Toxicity evaluation of tannery effluents using bioassays

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This research work was performed to evaluate the potential toxicity of tannery waste effluents and heavy metals contents which are usually present in excess amount in tanning industry discharge wastewater. Toxicity was evaluated using bioassays such as Ames test, hemolytic test, comet assay, brine shrimp assay and microbial test. The samples of tanneries wastewater were taken from two different cities (Sialkot and Kasur) of Pakistan. Ames test was used to evaluate tannery waste effect on agar. Hemolytic test was performed on human and bovine Rbc’s to find out the percent lysis of blood and for bovine Rbc’s sheep blood was taken as sample. Brine shrimp test was performed to check the effect of effluents on Naupliis of (artemia salina) as brine shrimp contains hemoglobin, so effects on artemia salina larva helps to study the effects on human beings. After this study, we concluded that Cr$^{+3}$ is less toxic than Cr$^{+6}$, so Cr$^{+6}$ should be eliminated into water channels only after changing it into Cr$^{+3}$ using some bio treatments of sludge. Present study is helpful to know about the level of toxicity caused by the effluents discharged from tanneries and their adverse effects on human beings. This study can be utilized by government authorities to regulate tanneries on their waste before discharging in the surrounding areas.

INTRODUCTION

The excess use of heavy metals and toxic compounds in industries has increased the ecological contamination and this thing has attracted active research in toxicological studies (Iqbal et al., 2015; Abbas et al., 2017; Angelucci et al., 2017). Industries produce tons of toxic waste every day, with growing knowledge and awareness about the environment, many governmental agencies in different parts of the world are enforcing laws for industries to recycle or reuse their waste products. One of the exceptions is leather tanning industry whose waste cannot be recycled. Different qualities of leather having different colours are produced by excessive use of various dyes and chemicals (Karci et al., 2014; Iqbal et al., 2019a, b; Khambhaty et al., 2017). Tannery waste water (TWW) is always discharged into the water channels which increase the amount of heavy metals (especially Cr$^{+3}$ and Cr$^{+6}$) in water along with total dissolved solids (TDS), chemical oxygen demand (COD), sulfates, chlorides, and other minerals. TWW is eliminated by tanning industrial units, which usually contains organic and inorganic complex
compounds, oil and suspended solids. Such direct discharge of untreated TWW into environment is casting negative impact on aquatic and terrestrial atmosphere and posing threat to number of aquatic species. Crops cultivated on contaminated lands, usually uptake heavy metals with water from soil and it can cause serious health problems to human. So toxicity and mutagenic effects of TWW eliminated by leather products manufacturing units should be strictly monitored (Dixit et al., 2015). Textile, dyes manufacturing units and tanneries are on the top of the list in waste producing industries throughout the world and contributes the most to pollute the aquatic eco system. Water pollution has become a global challenge for humanity due to serious health side effects (Sharma and Malaviya, 2007; Tigini et al. 2011).

Cr is highly toxic metals as compared to the other hazardous metals. About 40% of the used Cr is directly released into the water bodies. Unregulated techniques and direct release of Cr with effluents induces environmental pollution, water and soil contamination (Szulczewski et al. 1997). It was demonstrated that Cr set off chronic and acute toxicity including dermatome toxicity, neurotoxicity, immune toxicity, genotoxicity, and carcinogenicity along with environmental toxicity (Andleeb et al. 2018; Zahoor and Rehman, 2009). Nowadays, Cr tanning is preferred by the tanning industries due to the processing speed, leather colours, low cost and better stability of the leather (Firdaus et al. 2011). Environmental contamination by tanneries waste discharge which is the 5th largest source of water pollution and it’s a serious concern through the world. About 80% of Cr which is accumulated in nature in the form of pollution is being produced from these tanning industry units on daily basis (Shi et al., 2016). Higher level of COD, BOD, TDS and complete removal of specific pollutants, pentachlorophenol, Cr⁶⁺ and Cr³⁺, synthetic tannins, surfactants, chlorides, grease, sulphate, azo dyes and oil from TWW is impossible even after treatments. Genotoxicity and mutagenicity is also caused by the effluents of leather tanneries (Kumari et al. 2016; Khabhhaty et al. 2017). Toxic effects of TWW on living organisms and toxicity measurement through analytical methods provides non reliable results. For accurate results, set of bioassays are adopted throughout the world (Table 1). Bioassays are cost effective alternatives to comprehensive chemical analysis. The objective of this study was to assess the level of toxicity of TWW effluents by means of bioassay tests (Ames test, haemolytic and brine shrimp).

**MATERIAL AND METHODS**

**Chemicals and reagents**

All the reagents employed in this study were of analytical grade and were purchased from Merck and Sigma-Aldrich. It includes NaOH (sodium hydroxide), NaCl (sodium chloride), LMP and NMP Agaroze, sodium sarcosinate, Cyclophosphamide, EDTA, Lysing solution, Ethidium bromide, lymphocytes separation medium, HCl (hydrochloric acid), DMSO (dimethyl sulfoxide), Alcohol, HNO₃ (nitric acid), Acetone, Triton X-100, Brine, PBS (Phosphate buffered Saline), MMS (methyl methanesulfonate), Tri X-135, ASA-400, Triton X-100, Black and white films, Disodium salt, Tris base, Phosphated buffer saline (PBS), Ethidium bromide, Hydrogen peroxide (H₂O₂), RPMI-1600 medium.

**Sample collection**

The TWW samples (WW-1 to WW-4 includes colour dye mix waste, Cr treated water and Chrome soda treated neutralized waste respectively) were collected from three different leather tanneries from the vicinity of Sialkot and Kasur by following the standard sampling methods designed by Eaton et al. (2005). Fresh water samples (FW-1 to FW-4) were also collected for testing of toxicity level. Samples were collected in pre-cleaned plastic bottles. Plastic bottles were washed by using HNO₃ 1% v/v and after 24 h washed with distilled water three times. The collected wastewater samples were stored at 4 °C.

**Haemolytic assay**

Iqbal et al. (2014) procedure was chosen for haemolytic assay. Heparinized tubes were used for collection of blood samples of bovine and human. Isotonic saline solution of phosphate having pH 7.4 (pH. was adjusted using solution mixture of 1% HCl and 1 M NaOH) was used as buffer during collection of blood samples and phosphate buffer was already chilled up to 4 °C. For 4 minutes, blood samples were centrifuged at 4000 rpm for 3 times to separate the red blood cells (Rbc's). The Rbc's were suspended in PBS solution. Using Haemocytometer, 7.06 × 10⁶ cells/mL erythrocytes were taken for each test. 1.5 mL of sample was taken in Eppendorf tube. 0.1% triton X-100 and PBS was taken as Positive control and negative control respectively. Eppendorf tubes were incubated with agitation at 37 °C for 30 min. Tubes were kept on ice for 5 min. to cool down after removing from incubator and content of the tubes was centrifuged for 7 min. Then 100 μL supernatant obtained as a resultant of centrifuge and 4 °C chilled 900 μL of PBS was mixed and temperature of test tubes containing mixture was lowered by placing them over ice for five min. Absorbance at 578 nm was recorded. Percentage Rbc’s lysis was evaluated using following equation.

\[
\text{RBC's lysis (\%)} = \frac{(As/As_{100-100}) \times 100}{1} 
\]

Where, As is absorbance of sample and As_{100-100} means absorbance of Triton X-100. 0.1% Triton X-100 and phosphate buffer saline solution was used as positive control (PC) and negative control (NC), respectively.

**Brine shrimp assay**

The assay was performed by following the procedure described in Iqbal et al. (2014). Artificially prepared seawater (APS) was used to hatch the eggs of brine shrimp (Artemia salina L), and incubated at 30 °C for up to 48 h under continuous aeration. The eggs were completely hatched in 48 h and larvae (nauplii) when comes out from the eggs they were attracted towards one side of the container with bright light source these larvae were collected and transferred to the test solution.
### Table 1: Toxicity evaluation of industrial effluents using bioassays

<table>
<thead>
<tr>
<th>Samples</th>
<th>Bioassay</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannery effluents</td>
<td>Ames test TA98</td>
<td>Showed high mutagenic index</td>
<td>Alam et al. (2010)</td>
</tr>
<tr>
<td>Textile waste</td>
<td>Hemolytic test TA98</td>
<td>81% cell death</td>
<td>Iqbal et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Shrimp test TA98</td>
<td>68% cell death</td>
<td></td>
</tr>
<tr>
<td>Composite tannery effluent</td>
<td>Direct exposure to Pila Globosa for 96 hours.</td>
<td>62/96 agar plates affected</td>
<td>Bhattacharya et al., 2016</td>
</tr>
<tr>
<td>Wastewater samples</td>
<td>Standardized lettuce bioassay</td>
<td>Germination of seeds was suppressed</td>
<td>Elabbas et al., 2016</td>
</tr>
<tr>
<td>Sediment samples from industrial area</td>
<td>Comet assay</td>
<td>DNA damage</td>
<td>Frenzilli et al., 2009</td>
</tr>
<tr>
<td>Wastewater samples</td>
<td>Lumistox luminescent bacteria test A. cepa</td>
<td>Decrease in root tip length with increasing concentration of effluents</td>
<td>Katsoyiannis et al., 2007</td>
</tr>
<tr>
<td>Effluent samples from common effluent treatment plant (CETP)</td>
<td>Allium cepa bioassay</td>
<td>Decrease in root tip length with increasing concentration of effluents</td>
<td>Kumari et al., 2016</td>
</tr>
<tr>
<td>Tanneries wastewater</td>
<td>Salmonella mutagenicity test</td>
<td>Significant mutagenicity with TA98, TA97a and TA100 strains</td>
<td>Masood and Malik, 2013</td>
</tr>
<tr>
<td>Effluent samples</td>
<td>A. cepa test</td>
<td>Decrease in root tip length</td>
<td></td>
</tr>
<tr>
<td>Waste water of municipality of Santa Maria do Herval, Cadeia River industrial wastewaters</td>
<td>Salmonella microsuspension bioassay</td>
<td>Significant response for direct mutagenicity, with strain TA98 in the winter sampling, and for TA100 and TA102 during the summer.</td>
<td>Tagliari et al., 2004</td>
</tr>
<tr>
<td>Wastewater samples from Danube Riverside at Novi Sad, Serbia</td>
<td>Ames or Salmonella typhimurium assay in vitro gene bioassays</td>
<td>No increase of the revertants number compared to the negative control</td>
<td>Tigini et al., 2011</td>
</tr>
<tr>
<td>Wastewater samples</td>
<td>Daphnia magna test</td>
<td>Holistic risk assessment was done by individual and mixture of chemicals</td>
<td>König et al., 2017</td>
</tr>
<tr>
<td>The dyes Direct Black 38 and Reactive Blue 15</td>
<td>Brine shrimp toxicity assay</td>
<td>Toxicity potential was observed</td>
<td>Cortés et al., 2018</td>
</tr>
<tr>
<td>Untreated textile industrial effluent of disperse, direct and reactive dyes</td>
<td>Allium cepa test</td>
<td>Decrease in root tip length with increasing concentration of effluent. Moderate mutagenic activity.</td>
<td>Bilal et al., 2016</td>
</tr>
<tr>
<td>Raw effluent, activated sludge, dehydrated sludge &amp; treated effluent</td>
<td>Allium cepa test</td>
<td>Significant reduction of the mitotic index was observed</td>
<td>da Silva et al., 2018</td>
</tr>
<tr>
<td>Microbial dation</td>
<td>Allium cepa test</td>
<td>non-toxic azo dyes found to be cytotoxic</td>
<td>Rawat et al., 2018</td>
</tr>
<tr>
<td>Wastewater samples</td>
<td>Cytotoxicity test</td>
<td>Total bacterial count was 1×106</td>
<td>Iqbal et al., 2015</td>
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TWW samples were diluted three times by APS and new volume of the test solution was adjusted to 100 mL. Suspension of larvae (Naupliis) containing at least 30 larvae were incubated for 36 h. These larvae and TWW containing tubes were examined after 36 h with a magnifying glass and the number of dead Naupliis was counted. For a positive control (PC) during the experiment cyclophosphamide 10 μg/mL was used. The % lethality was calculated by comparing the number of dead and mean survived larvae of the test solution under control.

Ames test

Mutagenicity potential of tannery wastewater was analyzed (Iqbal et al., 2014) with the help of genetically modified Salmonella typhimurium strains (TA 98 and TA 100). Ames test was done by the methodology reported in Kaplan et al. (2004). Freshly prepared cultures of Salmonella typhimurium containing 1 billion to 10 billion cells were used for this study. Different test samples of treated and untreated TWW samples were prepared. Positive mutagens controls for TA 100 strain, sodium azide (0.5 μg/plate) and for TA 98 mutant strain, 4-Nitro-o-phenylene diamine (0.3 μg/plate) were also prepared. Freshly prepared Vogel-Bonner medium E (50X) was placed on petri plates and air dried the plates, and in glass tubes 0.1 ml of different TWW were added, 1 ml amount of molten top agar, and just 0.1 ml each strains i.e., TA98 and TA100 was added in each of the glass tube. Glass tubes material was gently mixed and poured on the surface of minimal glucose agar (GM plates). The plates were incubated for 48 h at 37 °C on solidification of top agar. The results were noted down as number of reverted colonies per plate, mean value and standard deviation of result value was also reported.

Microbial test

Microbial test was performed on wastewater samples to evaluate the toxicity. Method and standard protocols was adopted as per guide of (Bartram et al., 1996). Isolated gram positive (G.P) E. coli colony, nutrient agar and Lactose broth were incubated at 37 °C for 24 h. After incubation time, test tubes were analyzed for gas and acid presence or absence and total number of coliform was also noted microscopically precisely. Microbial test allows rapid, cost saving and precise approach to find the potential toxicity level of food and wastewater.
RESULTS AND DISCUSSION

Cytotoxicity

Toxicity evaluation is useful study to protect our environment from hazardous and toxic contaminants. In present study toxicity and mutagenicity of fresh water and wastewater samples taken from different tanneries was evaluated using different bioassays such as heamolytic test with human and bovine RBC’s, Brine shrimp test, Ames test and microbial test. For toxicity evaluation Heamolytic assay with human Rbc’s was performed in carefully controlled conditions. Wastewater sample 1 which had color dyes of tannery caused cell death up to 86.33±2.62 which proved dye containing waste as highly cytotoxic. Wastewater sample 2 containing Cr treated wastewater caused cell death up to 48.50±1.70 which was proved to be mild cytotoxic in nature. Wastewater sample 3 containing Cr treated wastewater caused cell death up to 47.33±2.49 and 43.50±1.50 respectively. On the other hand, drinking water sample collected from area near to tannery was also tested and result for the freshwater sample 5 to 8 were 17.00±1.25, 20.50±1.70, 18.67±2.05 and 16.67±1.25 respectively. Graphical representation of the results is presented in Fig. 1 which shows the death rate of human blood cells in samples fresh water and wastewater of tanneries. The result of drinking water proved that it was not cytotoxic in nature but wastewater is highly toxic. PC and negative control (NC) were titronX-100 (0.1%) and phosphate buffer saline, respectively. Tigini et al. (2011) also reported the toxicity of tannery and textile wastewater by using a battery of bio-tests with higher and lower organism.

Bioassays included were bacterial test, Algal test, D. magna test and lemma test. Results revealed that D. magna test was the most sensitive for toxicity evaluation. Meric et al. (2005) reported that leather tanning wastewater is highly cytotoxic and harms the growth of marine Algae. Toxic effects were also studied on sea urchin and marine Microalgae under polluted and treated wastewater conditions. Results revealed that biological treatment of wastewater is useful technique to reduce the toxicity of tannery wastewater.

Similarly, Heamolytic test with Bovine Rbc’s was also performed to study toxicity of tannery wastewater. Wastewater Sample 1 containing colour dye wastewater of tannery caused cell death up to 83.33± 3.09 which was proved highly cytotoxic in nature. Cr treated wastewater of tannery also caused cell death about 46.00± 0.82 which proved to be mild cytotoxicity. Wastewater Sample 3 and 4 having the Cr soda treated wastewater samples killed Bovine RBC’s up to 83.33± 3.09 and 46.00± 0.82 respectively.
Rbc’s up to 42% ± 1.25 and 40% ± 0.82 respectively. Such result proved that Cr treated wastewater samples were mild cytotoxic. Fresh water or tap water inside the tannery was also tested and results for the fresh water sample 5 to 8 were 17.50%±0.82, 18.00%±1.25, 16.33% ± 1.25 and 15.00% ± 1.63 respectively in terms of cell death. The results of fresh water revealed that it was non cytotoxic. TitronX-100 (0.1%) was used as PC during bovine hemolymic test and NC was phosphate buffer saline. Fig. 2 shows the death rate of bovine cells in samples fresh water and wastewater of tanneries. Sharma and Malaviya, (2016) carried out toxicity evaluation of tannery wastewater with help of Triticum Aestivum bioassay. Chemical methods are extensively used to evaluate toxicity (Pantazopoulou and Zouboulis, 2018). Papageorgiou et al. (2017) used ladle furnace slag to measure the toxicity of tannery sludge. Oral et al. (2007) evaluated the toxicity of Cr containing wastewater of tannery by using sea urchin and Daphnia Magna tests. Results of both test revealed that Cr containing wastewater of tannery is highly cytotoxic in nature. For further evaluation of toxicity brine shrimp assay was also performed. Wastewater Sample 1 containing the dyes mixed wastewater of tannery showed alarming results and caused Nauplius death up to 71.33%±4.92.

Results proved dye containing wastewater of tannery highly cytotoxic in nature. Cr treated wastewater sample 2 showed result as 40.00%±1.70 in term of Nauplius death, which revealed this sample as mild cytotoxic as compare to dye containing wastewater. Cr soda treated wastewater samples 3 and 4 of different tanneries were also tested and results were 35.00% ± 0.82 and 35.50% ± 0.41 respectively. Results of Cr soda treated samples show potential mild cytotoxic effects. Drinking tap water samples which were collected from inside the different tanneries were also tested and results for freshwater sample 5 to 8, were 17.17% ± 0.62, 16.00% ± 0.82, 15.17% ± 0.85, 17.17% ± 1.31 respectively in terms of cell death respectively. The results of drinking water samples revealed that water samples were non cytotoxic in nature. In Brine Shrimp test cyclophosphamide (10µg/mL) was used as PC and saline water as NC. Fig. 3 shows cytotoxicity of TWW and freshwater samples. Similarly, tannery waste contaminated soil samples were studied by Masood and Malik, (2013). Toxic and mutagenic effect were highlighted which were determined by employing Ames test, A. cepa test and E. coli test k-12 mutants. Ames test was performed using bacterial strains TA-97a, TA-98, and even TA-100. TA-98 strain proved to be the most sensitive strain among all other. Heavy metals are frequently used in several industries to produce near products on the other hand they contribute to make environment toxic and pollutant. Islam et al. (2017) reported the toxicity of heavy metals such as Cr, Ni, Cd, Pd and Cu. Their concentrations were 239, 206, 16, 195 and 267 mg/kg in contaminated soil.

Bioassays are extremely sensitive and excellent technique to measure the toxicity of industrial wastewater of tannery, textile, paint, pigment and ink industries. In present work toxicity of tannery wastewater containing Cr and Cr soda treated wastewater was evaluated by using different bioassay. Hemolymic test with bovine and human Rbc’s and brine shrimp test was used to measure toxicity. It was concluded that these bioassays are better tool to evaluate toxicity of tannery wastewater. Results of Heamolytic and Brine Shrimp test proved had proved that tannery wastewater containing dye is highly cytotoxic and can cause serious health hazards to aquatic organisms. Proper regulatory Procedure should be followed to save toxicity sensitive species of aquatic environment.

**Mutagenicity**

For mutagenicity potential effect evaluation of tannery wastewater Ames test was used with test strain TA-98 and TA-100. The results of Ames test TA-98 are shown in Fig. 4. The results of Ames test were obtained in terms of number of revertant colonies. The number of revertant colonies for color dye containing wastewater sample 1 were 2425±62.36 out of 3350. Such results proved this sample highly mutagenic that is a serious health hazard for aquatic life. Similarly, Cr treated wastewater sample 2 was also tested and number of revertant colonies were 1725±73.60 which is the indication of mild mutagenicity. The number of revertant colonies for chrome soda treated wastewater samples 3 and 4 were 1903.33±130.21 and 1725±40.82 respectively, result showed that such sample were mild mutagenic as compare to dye containing wastewater of tannery. Mutagenicity of drinking water near tannery was also investigated by using same test. The results of fresh water sample 5-8 were 896.67±44.97, 776.67 ± 61.28, 925.00±73.60 and 805.00±49.67, respectively. The results of drinking water revealed that it was not mutagenic in nature on TA-98. PC for Ames test TA-98 was 4-nitro-o-Phenylenediamine (0.25µg/plate) with 3350 ± 40.82 average revertant colonies and NC solvent without sample with average of 430±21.60 revertant colonies were used as negative control. Tagliari et al., (2004) used Ames test for evaluating mutagenicity of tannery waste by employing test strain TA 98, TA 100, TA 102 and TA 97a. The target species in this research were fishes of polluted area. The results were obtained in terms of number of revertant colonies. Kumai et al. (2016) used *Bacillus cereus* Cr-I strain to reduce genotoxic effects of tannery effluents. Result revealed that this train reduces genotoxicity up to a considerable amount which were tested over *A. Cepa* in terms of growth rate. The mutagenicity of tannery wastewater samples was also determined by using Ames test with test strain TA-100. In the same way as previously described and results are shown in Fig. 5. The revertant colonies were 2740 ± 64.81 for colour dye containing waste sample 1. Results show that this sample was highly mutagenic in nature. On the other hand, Cr treated wastewater sample 2 was mild mutagenic because for this sample the number of revertant colonies were 207±37.42. Chrome soda treated wastewater sample 3 and 4 were also investigated and results were 1875±49.22 and 1883.33±33, respectively. The results of these samples proved them as mild mutagenic as compare to dyes containing wastewater. Mutagenicity of drinking water near tannery was also investigated by using same test. The results of freshwater sample 5 to freshwater sample 8 were 1405±62.85, 1303±44.97, 1390±29.44 and 1186.67±49.89, respectively.
**Fig. 3:** Brine shrimp death rate observed in fresh water and wastewater samples from tanneries

**Fig. 4:** Cytotoxicity of freshwater & wastewater samples of different tanneries evaluated through Ames test (TA 98)
Positive control for Ames test TA-100 was sodium Azide (0.5 µg/plate) with 4216.67±62.36 average revertant colonies and NC solvent without sample with average of 593.33±33 revertant colonies were used as negative control. Bhat et al. (2017) evaluated the toxic of industrial waste using different bioassay such as A. cepa, and Vicia faba. These two bioassays are cheaper and sensitive as well as compared to other in-vitro assays. On getting positive result they had suggested the possible use of earthworms and vermin technology as effective biodegradation method to reduce the harmful effects of industrial sludge.

Dixit et al. (2015) highlighted the toxic hazards of leather tanning wastewater. It was found that TWW contain several toxic chemical including Benzyl Butyl Phthalate, Dibutyl Phthalate and anthracene along with nonyl phenol. TWW contains several organic compounds which induce mutagenicity in living species. Because of Biological activity, mutagenicity is always evaluated by using bioassays. In present research mutagenicity was evaluated using TA 98 and TA 100 by following Ames test methodology. The results of Ames test revealed that TWW is highly mutagenic due to the presence of chromium and chrome soda in it. Ames test

![Graph](image-url)

**Fig. 5:** Cytotoxicity of freshwater & wastewater samples of different tanneries evaluated through Ames test (TA100)

### Table 2: Total coliform in tannery wastewater evaluated through microbial test

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total Bacterial count (CFU)</th>
<th>Total coliform (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW-1</td>
<td>&gt; 10^6</td>
<td>&gt; 10^5</td>
</tr>
<tr>
<td>WW-2</td>
<td>&gt; 10^3</td>
<td>&gt; 10^2</td>
</tr>
<tr>
<td>WW-3</td>
<td>&gt; 10^3</td>
<td>&gt; 10^2</td>
</tr>
<tr>
<td>WW-4</td>
<td>&gt; 10^3</td>
<td>&gt; 10^2</td>
</tr>
<tr>
<td>FW-1</td>
<td>Not Detected</td>
<td>Not Detected</td>
</tr>
<tr>
<td>FW-2</td>
<td>Not Detected</td>
<td>Not Detected</td>
</tr>
<tr>
<td>FW-3</td>
<td>Not Detected</td>
<td>Not Detected</td>
</tr>
<tr>
<td>FW-4</td>
<td>Not Detected</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>
was selected due to its diversity because it can be applied with TA-104, TA-102 and TA-97a. It was concluded that Ames test is the best way to monitor mutagenicity even for small concentration of pollutants. Ames test results were compared with chemical tests and it seems that Ames test provides more precise and accurate results.

Microbial load

Microbial test was performed for detection of coliform bacteria in wastewater (Table 2). Total coliform (CFU) in waste sample 1 containing color dyes was >10^5 and it should be around 10^3 so sample 1 was classified as highly cytotoxic and mutagenic. Similarly, Cr treated wastewater sample 2, Cr soda treated wastewater samples 3 and 4 showed average CFU >10^2 and are classified as mild or nontoxic. Fresh water samples 5 to 8 was also tested thorough microbial test and result collected after test showed that drinking water samples were nontoxic. Microbial load was evaluated and it was observed that wastewater sample 1 was highly contaminated whereas wastewater sample from 3 to 4 are mild contaminated and no bacterial contamination was observed in sample 5 to 8. The findings are in line with previous studies e.g. Megharaj et al., (2003) found that soil contaminated with tannery wastewater usually enriched with Cr^3+ and Cr^6+ ions contents that’s why Bacillus Sp. and Arthrobacter Sp., were unable to grow in Cr contaminated soil. Sponza, (2006) has done a similar work on dye industry wastewater and tried to culture many microbes like Coliform- Escherichia coli in contaminated water but the number of colonies was too low because of high contamination. Abovementioned stats clearly show that treated tannery wastewater are less toxic as compared to untreated wastewater. So all government and public institutes make sure that wastewater should be discharged only after treatment, as it will help to control further damage to environment (Ali et al., 2019; Bilińska et al., 2019; Caicedo et al., 2019; Gao et al., 2019; GillPavas et al., 2019; He et al., 2019; Osawa et al., 2019; Pantelaki and Voutsa, 2019; Tang et al., 2019; Tian et al., 2019; Vicente-Cera et al., 2019; Zhan et al., 2019).

CONCLUSIONS

Present work was designed to evaluate the potential toxicity and mutagenicity of tannery waste and heavy metals present in wastewater discharged by tanneries. The purpose of whole work was to analyze the potential effects on the living organisms of aquatic ecosystem and also on human health. Different bioassays were employed to evaluate the toxicity and mutagenicity due to tannery wastewater. From result of each bioassay it can be found that sample 1 which contains non treated, dye mix wastewater of tannery is highly toxic and damaging to human health whereas later Cr treated wastewater sample 1 to 3 are mild toxic. Fresh water samples show non-toxicity behavior towards all the tests. It can be concluded that dye mix non treated wastewater of tanneries is highly toxic for human health as compared to treated wastewater. Results of bioassays revealed that it's a better option to use bioassay rather than chemical test due to easy handling, cost effectiveness and accuracy of results. It is suggested that proper methods and procedures should be adapted to control the toxicity generation from and tanning units and to save the environment from toxic effects of waste effluents of tanneries.

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