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.

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 cladocerons 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 subspicatus*. 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) | Daphnia Toxicity (Gd) | COD (mg/l) | Suspended Solids (mg/l) |
|---------------------------------|-----------------------|------------|------------------------|
|                                 | I P S E %             | I P S E %  | I P S E %               |
| Dec                             | 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                             | 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                             | 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                           | 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.

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.
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.

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,

\[
\text{Gd} = 0.0631 \text{COD} - 5.9436 \quad (r^2 = 0.899) \quad \ldots \quad (i)
\]
\[
\text{Gd} = 0.0978 \text{SS} - 2.4465 \quad (r^2 = 0.801) \quad \ldots \quad (ii)
\]

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.

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 upgradation 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.

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 respect 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 tests 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.
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Fig 6: Interrelationship between Daphnia Toxicity (Gd) and Suspended Solids