Ecotoxicological characterization of tannery wastewater in Dhaka, Bangladesh

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Abstract: Tanning industries are one of the main economic activities in Bangladesh. It has been well documented that wastewater discharged from tanneries without appropriate treatment results in detrimental effects on the ecosystem. No ecotoxicity evaluation of any aquatic environment in Bangladesh has been conducted so far. In this study, a battery of toxicity bioassays and chemical analysis were carried out from water samples obtained from three sampling points: upstream from discharging site on River Buriganga (S1), raw wastewater effluent (S2), and downstream the discharging sluice gate (S3), in the Hazaribagh tannery area of Dhaka City, Bangladesh. While S1 and S3 water samples did not show significant toxicity in the bioassays tested, S2 exhibited high acute toxicity to the bacterium Vibrio fischeri (15-min Microtox® test, EC₅₀ = 9.8%), the higher plant Lactuca sativa (5-day root elongation inhibition test, EC₅₀ = 14.2%), and the microcrustacean Daphnia magna (24-hour mobility test, EC₅₀ = 31.5%). The results suggested that the raw wastewater effluent had detrimental effects on broad spectrum of organisms in the aquatic ecosystem and bacterium was the most sensitive. The chemical analysis revealed that sample S2 contained an extremely high concentration of chromium (47 g l⁻¹). Additionally, microbiological analysis indicated that the sampling area is impacted by fecal pollution, increasing the environmental health risk for its inhabitants.

Keywords: Buriganga River, Ecotoxicity, Chromium tanning

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Introduction

Located in the capital city of Bangladesh, Dhaka, the densely populated Hazaribagh tannery industrial zone constitutes 90% of the total 270 tanneries in the country. Approximately 15000 m³ of untreated chemical wastes are discharged to the low-lying areas, natural canals and other water bodies such as the Buriganga and Turag rivers, which are major sources of water supply for agricultural, livestock and fishing activities (UNIDO, 2000). Chrome tanning is the most common type of tanning where large amounts of chrome powder and liqueur are used (Gain, 2002; Islam et al., 2004; Nath et al., 2009). Furthermore, several other studies have been undertaken describing the pollution state of the Hazaribagh area (Kashem and Singh, 1999; Zahid et al., 2006; Shams et al., 2008).

In the evaluation of the environmental impact of complex industrial wastewater, physicochemical analysis alone does not provide sufficient information about the toxicity of the sample. Whereas chemical analysis is mainly used to identify the chemical pollutants, bioassays address pollutant bioavailability, as well as a more comprehensive understanding of joint toxicity effects. Integration of both chemical and biological approaches is therefore crucial to corroborate ecotoxicity testing (Persoone et al., 2000; Kuczynska et al., 2005; Mwinyihija et al., 2006).

Ecotoxicological studies can be conducted applying laboratory methods with the use of different experimental models (e.g. whole organism) in an attempt to extrapolate obtained results to evaluate the risk of exposure (Kuczynska et al., 2005). Nowadays, these simple and cost-effective bioassays such as the ones that use the bioluminescent bacterium Vibrio fischeri or the microcrustacean Daphnia magna can provide a direct quantitative measure of actual environmental toxicity of industrial effluents and/or the receiving water bodies. Moreover, there has been an increasing trend towards the use of such assays for biomonitoring (Nohava et al., 1995; Liu et al., 2002; Talapatra and Banerjee, 2004; Mwinyihija et al., 2006).

Although the physicochemical properties of the water bodies in the Hazaribagh area, including its aquifer environment, have been previously studied (Karn and Harada, 2001; Zahid et al., 2006), this is the first ecotoxicological study of an industrial effluent in Bangladesh.

The Hazaribagh area in Dhaka was selected because of its long history of widely known untreated discharge of chromium-based tanning effluents (Gain, 2002). Therefore, the general objective of this work was to evaluate ecotoxicity of wastewater in the Hazaribagh area. This study is the first paper reporting ecotoxicity of wastewater from Hazaribagh and may be the first one from Bangladesh. Additionally, we determined the general degree of biological pollution to show the risk at which the residing population is exposed to.
Materials and Methods

Sampling: Water was sampled directly from three different sites in Hazaribagh, located in Dhaka, Bangladesh (23°43'N, 90°21'E). Sample (S) collection was performed on October 2005 in the upstream of the tannery effluent discharging point, to the Buriganga River (S1), the raw wastewater effluent (S2) and 10-meter downstream location from the sluice gate that connects the effluent discharge stream directly to the river (S3). Samples were immediately transported to the laboratory where the physicochemical analysis was performed, and stored subsequently at 4°C to perform ecotoxicity evaluation with bioassays within 4 weeks. Prior to samples for bioassay were filtered using cellulose nitrate membrane filters with 0.45 mm pore size.

Physicochemical analyses: Physicochemical parameters included pH, TDS (Total Dissolved Solids), EC (Electric Conductivity), salinity, dissolved oxygen and turbidity, which were carried out according to the methods previously described (APHA, 1985). The water samples were not filtered during collection and were subsequently stored in polyethylene bottles which were pre-washed with concentrated nitric acid. The analyses represented the total load of heavy metals (dissolved plus colloid-bound) in the water samples. Total concentrations of Cr, Zn, Cu, Ni, Cd and Pb of unfiltered water samples were determined by induction-coupled plasma mass spectrometry (SPQ9000, Seiko Instruments Inc, Tokyo), essentially according to the published method by El Samrani et al. (2007).

Bacterial bioassay: The acute toxicity to bacteria was examined using the luminescent bacteria Vibrio fischeri strain NRRBL-11177 (Microtox® Acute Toxicity Reagent, SDI, Newark, USA). Intensity of the luminescence at 490 nm was measured by the Microtox® analyzer (Model 500, Microbics Corporation, Carlsbad, California, U.S.A.) at time 0 and after 15 minutes of the addition of the bacterial suspension to the test sample. The inhibition rate (%) of each sample (n=5) was calculated using the following equation

\[
\text{% I} = \left( \frac{L_s - L_c}{L_s} \right) \times 100, 
\]

where \(L_s\) and \(L_c\) are luminescence intensity of the control and the test sample, respectively (Microbics Corporation, 1998). EC\textsubscript{50} (half maximal effective concentration) was calculated by means of logistic regression analysis using TRAP (Toxicity Relationship Analysis Program) v.1.0 software (Environmental Protection Agency, U.S.A., 2002). Sample dilution was made with 2% NaCl that was also used as the negative control. Internal quality control tests using sodium dichromate (K\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7}) were run during the study.

Plant bioassay: Phytotoxicity of the water samples was assessed by the 5-day lettuce seed root elongation inhibition test (Dutka, 1989). Twenty lettuce seeds (Lactuca sativa) were placed on a filter paper (Type 2, Toyo Roshi, Tokyo, diameter = 84 mm) wet with 2 ml of sample dilutions in petri dishes and incubated for 5 days at 22 ± 2°C in the dark. Dilutions and the control were conducted with pure water. The test was performed with five replicates. At the end of the incubation period, the number of germinated seeds was determined, and length of roots was measured. Non-germinated seeds account as 0 cm in root length. Inhibition rate of root length compared to the water control at day 5 was expressed as percentage and EC\textsubscript{50} was calculated as described above.

Microcrustacean bioassay: Acute toxicity to the microcrustacean Daphnia magna was examined using the DAPHTOXKIT F\textsubscript{TM} magna (Microbiotests, Creasel, Belgium) according to the manufacturer’s manual based on ISO norm 6341 (ISO, 1996) and OECD test guideline 202 OECD (OECD, 1984). In brief, twenty freshly hatched neonates were tested for each dilution, made with standard freshwater (ISO formula), which was also used as control. The number of dead plus immobile organisms in the controls was less than 10% as the guidelines stated. Survival rate was evaluated and the number of immobile D. magna after a 48 hr exposure period was scored. At least three to four replicates were carried out. Validity of the results was confirmed with a K\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7} internal control. EC\textsubscript{50} was calculated by logistic regression analysis as described above.

Microbiological analysis: Microbiological analyses including total coliform, fecal coliform and fecal streptococci were performed according to guidelines based on ISO norms 7899-2 (1984) and 9308-1 (1990).

Results and Discussion

Table 1 shows the result of heavy metal analysis. While Cu and Ni concentrations were below the detection level in all samples, relatively low concentrations of Pb (31.6, 34.8 and 10.3 mg l\textsuperscript{-1}) and Cd (0.6, 0.6 and 0.04 mg l\textsuperscript{-1}) were found in S1, S2 and S3, respectively. On the other hand, whereas S1 showed a low concentration of Cr (13 mg l\textsuperscript{-1}), extremely high concentrations of Cr (46.848 and 3.728 mg l\textsuperscript{-1}) were found for S2 and S3, respectively. The S1 value for Cr is comparable to the surface concentration in the site termed “Profile 2”, sampled from an identical location in the Hazaribagh area previously reported by Zahid et al (2006). Accordingly for Zn, high values were detected for S2 and S3 (608.1 and 88.9 mg l\textsuperscript{-1}, respectively), and S1 values were below the detection level.

These results suggest chromium and zinc were released from the surrounding tanneries and that the streamwise attenuation in the metal concentrations along the creek seems to be caused by sorption to the suspended or bottom sediments.

The hydrological budgets are roughly estimated as follows:

\[
Cr_\text{i} = (1 - a) \ Cr_\text{r} + a \ g_{\text{oC}} \ Cr_\text{r}
\]

where \(a\) = discharge ratio of wastewater, and \(g_{\text{oC}}\) denote the individual fraction of the dissolved matter Cr released from the tanneries. The release of wastewater is about 76% of the total discharge (\(a = 0.76\)) and an effective dilution is not expected. Here, equation for Cr is show as the representative while hydrological budget of Pb and Zn were also estimated identically. The Zn concentration at S1 is assumed
renders deleterious effects on aquatic organisms (Persoone et al., 1993). Adsorption and partial sedimentation explain the reduction in dissolved concentrations of Pb, Cr and Zn, only 10 to 17% of the total in the downstream sample. The situation of dissolved and particulate matters is described thoroughly in Zahid et al. (2006).

Physicochemical parameters of collected samples are shown in Table 2. Values such as dissolved oxygen, total dissolved solids and electric conductivity denote the low quality of water sample S2. When compared to S1 and S3. Turbidity in S2 did not show a very high difference when compared to the upstream sample S1, but did so with the downstream sample S3. Steady settling and sedimentation can account for the decrease in turbidity. Levels of pH remained within biocompatible levels for freshwater fish and bottom dwelling invertebrates (6.0-9.0).

Since electric conductivity is proportional to the total dissolved solids, it should remain conserved after the dilution (S1+S2), where the discharge ratio of the natural stream to the effluent is approximately 24. This enables us to estimate the resultant downstream chromium concentration as 1886 mg l⁻¹; however this value is just half of the actual concentration (3728 mg l⁻¹). This is partly due to the hyper-concentration of suspended solids, where the bottom concentration is much higher than the surface one, resulting in an underestimation. Thus the vertical profile of particulate matters, including coliform colonies (shown in table 4), can be accounted as one of the reasons of budget imbalance.

S1 and S3 did not show a significant toxicity in any of the three bioassays, hence EC₅₀ values could not be determined for samples S1 and S3. On the other hand, S2 exhibited strong toxicity to all three bioassays. Table 3 shows that amongst the three bioassays performed, the 15' Microtox® test rendered the most sensitive with an EC₅₀ of 9.8% in sample S2. This was followed by an EC₅₀ of 14.2% obtained with the toxicity test for the higher plant Lactuca sativa, and 31.5% for Daphnia magna with the 48-h acute toxicity test. This is in accordance with previous results which have proven the Microtox® assay to be very sensitive technique for detecting toxicity of metals and aquatic contaminants ( Dutka and Kwan, 1981; Coleman and Qureshi, 1985). Those results indicate that the raw wastewater effluent from tannery of Hazaribagh possessed detrimental effects on the broad spectrum of organisms in the ecosystem, implying that the ecosystem of Buriganga basin was disturbed by discharge of the effluents.

Heavy metal content may play an important part in the toxicity observed in the bioassays. The extremely high concentrations of chromium, which is a known carcinogen also renders deleterious effects on aquatic organisms (Persoone et al., 1993), appears to be at first hand the main cause of the observed effects on the tested organisms. Nevertheless, an interaction between heavy metals is expected due to the high content of Zn and Pb observed, which have shown low EC₅₀ values for the Microtox system in previous reports (Codina et al., 1993; Ishaque et al., 2006).

### Table - 1: Total heavy metal concentrations (mg l⁻¹) in water samples from the Hazaribagh tanning area. Values are mean ± standard deviation of 3 samples, ND = not detectable

<table>
<thead>
<tr>
<th>Metal</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>13±2.1</td>
<td>46848±3434</td>
<td>3728±255</td>
</tr>
<tr>
<td>Zn</td>
<td>ND</td>
<td>608.1±66.3</td>
<td>80.9±10.2</td>
</tr>
<tr>
<td>Pb</td>
<td>31.6±4.5</td>
<td>34.8±4.3</td>
<td>10.3±1.1</td>
</tr>
<tr>
<td>Ni</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cu</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cd</td>
<td>0.6±0.1</td>
<td>0.6±0.2</td>
<td>0.04±0.01</td>
</tr>
</tbody>
</table>

### Table - 2: Physicochemical characterization of water samples from the Hazaribagh tanning area. (NTU = Nephelometric turbidity unit)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Dissolved oxygen (mg l⁻¹)</td>
<td>4.7</td>
</tr>
<tr>
<td>pH</td>
<td>8.12</td>
</tr>
<tr>
<td>TDS (mg l⁻¹)</td>
<td>254</td>
</tr>
<tr>
<td>EC (mS cm⁻¹)</td>
<td>525</td>
</tr>
<tr>
<td>Salinity (%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>180</td>
</tr>
</tbody>
</table>

### Table - 3: Acute toxicity of sample S2 to Vibrio fischeri, Lactuca sativa and Daphnia magna

<table>
<thead>
<tr>
<th>Species</th>
<th>Exposure time</th>
<th>EC₅₀ (95% confidence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. fischeri</td>
<td>15 minutes</td>
<td>9.76 (8.6 - 11.1)</td>
</tr>
<tr>
<td>L. sativa</td>
<td>5 days</td>
<td>14.16 (9.4 - 21.3)</td>
</tr>
<tr>
<td>D. magna</td>
<td>2 days</td>
<td>31.58 (21.4 - 46.6)</td>
</tr>
</tbody>
</table>

### Table - 4: Microbiological tests. Total coliforms are expressed as colony forming units per ml. Faecal coliforms and faecal streptococci are expressed in colony forming units per 100 ml.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Stations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Total coliforms</td>
<td>3.6x10⁵</td>
</tr>
<tr>
<td>Faecal coliforms</td>
<td>2.0x10⁵</td>
</tr>
<tr>
<td>Faecal streptococci</td>
<td>2.0x10⁵</td>
</tr>
</tbody>
</table>

High values of electric conductivity and salinity in S2 were also accounted for the toxicity to the three different ecological strata evaluated, specially on the root elongation test, where lettuce root growth is heavily impaired under saline stress conditions (Kaya et al., 2002).

As seen in Table 1, S2 contained high concentrations of Cr and Zn. In this study, it was observed that the EC₅₀ value of sample S2 was higher than what was expected along with standard experiments ECOTOX database (US EPA, 1999), indicating that apparent toxicity was reduced by environmental matrix. This phenomenon is due to the occurrence of several physicochemical and environmental factors which affect the environmental sample, generally altering the bioavailability of metals (Codina et al., 1993). As such, humic and fulvic substances can provide a plausible
Microbiological analysis (total coliform, faecal coliform and faecal streptococci tests) displayed the greater number of colony forming units (CFU) on S2 (Table 4). Sample S2 showed 32 to 55-fold of microbial presence than in S1. In terms of total coliforms and faecal coliforms, the downstream sample S3 demonstrated 11 to 12-fold higher CFU than the upstream river water sample, S1. Those results indicate that water from S2 suffers from bacteriological contamination, related to the lack of an appropriate wastewater treatment system in the area.

Based on these results, we can conclude that the degree of environmental hazard in this particular area of Hazaribagh is high. It was suggested that elevated concentrations of heavy metals may be posing a considerable risk to the ecosystem surrounding the tanneries. It has been previously suggested that the presence of chromium in the environment can cause detrimental human and environmental effects (Pellerin and Booker, 2000; Shanker, 2005; Kuykendall et al., 2006). This study provides biological evidence that the tannery wastewater of the area of Hazaribagh exhibits detrimental impacts on broad aquatic and land organisms, which in turn destruct the ecosystem.

It is thus clear based on this study that the posed risk for the environment in the area is of serious concern, and as such, it is important to enhance the environmental monitoring capabilities of Dhaka’s water environment through the use of environmental bioassays. Further studies such as TIE (Toxicity Identification Evaluation), in which chemical and biological assay are combined to identify the chemical nature of toxicants, are needed. They would contribute to clarify in detail the full extent of the ecotoxicological impact of the tannery wastewater discharge.

Finally, this work contributes to provide information about the requirement of simple toxicity bioassays guided by chemical analysis, are a need to better understand the ecological impacts of effluent discharges on a receiving water body.

Acknowledgments

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