Sustainable systems

Assessment of acute toxicity of Modjo tannery effluent using *Daphnia magna* bioassay

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ABSTRACT

Tannery wastewater contains large quantities of organic and inorganic compounds, including toxic substances such as sulfides and chromium salts. There are many established facts about the effects of tannery effluent on water quality, on living biota and to the environment. Employing bioassay for evaluation of industrial effluent helps better understanding and picture of possible effects of industrial effluents. In Ethiopia, chemical analysis based factory effluent evaluation and monitoring has been commonly employed. The aim of this research was to determine the toxicity of Modjo tannery effluent to the receiving system (Modjo river) (also known as Mojo River) using Daphnia bioassay at 24-h and 48-h acute toxicity test, and to suggest the possibility of employing Daphnia bioassay for evaluation and monitoring of factory effluents in the country. Two effluent samples from two different tannery stages (beam-house stage and tanning stage) were considered. The studyresult showed that both effluents caused moderately acutely toxic effects on D. magna at both 24-h and 48-h of exposure with LC50 values ranging from 4.51% to 8.36% dilution. Therefore, proper treatment of the currently discharged effluent concentration to <4.51 % is recommended before direct release into the river. The result also showed that the effluent from tanning stage caused more sensitivity to the test organisms at both 24-h (EC50= 4.65%) and 48-h (EC50= 4.51%) tests than the effluent from beam-house stage, which has EC50= 8.36% at 24-h and EC50= 7.91% at 48-h tests. This toxicity level difference might be attributed to the variation in chemical composition of tanning stage and beam-house stage effluents.

Keywords: acute toxicity, bioassay, Daphnia magna, LC50, tannery effluent, Modjo (Mojo) River, water quality

Introduction

Leather tanning, in comparison to other industries, stands out as one of the most environmentally detrimental activities with a notably high toxic intensity per unit of output (Khan *et al*., 1999). This intricate process involves the conversion of animal hides into leather through a series of complex stages, utilizing substantial amounts of water and a range of chemicals, including lime, sodium sulfide, ammonium sulfate, sodium chloride, bactericides, vegetable tannins, and chrome salts (Cooman *et al*., 2003).

The culmination of tannery operations results in the discharge of effluents into water bodies and onto land, presenting significant environmental challenges. The effluent contains dissolved and suspended solids, as well as various chemicals and compounds that adversely impact the quality of nearby streams, rivers, groundwater, and aquatic habitats, leading to acute toxicity for diverse organisms (Tudunwada *et al*., 2007). Notably, the high sulphide content of tannery sludge poses environmental odor issues (Tudunwada *et al*., 2007). Moreover, chromium, an inherent byproduct of the tanning process, is identified as a human carcinogen and a stressor for plants (Cristina *et al*., 2008).

Presently, Ethiopia hosts over 20 tanning industries, and a concerning 90% of these establishments release untreated wastewater into nearby water bodies and open land, highlighting the severe threat posed by the tanning industry to the aquatic environment, particularly in major cities, rivers, and lakes (EPA, 2001). Notably, chromium levels exceeding permissible limits have been reported in Akaki areas, indicating a critical environmental concern (Fisseha Etana, 1998). Additionally, Akaki and Modjo Rivers, situated in areas with clusters of tannery industries, face extreme degradation threats, impacting a densely populated region where the majority relies on water from these rivers for various purposes (Seyoum Leta *et al*., 2003).

Effluents from tannery operations constitute a complex mixture of compounds with a highly variable composition and bioavailability, making a comprehensive ecotoxicological evaluation challenging (Barata *et al*., 2008). Although traditional assessments rely on recognized physical and chemical parameters, the incorporation of bioassays alongside chemical tests is increasingly recommended to provide a more holistic understanding of the

potential effects of industrial effluents (Cristina *et al*., 2008; Martins *et al*., 2007). Biological tests, as highlighted by COHIBA (2010), serve as valuable tools for early warning of potential wastewater effluent hazards, leading to a global trend in combining both chemical tests and bioassays for industrial effluent evaluation.

In Ethiopia, the evaluation and monitoring of factory effluents have primarily relied on chemical tests, with less attention given to the assessment of their effects on aquatic biota using bioassays. For instance, previous studies focused on characterizing downstream pollution profiles along Modjo River, but the potential effects of Modjo Tannery effluent on aquatic biota using Daphnia bioassay remain unexplored (Seyoum Leta *et al*., 2003).

Daphnia magna is widely employed in aquatic toxicology due to its ease of culture, high sensitivity to toxicants, and its ability to reproduce by cloning (Donald *et al*., 1989). Recognized by Environmental Protection Agencies (EPA) as a reliable toxicity indicator, D. magna bioassay has been utilized to assess risks and establish guidelines for waste discharges (Rossini and Ronco, 1996). Moreover, Daphnia species tests are endorsed by international organizations such as ASTM, USEPA, EEC, and OECD as the preferred freshwater invertebrates for bioassays (Talapatra and Banerjee, 2004).

This study aims to assess the toxicity of Modjo tannery effluent using Daphnia magna bioassay and advocates for the incorporation of Daphnia magna bioassay in tannery effluent monitoring practices in the country.

2. Materials and methods

2.1. Study Area

Modjo town, located at 8°35' N latitude and 39°07' E longitude with an elevation between 1788 and 1825 meters above sea level, is situated approximately 80 km south of Addis Ababa. Modjo Tanning, a medium-sized leather industry in the same town, processes 844,000 sheep and 1,656,000 goat skins annually, utilizing water from Modjo River and discharging effluents directly into the downstream. The daily volume of wastewater discharge into Modjo River ranges from 3500 to 5500 cubic meters (Seyoum Leta, *et al*., 2003).

2.2. Culturing D. magna

Test organisms underwent continuous laboratory culture under specified conditions outlined in Table 1.

Table 1. Summary of Culture Conditions of D. magna (Modified from APHA, 1992, and APHA, 1998)

Culture Condition	**Details**
Source of Daphnia	Pond located at Addis Ababa University, 'Arat kilo' campus, Ethiopia
Number Introduced/Container	20
Age of D. magna	< 24 hr
Aeration of Culture Vessels	None
Culture Water	River water aerated for 24 hrs before use
Feed of Daphnia magna	Bacteria
Bacterial Feed	20 gm of cow dung mixed with 500 ml of river water, stored for 3 days before use
Bacterial Feeding Frequency	Twice/day
Light Quality	Cool white fluorescent light (>600 Lux)
Photoperiod	14 hr. light: 10 hr. dark
Temperature	23 ± 2.0 °C
Dissolved Oxygen (DO)	60 and < 100% saturation
pH	6.0 to 8.5
Culture Vessel	1000 ml glass jar
Total Culture Volume	800 ml
Contaminating Chemicals	None

Culture Condition	Details
Culture Health Criteria	Batches with no ephippia
Pre-test Treatment	None

2.3. Sampling and Acute Toxicity Test

Effluent samples were obtained from the beam-house and tanning stages, recognized as the most contaminating stages. The samples were collected from Modjo Tannery effluent outlet and transported to the laboratory for testing under conditions specified in Table 2.

Table 2. Summary of Test Conditions for 24 and 48 Hours Acute Toxicity Test (Modified from APHA, 1992, and APHA, 1998)

Test Condition	Detail
Organism	D. magna
Number/Container	10 organisms
Age	< 24h
Test Type	Static (non-renewal)
Temperature	23 ± 2.0 °C
Aeration During the Test	None
Feeding During the Test	None
Photoperiod	14 h light: 10 h dark
Light Quality	Cool white fluorescent light (>600 Lux)
Test Concentration (in %)	0, 1, 2, 3, 4, 5, 6, 7, 8, and 9
Number of Replicates/Test	3
Dilution Water	Modjo River water
Test Vessel	1000 ml glass jar
Test Duration	48 hours
Observation Period	4

Test Condition	Detail
Assessment Endpoint	Mortality/immobilization
pH Adjustment	None
Handling	Using a 4 mm wide plastic pipette
Test Validity	Control organism must have mortality <10%

2.4. Data Analysis

D. magna mortality responses were transformed into predicted dose/response data using probit in SPSS Version 15 Program. The predicted dose/response data were then analyzed in Excel to determine median effect concentrations (EC50), representing the concentration estimated to immobilize 50 percent of the D. magna population at 24h and 48h of exposure. Additionally, a best-fit dose-response curve was fitted using the predicted dose/response data of 24h exposure in Excel to determine the slope.

3. Results and discussions

3. Results and Discussions

The study determined 24h and 48h EC50 values for both beam-house and tanning stages effluent samples, as presented in Table 3. The toxicity classification used, following Tonkes *et al*. (1999), Sponza (2002), and Verma (2008), categorizes effluents with an EC50 value between 1-10% for D. magna bioassay as "moderately acutely toxic" (Table 4). Consequently, the effluents from both stages in this study were classified as moderately acutely toxic.

Table 3. 24h and 48h EC50 Values, 95% Confidence Limits, and Slopes for Tanning and Beam-house Stages Effluent Samples

Effluent Stage	Beam-house	Tanning
24h EC50	8.36	4.65
95% Confidence Limit at 24h	7.87-8.9	4.18-5.07

Effluent Stage	Beam-house	Tanning
48h EC50	7.92	4.51
95% Confidence Limit at 48h	7.24-8.5	3.99-5.01
Slope at 24h EC50	6.45	44.21

The results indicated that the tanning stage effluent caused higher sensitivity in the test organisms compared to the beam-house stage effluent for both 24h and 48h exposures (Table 3). This variability can be attributed to the different chemical compositions in various tannery processes. Notably, heavy metal content, particularly chromium from the tanning stage, played a significant role in the observed toxicity response in D. magna bioassay (Arias *et al*., 2010). A similar study by Talapatra and Banerjee (2004) reported higher mortality in D. magna exposed to tanning stage effluent compared to other stages of tannery effluent.

Table 4. EC50 Toxicity Rating for Different Concentration Ranges (Modified from Tonkes et al., 1999; Sponza, 2002; Verma, 2008)

EC50 Percent of Effluent	Toxicity Rating
>100%	Relatively not acutely toxic
10-100%	Minor acutely toxic
1-10%	Moderately acutely toxic
<1%	Very acutely toxic

In toxicological experiments, the length of the exposure period influences the biological responses of test organisms. Consistent with this principle, the study demonstrated that longer exposure periods resulted in lower EC50 values, indicating greater toxicity (Verma, 2008).

4. Conclusions and Recommendations

Studies on wastewater effluents emphasize the practical role of toxicity tests using bioassays in water quality monitoring. This research validates the efficacy of toxicity

testing using D. magna bioassay and suggests its adoption for establishing effluent discharge permits and water quality monitoring internationally.

The study concludes that Modjo Tannery effluent is moderately acutely toxic. Therefore, it recommends that the effluent should undergo proper treatment before discharge into Modjo River, aiming for concentrations below 4.51% of the current discharge concentration.

Given the high sensitivity of D. magna to toxicants, the study highlights the significant impact of Modjo Tannery effluent on Modjo River. The recommendation includes the formulation of strict legislation to control effluent discharge limits.

The study confirms that tanning stage effluent is more toxic than beam-house effluent due to differences in chemical composition. However, further detailed studies are needed to identify the specific causative agents for the observed higher mortality of D. magna and to implement appropriate measures.

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