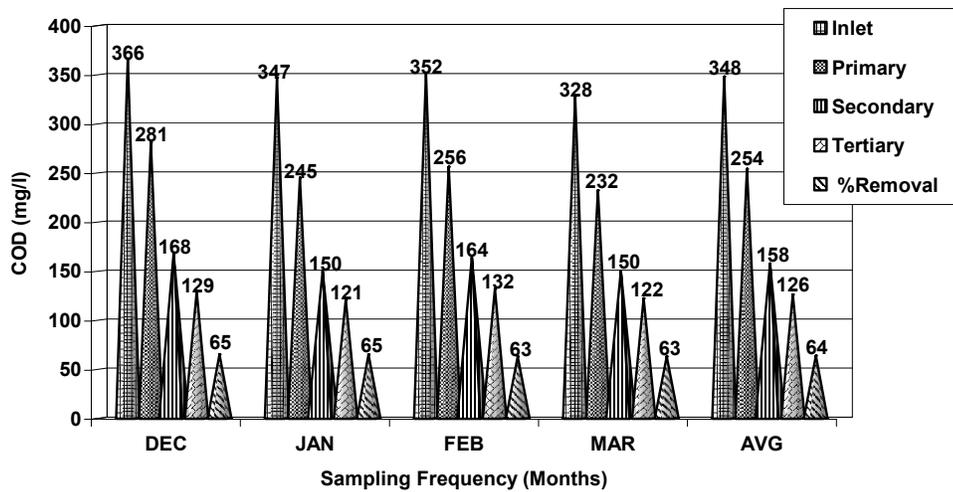


Fig 2: Status of Chemical Oxygen Demand (COD) at different treatment levels of CETP

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.

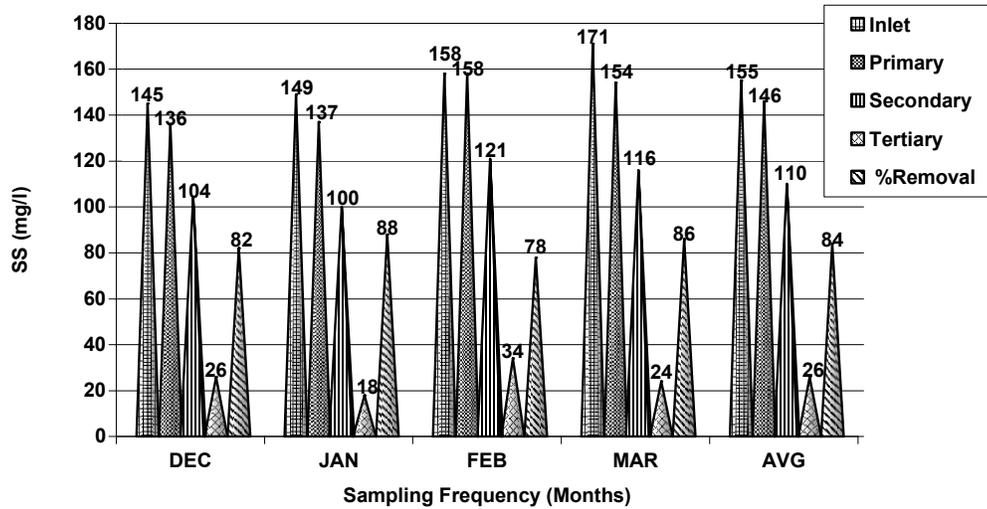


Fig 3: Status of Suspended Solids (SS) at different treatment levels of CETP

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 ($r^2 = 0.899$).... (i)
 Gd = 0.0978 SS - 2.4465 ($r^2 = 0.801$).... (ii)

Both the coefficient of correlation values were found to be varying between 0.8- 1, which indicates

relatively good interrelationship between the concerned parameters. For COD there was a positive correlation between *Daphnia* Toxicity (Gd) removed and COD ($r^2 = 0.899$) as shown in fig.5. Suspended solids removal also shows significant correlation to the toxicity removal ($r^2 = 0.801$) as depicted in fig.6. It shows that Gd value could be more strongly correlated to COD than SS. Thus these interrelationships could be helpful in routine monitoring of STPs efficiency.

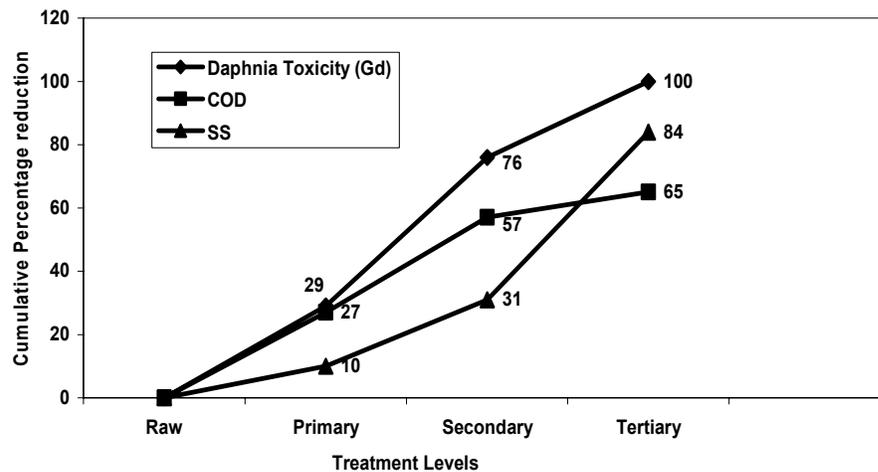


Fig 4: Cumulative reduction of performance parameters at different treatment levels of CETP

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. 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.

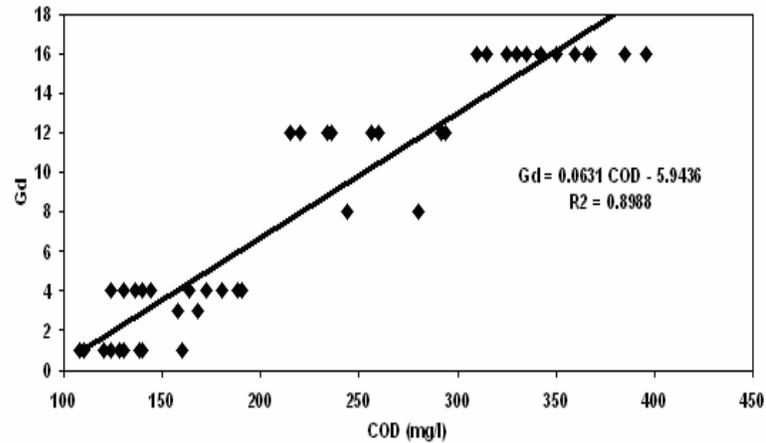


Fig 5: Interrelationship between *Daphnia* Toxicity (Gd) and Chemical Oxygen Demand

Conclusion: This paper investigates the utility and validity of bioassay toxicity testing for monitoring of wastewater treatment plant. The crustacean *Daphnia magna* was used as the sensor organism. The validation studies indicated that the acute toxicity test can be considered as high sensitivity analytical tools to detect common environmental concentrations of the pollutants. 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 mean percentage removal in toxicity after PST, SST and tertiary treatment units was observed as 25%, 75% and 100% respectively during all the round of sampling. It revealed 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. It means that CETP technically reduced the toxicity of wastewater to maximum extent. The results clearly showed that the use of bioassay tests produced additional information about the toxicity potential of industrial discharges and effluents.

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). Of the more common physicochemical parameters, the COD and SS showed a statistically significant correlation with toxicity reduction at different treatment units and 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). Our investigation showed that toxicity test with the studied species may be used as an effective tool for the assessment of water purification process effectiveness at the wastewater treatment plants. Thus the concerned study sounds that knowledge of the toxicity of effluents can benefit treatment plant operators in optimizing plant operation, protecting receiving water quality, establishing sewer discharge permits and control of toxicity can give more objective baseline data for environmental risk assessment of polluted waters dumped into natural ecosystem.

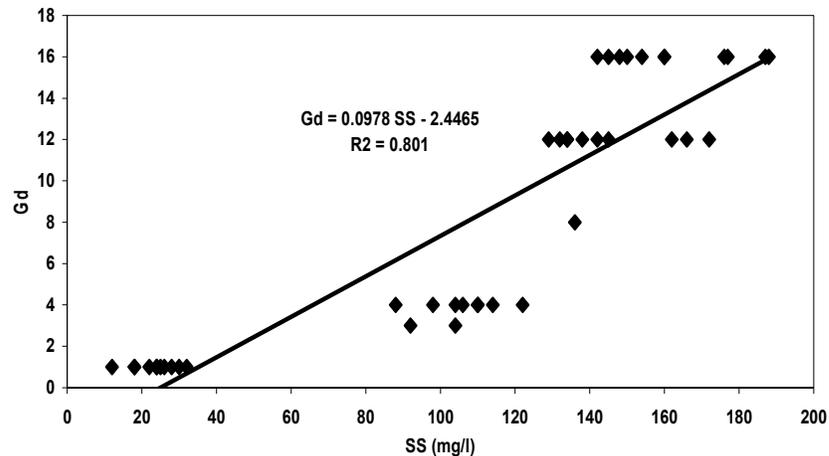


Fig 6: Interrelationship between *Daphnia* Toxicity (Gd) and Suspended Solids

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