Toxicity Evaluation of Effluents from Dye and Dye Intermediate Producing Industries Using Daphnia Bioassay

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Citation

Abstract
Wastewater from seven dye and dye intermediate producing industries and a common effluent treatment plant was assessed to characterize the acute toxicity using Daphnia bioassay. The 24 and 48 h EC50 values and its confidence limits were estimated applying the probit analysis method. The 24 h EC50 values for different dye effluent samples A, B, C, D, E and F were 1.35, 17.32, 17.67, 29.18, 46.49 and 25.11%, and 48 h EC50 values were 1.23, 14.12, 15.52, 24.53, 29.69 and 23.22%, respectively. Result showed linear relationship with high degree of confidence (0.84<r2<0.98) between end point (immobilization) and test concentrations. Effluent of two dye industries 'G' and 'H' had EC50 value >100%. Effluent of industry 'A' exhibited highest toxicity causing 100% immobility at 3.13% effluent concentration. Effluent of industry 'G' and 'H' had shown least toxicity, and was found toxic at the concentration of full strength (100%) causing ≤ 20% and 10% immobility in 24 h. Out of 8 industries effluents tested, 5 showed 100% immobility of daphnia at 50% of effluent concentration, while 1 showed highest toxicity (100% immobility) at a very low concentration (3.13%) of the effluent, and 2 showed lowest toxicity. These results clearly indicate that the bioassay reflects the overall toxicity potential of indusial discharge as it provides the complete response of test organisms to all components of water.

INTRODUCTION
Effluents are very important source of chemicals entering into aquatic ecosystem, deteriorating the water quality and thereby affecting the flora and fauna. There have been increasing trends towards use of biomonitoring to assess the water quality of resources/receiving water bodies [12]. The analysis of a recognized set of water chemistry parameters is the most commonly used method of assessing water quality by pollution control boards/regulatory authorities. However, these parameters provide information about the chemical complexity but not their toxicity. Moreover, study of physico-chemical parameters of effluent does not throw any light on the synergistic or antagonistic effects on biological systems. Thus an adequate monitoring system should assess both the effects and distribution of pollutants [13]. The chemical analysis identifies only the main chemical pollutants available while a bioassay (toxicity test) addresses pollutant bioavailability and response to total effect of actual potential [14].

The traditional acute multi-concentration toxicity tests when applied for the determination of effluent is often recognized as Whole Effluent Toxicity (WET) test. The WET test is widely used in the USA, Canada and European countries, and is an important component of the environmental protection agencies of these countries for detecting and addressing the toxicity of industrial effluents/wastewaters. Determination of acute toxicity of the chemical products is one of the cornerstones of the general hazard assessment of the programs laid down by various environmental protection agencies. However, most of the regulatory agencies and research organizations are using the well-established conventional acute toxicity test with fish and daphnids in which survival/death/immobility is the most frequently monitored end point [507].

Daphnids are routinely used as standard test organism in aquatic toxicity bioassay (acute and chronic) because of their rapid reproduction, sensitivity to chemical environment, and play key ecological role in the aquatic food chain by serving as an intermediate between primary producers and fish [8]. Daphnia magna has been evaluated as a good organism to test effluent toxicity in textile wastewater [909], dye and dyes wastewater [11213].

India is among one of the major producers of dyes and dyes intermediate from Asian region and meets the requirement of the world at large. There are about 900 dyes and dyes
intermediate producing industries in India. Most of them are
in small-scale unorganized industrial sector, and about five
thousand dyes and dyes intermediate are in commercial
production. The wastewater generated from dye and dye
intermediate industries mainly have intense color having
various shades of red, blue green, brown and black through
the production of different color containing dyes and usually
have high level of COD, BOD, acidity, chlorides, sulphates,
phenolic compounds and various heavy metals viz. copper,
cadmium, chromium, lead, manganese, mercury, nickel, zinc
etc. [14]. Although it seems logical that dyes should be safer
to the environment than neurotoxic insecticides [4], little
work has been done to determine the potential impact of
dyes on the ecosystem or selected indicator sps. in the
laboratory [15161]. Dyes, as they are intensively colored,
cause special problems in effluent discharge and even small
amount is noticeable. The effect is aesthetically more
dispersing rather than hazardous, and can prevent sunlight
penetration decreasing photosynthetic activity in aquatic
environment. Although, some azo dyes that causes the
effluent color have been implicated as being
mutagenic/carcinogenic as well as toxic to aquatic life [1718].

Therefore in order to assess the effluent toxicity status of
polluting industries traditional acute toxicity test (WET) was
applied for dyes and dyes intermediate producing industries
using Daphnia magna as a test model. The overall objective
of this study was to assess the acute toxicity of industrial
effluents of which seven are dyes and dyes intermediate
producing industries and one is common effluent treatment
plant which receives dyes effluents from more than fifty
dyes producing small scale industries. Further, our aim was
also to contribute towards the development of a more
effective policy of pollution control and effluent regulation
using bio-criteria derived from acute WET test results.

MATERIALS AND METHODS

Grab samples of the effluents were collected at the point of
outlet discharge from the wastewater treatment plants
(WTPs) of seven different dyes and dyes intermediate
producing factories (labeled as A, B, C, D, E and H) and
a common effluent treatment plant (labeled as F) within the
plant premises. Factory-A produces cuphtohalosyanine
complex (CPC crude), factory-B produces azo pigments,
auxiliaries and intermediates, factory-C produces synthetic
organic dye stuff and pigments dye stuff, auxiliaries and
intermediates, azo and diazo reactive dyes, disperse dyes and
intermediates, factory-D produces G Salt and R salt,
Factory-E produces acid black, red, blue violet, yellow. F-
(WTP) Common effluent treatment plant receives
wastewater (primarily treated) from >50 dye producing
units, factory G - produces reactive dyes, and factory - H
produces tartrazine, indigo carmine, allura red. All of the
effluent samples of these factories were stored in amber
glass bottles (2.5 L) and transported to the laboratory at the
earliest in a lightproof ice chest, and stored under
refrigeration (at 4 ° C) in darkness until analysis and
bioassay. Dissolved oxygen (DO) (WTW OXI-96 oxy-
meter), conductivity (TD Scan conductivity meter) and pH
(W T W pH - meter) were also measured as soon as samples
arrived to the laboratory. Temperature ( ° C) was recorded at
the sampling site.

Daphnia magna Straus used in the experiment were selected
from the laboratory stock culture maintained at 25±2 ° C, in
5-litre borosilicate glass beakers and were fed on green algae
Selenastrum sps. and yeast powder. The daphnids were
reared in photoperiod of 12 h light/12 h dark cycle, with
light > 600 Lux (Metrix AV0 LM 4 Lux-Meter, France).
Reconstituted dilution water (RDW) was used for dilution of
effluents to which Daphnia culture was maintained.

To evaluate the aquatic toxicity of dye effluents author
performed single species laboratory toxicity test with
Daphnia magna, and the 48 h acute toxicity test was
conducted after exposing them to various concentrations of
the effluent in RDW within 48 h after the effluent sample
had arrived in the laboratory. The experimental
concentrations were 100, 75.0, 66.6, 50, 33.3, 25, 12.5, 6.25,
3.12, 1.56, 0.8 and 0.4 % of the effluent. Control water and
effluent dilutions were prepared with reconstituted dilution
water (RDW) with the same formulation used for culture
water. Reconstituted water was aerated 12 h before effluent
dosage. All the toxicity tests were run at 25±2 ° C. Test
chambers were not aerated during experimentation. Twenty-
four hours prior to the test adult daphnids (gravid females)
were sorted and the young one (neonates) produced from
these adults were used in the experiment. Batches of five
neonates (≤ 24h) received from gravid females were placed
in glass beaker of 100 ml containing 50 ml test volume with
two set of control group. Each concentration was replicated a
minimum of 4 times. The numbers of immobilized daphnids
were recorded after 24 h and 48 h exposure. Daphnids
unable to swim for 15 s after gentle stirring were considered
as immobile/dead [8]. The test protocol is summarized in
Table 1, and details of the methodology is described
elsewhere [10]. The experiment was validated with positive control test using reference substance potassium dichromate (K$_2$Cr$_2$O$_7$). The 24 h EC$_{50}$ was 0.813 (0.70–1.01) mg/L, after ISO [11].

**Figure 1**

Table 1: Summary of test protocol for

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>Daphnia magna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt; 24 hr</td>
</tr>
<tr>
<td>Test type</td>
<td>Static (non-renewal type)</td>
</tr>
<tr>
<td>Temperature</td>
<td>25 ± 2.0 °C</td>
</tr>
<tr>
<td>Light quality</td>
<td>Cool white fluorescent light ≥ 0.0 Lux</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>12 hr</td>
</tr>
<tr>
<td>Feeding regime</td>
<td>24 hr before experiment</td>
</tr>
<tr>
<td>Dilution water</td>
<td>Reconstituted dilution water (RDW)</td>
</tr>
<tr>
<td>Test vessel</td>
<td>100 mL glass beaker</td>
</tr>
<tr>
<td>Test solution volume</td>
<td>50 mL</td>
</tr>
<tr>
<td>Test specimens/vessel</td>
<td>5 daphnids in each vessel</td>
</tr>
<tr>
<td>Test vessel/concentration</td>
<td>Four</td>
</tr>
<tr>
<td>Effluent concentrations</td>
<td>100, 75, 50, 25, 12.5, 6.25, 3.13, 1.60, 0.80, 0.40, %</td>
</tr>
<tr>
<td>Test duration</td>
<td>48 hrs</td>
</tr>
<tr>
<td>Observation period</td>
<td>24 and 48 hrs</td>
</tr>
<tr>
<td>End point</td>
<td>Immobilization</td>
</tr>
</tbody>
</table>

The acute toxicity data were analyzed when two or more partial immobilizations were observed. The EC$_{50}$ and its 95% confidence limits were calculated by probit analysis method to using SPSS software 6.1. The regression analysis was also performed to determine the correlation between x and y variables for regression equation and $r^2$ values. $r^2$ is the correlation coefficient and reflects statistical significance between dependent and independent variables. Toxicity unit (TU) value was calculated using EC$_{50}$ (100/EC$_{50}$ expressed as volume percentage). Ratio of acute toxicity was calculated by dividing 48 h EC$_{50}$ with 24 h EC$_{50}$.

**RESULTS AND DISCUSSION**

The physico-chemical characteristics of the reconstituted dilution water (RDW) used in the experiment showed that water used in this study had pH 7.8, calcium hardness (as CaCO$_3$) 180.0 mg/L, conductivity 0.7 mS. The physico-chemical characteristics of the effluents differed substantially from one another with respect to chemical characteristics, as expected due to a relatively wide spectrum of dyes manufacturing sources. Dissolved oxygen concentration of the effluents ranged from 3.5–5.2 mg/L, the pH ranged from 7.2–8.0 and conductivity from 0.8–1.5 mS and temperature 26.0–31.0 °C. Acute toxicity test results of 8 dyes industries (24 and 48 h calculated EC$_{50}$ and its 95% confidence limit) are given in Table 2. Effluent-A showed highest toxicity causing 100% mortality at 3.13% effluent concentration and more than 50% mortality in 1.5% effluent concentration. The effluent of industry ‘G’ showed very low toxicity and was found toxic at the concentration of full strength (100%) causing ≤ 20% mortality in 24 h and ≤ 25% mortality in 48 h. However, the effluent of industry ‘H’ had lowest toxicity causing only 10% mortality at 100% effluent. Hence, the EC$_{50}$ value could not be derived for both effluents. The 24 and 48 h EC$_{50}$ value for dye industries G and H were always >100, and $r^2 = 0.56$.

The 24 h EC$_{50}$ values and the confidence limit for the effluent of dye industry A, B, C, D, E and F were 1.35 (1.13–1.63), 17.32 (13.94–21.82), 17.67 (14.45–21.61), 29.18 (25.93–33.27), 46.49 (33.43–73.59) and 25.11 (22.55–27.62)%, respectively. The linear regression analysis performed reflected statistical significance between dependent and independent variables ($r^2 0.85 – 0.95$). The 48 h EC$_{50}$ values for effluent of dye industry A, B, C, D, E and F were 1.23 (1.02–1.48), 14.12 (11.53–17.42), 15.52 (12.68–18.86), 24.53 (21.48–28.18), 29.69 (26.54–33.55) and 23.22 (20.70–25.94)%, respectively (Table 2).

**Figure 2**

Table 2: 24 h and 48 h EC50 values, its 95% confidence limits and ratio, and toxicity units (TU) for dyes industrial effluents

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dyes and dye intermediate producing industries</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h EC$_{50}$</td>
<td></td>
<td>1.35</td>
<td>17.32</td>
<td>17.67</td>
<td>29.18</td>
<td>46.49</td>
<td>25.11</td>
<td>&gt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Confidence Limit (95%)</td>
<td></td>
<td>1.13</td>
<td>13.94</td>
<td>14.44</td>
<td>25.93</td>
<td>33.43</td>
<td>22.55</td>
<td>22.55</td>
<td>&lt;100</td>
</tr>
<tr>
<td>48 h EC$_{50}$</td>
<td></td>
<td>1.23</td>
<td>17.67</td>
<td>21.27</td>
<td>23.59</td>
<td>27.61</td>
<td>25.11</td>
<td>&gt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Confidence Limit (95%)</td>
<td></td>
<td>1.00</td>
<td>12.53</td>
<td>15.22</td>
<td>26.34</td>
<td>33.54</td>
<td>25.94</td>
<td>25.94</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Rate of</td>
<td></td>
<td>1.10</td>
<td>1.22</td>
<td>1.13</td>
<td>1.13</td>
<td>1.56</td>
<td>1.10</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Toxinity Unit</td>
<td></td>
<td>0.13</td>
<td>7.58</td>
<td>6.36</td>
<td>4.35</td>
<td>4.35</td>
<td>4.35</td>
<td>4.35</td>
<td>4.35</td>
</tr>
</tbody>
</table>

The linear regression analysis performed reflected statistical significance between dependent and independent variables (0.84 < $r^2$ ≤ 0.98). The 24 and 48 h EC$_{50}$ value for the effluent of dye industries G and H were >100%, and $r^2 = 0.56$, showing least toxic response. The regression equations and correlation coefficients were also worked out. The relationship is a sigmoid curve, but can also be expressed in linear fashion with a high degree of confidence ($r^2$). The coefficient of determination was as high as $r^2 = 0.98$. Out of 8 effluents tested 5 showed 100% immobility of daphnia at 50% of the effluent concentration, while 1 showed heights toxicity (100% immobility) at 3.13% concentration of the effluent and remaining two showed lowest toxicity at full strength (100% effluent concentration).

In toxicological experiments, the time of exposure has large
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Effect on biological response. The general rule of thumb is that the longer the exposure time lesser the EC50 value and greater is the toxicity. In our study too, results showed similar pattern having lesser 48 h EC50 value than 24 h EC50 (Table 2). The ratio of EC50 (24 h to 48 h) was found in between 1.1–1.2 except for one sample (1.5), which showed the delayed acute toxic response (Table 2). Kim et al. [20] reported the acute toxicity ratio (24 h to 48 h) for hexavalent chromium 1.2. Our results are in accordance with the above report.

The classification of effluent [222] based on general criteria (Table 3) showed that out of 8 effluent tested one was moderately acutely toxic (48 h EC50 >1.2%), 5 were minor acutely toxic (48 h EC50 >14.12 –29.69%) and the remaining 2 were not acutely toxic (48 h EC50 >100%). Researchers [3123] used the toxicity classification based on Toxicity Unit to classify the effluents toxicity. Based on this classification two effluent sample (G and H) had TU < 1 and remaining 6 were having TU >1.

Figure 3

Table 3: General criteria of toxicity classification and relationship between LC, LD and EC and toxicity rating

<table>
<thead>
<tr>
<th>LD50 (mg/kg)</th>
<th>LC50 (mg/L)</th>
<th>EC50 % of effluent</th>
<th>Toxicity rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;500</td>
<td>&gt;100</td>
<td>&gt;100%</td>
<td>Relatively not acutely toxic</td>
</tr>
<tr>
<td>500-5000</td>
<td>10-100</td>
<td>1-100%</td>
<td>Minor acutely toxic</td>
</tr>
<tr>
<td>50-500</td>
<td>1-10</td>
<td>1-1%</td>
<td>Moderately acutely toxic</td>
</tr>
<tr>
<td>&lt;50</td>
<td>&lt;1</td>
<td>&lt;1%</td>
<td>Very acutely toxic</td>
</tr>
</tbody>
</table>

Despite the recent developments in chronic toxicity test, traditional acute toxicity (WET) test are still widely used as standard eco-toxicological procedure by environmental agencies/toxicology laboratories to assess the potential impact on ecological system [31]. Hence the acute toxicity test was conducted exposing the Daphnia magna to treated wastewater of eight dye producing industries. The acute multi-concentration toxicity test yielded a range of response values as a function of effluent concentration, which causes some difficulty in assessing toxicity for effluent classification. However, assessment of industrial effluent by means of acute toxicity test seems to be sufficient for preliminary classification of effluent whether toxic or non toxic. Some authors have already used this idea to assess effluent in terms of their acute toxicity. If the sample whose 48 h EC50 >100% or show null or poor response of the test organism in a bioassay could be classified as non-toxic [222].

Eco-toxicity is the conservation of living communities in receiving aquatic ecosystems, it seems reasonable that those effluents having >1 TU be considered as Toxic, since this would imply that full-strength effluent would adversely affect 50% or more of the exposed organisms. Accordingly, samples having a 48-h EC50 >100% or which show null or poor response of the test organisms in the bioassays could be classified as Non-Toxic. In accordance with this criterion, only two effluent samples (G and H) in the present study were classified as Non-Toxic. Criteria to classify effluents into toxicity categories (from Low Toxic to Very Toxic) are more difficult. Calleja et al. [25] have proposed for leachates assessed with Daphnia magna acute toxicity tests the category of Low Toxic when 100 > EC50 > 75%, Toxic for 25 < EC50 < 75%, and Very Toxic for EC50 < 25%. Based on these criteria, it is shown that among the 8 industrial effluents 2 had 48-h 100 > EC50 > 75%, 1 had 25Ec 50 75%, 5 had EC50 < 25%. Thus they can be considered as being Very Toxic. In toxicity units, Very Toxic effluents have >4 TU, only 2 samples (G and H) could be considered to be Low Toxic (TU 1) following the guidelines by Calleja et al. [25]. Remaining 6 samples are considered to be Toxic >4 TU (25 < EC50 < 75%), i.e. A, B, C, D, E and F.

The results of low exposure concentration range are suitable for determining the no effect concentration (NOEC). The NOEC is the concentration at which a substance does not cause significant effect in comparison with control sample. It has an important ecological implication because it indicates the concentration above which adverse effect can be expressed. The use of ECx values (EC10/20) for responses <50% has often been chosen in effluent assessment, since these values are usually closer to the acute no-effect concentrations (NOEC). The observed acute no effect concentrations were 0.4, 6.25, 6.25, 12.25, 16.67, 12.25, 66.67 and 75% for effluents A, B, C, D, E, F, G and H, respectively. The EC10 values derived by probit analysis showed closer to acute NOEC. The EC10 values were 0.75, 7.39, 8.59, 14.24, 19.89 and 15.65 for effluent A, B, C, D, E and F.

Toxicological criteria, such as ECx, acute or chronic NOEC, which are used as parameters for effluent toxicity assessment, should be regarded as 'hypothetical' doses that will not cause adverse effects in the communities of the receiving aquatic systems, since the real environmental effects are subject to abiotic and biotic modifying factors which cannot be precisely predicted [7]. However,
assessments of industrial effluents by means of acute toxicity tests seem to be sufficient for a preliminary classification of effluents. Some authors have already used this idea to assess effluents in terms of their acute toxicity. Thus, samples whose 48-h EC_{50} >100% or which show null or poor response of the test-organisms in the bioassays could be classified as Non-Toxic \cite{2526}. However, when the EC_{50} >100%, there can be a risk of long-term toxicity which should be evaluated by means of chronic toxicity tests. Therefore, it is important that effluent assessment involves initial screening based on acute tests followed by chronic bioassays. However, NOEC values are dependent on the dilution factor and NOEC is not a statistical estimation like EC_{x}, which implies that the use of limits for this parameter is not appropriate. Chapman \cite{1} has argued against the use of NOECs in regulations and they consider the EC_{x} to be a more consistent and reliable parameter, less subjected to inter and intra laboratory variability. It is not the purpose of this paper to discuss possible alternatives to the classic toxicological parameters, but it is interesting to note that this is currently a subject of debate in the scientific community.

A study conducted by Lanciotti et al \cite{14} showed that textile wastewaters besides containing dyes have considerable amount of metals viz. chromium, copper, lead and zinc leading to toxicity to aquatic organism. Only 3\% of the samples showed considerable acute toxicity (EC_{50} < 100; TU.1) to Daphnia magna. The EC_{50} of paper mill wastewater containing dyes had value between 50-100\%, and EC_{50} were >25\% \cite{27}. In another study Tonkes et al. \cite{22} tested the effluent of 10 chemical industry using traditional acute toxicity test with Daphnia magna showed that out of 10 only 6 had acute toxicity < 100\% and 4 had >100. These studies also showed that dyes effluents had acute toxic properties, and therefore should be discharged into the aquatic environment after proper treatment. Finally it is concluded that effluents should always be subjected to acute multi-concentration test, yielding EC_{x} values, which are useful for effluent characterization and ranking in the scale of toxicity classes from non-toxic to very toxic either in v/v\% value or by using Toxicity Unit (TU). Result of this study showed that it is not possible to speculate on the mode of the toxic action due to difficulties in characterization of the chemical content of the effluent. Therefore, effluent composition should also be characterized. From the ecotoxicological view point single species toxicity provides limited data, hence a battery of test sps. should be included representing different tropic levels. Studies on wastewater effluents indicated that toxicity test have a viable role to play in water quality monitoring. Our results are intended to be basis for further environmental risk assessment.

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