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Toxicity assessment of textile wastewater treated by constructed wetland augmented with a consortium of endophytic bacteria using *Daphnia magna*

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ABSTRACT

Constructed wetlands (CWs) augmented with endophyte bacteria, well known as a sustainable and low-cost technological approach, have been widely applied to treat textile wastewater. However, the remaining dyes and other pollutants in treated textile wastewater are not guaranteed to impact the biotic environment negatively. Validating the toxicity level of treated textile wastewater is critical to ensuring aquatic environmental safety. Therefore, this study evaluated the acute toxicity of treated textile wastewater from CW reactor integrated with *Vetiveria zizanioides* and consortium endophytic bacteria. Acute toxicity (LC_{50}) represents the concentration causing 50% mortality in the aquatic organism population within 96 hours. Whole effluent toxicity (WET) testing using *Daphnia magna* as a standardized bioassay was conducted to determine LC_{50}. Three variations of treatments, untreated wastewater (K), constructed wetland without bacteria (C1), and constructed wetland with bacteria (C2), were monitored for treating three loading concentrations (25%, 50%, and 100%) of textile wastewater. As a result, the untreated wastewater had LC_{50} 1.52–200% and TUa 0.50–65.62, indicating slight to high acute toxicity. In contrast, CWs with and without consortium bacteria exhibited LC_{50} > 100% and TUa < 1, indicating that both treatments raised effluent quality and became non-acute toxicity. Moreover, the *D. magna* that survived in treated textile wastewater from CWs with and without bacteria exhibited less morphological and physiological impact on the organs, particularly heart rate and thoracic limb movement. These findings demonstrate that CWs augmented with endophytic bacteria can eliminate acute toxic risks in textile wastewater and reduce its hazardous potential to the aquatic environment.

Keywords: acute toxicity; textile effluent; *Daphnia magna*; constructed wetland; LC_{50} assessment.

INTRODUCTION

The traditional woven industry in Indonesia faces significant challenges related to environmental pollution. This issue becomes critical, because the owners of these industries often lack the financial resources and knowledge needed to manage and treat the wastewater generated during textile production. Synthetic dyes, such as naphthol, remazol, and indanthrene are commonly used in the dyeing processes of Indonesia's traditional woven industry and contain high levels of pollutants, including COD, TSS, colour, and heavy metals, which are difficult to biodegrade (Fajri et al., 2024; Ramadhani & Subandi, 2015). Textile effluents contain chemical compounds categorised as PBTs (persistent, bioaccumulative, and toxic substances), often known as persistent organic pollutants (POPs). Those pollutants can lead to acute toxicity effects, such as mortality to aquatic organisms (Akeredolu et al., 2023). These chemicals also pose significant environmental and ecological risks, such as invertebrate taxa richness and abundance declining near the effluent discharge site, significantly changing their life modes and feeding groups (Gómez et al., 2008). Therefore, it is crucial to assess the acute toxicity of textile effluent to protect aquatic ecosystems from these harmful effects.

Constructed wetlands are an increasingly adopted technology for treating textile effluents due to their eco-friendly and cost-effective approach, relying on natural processes involving vegetation, microbial communities, and soil filtration (Vymazal, 2010; Wei et al., 2020). Several studies have reported that wetland treatments have effectively reduced organic, nutrient and heavy metal contamination in textile effluents (Hussain et al., 2018b; Shehzadi et al., 2016; Tara et al., 2019). Despite that, the residual can remain even at very low concentrations and may pose potential risks to aquatic organisms (Berradi et al., 2019; Choudhury, 2014). The incomplete degradation of complex dye molecules and the persistence of heavy metals may contribute to residual toxicity, highlighting the need for post-treatment toxicity evaluations. The WET method has been widely applied to measure the acute toxicity level of wastewater exposure in surface water. This method provides a more comprehensive understanding of the wastewater potential hazard to aquatic organisms compared to testing individual chemical substances (Jin & Kusui, 2019). Moreover, WET is considered a more practical approach to analysing the extensive impact of whole pollutants than measuring every possible chemical and heavy metal pollutant (Marshall, 2016). Acute toxicity assessment evaluates mortality as the endpoint, with results expressed as LC_{50} (lethal concentration affecting 50% of test organisms) over an exposure period ranging from 24 to 96 h (US EPA, 2002).

Previous studies have reported acute toxicity levels in various treated textile effluents significantly affecting aquatic organisms (Alderete et al., 2021; Lach et al., 2022; Zhang et al., 2012). Certain levels of pollutants in textile effluents can negatively impact aquatic life behaviour, such as immobilisation, reduced swimming capacity, abnormal movement, sluggishness, decreased survival, and loss of muscular function (Carney Almroth et al., 2021; Parmar & Shah, 2021). These findings emphasise the importance of conducting bioassays, including lethal concentration 50 (LC_{50}) tests, to accurately assess the impacts of treated textile effluents on aquatic organisms.

Aquatic bioassays using model organisms are essential to evaluate the toxicity level of textile effluents. Among aquatic organisms, *Daphnia magna*, a type of zooplankton, was frequently used in ecotoxicological tests due to its high sensitivity to pollutants, central role in the lentic food chain, and ease of handling in laboratory settings (Bownik, 2017; Kakka et al., 2021). Compared to other aquatic species, such as fish or algae, *D. magna* offers practical advantages, including short life cycles and rapid reproductive rates, making it highly suitable for acute toxicity tests (Gyllström & Hansson, 2004).

Textile wastewater is characterised by a high concentration of pollutants, such as dyes, heavy metals, and organic chemicals, which can remain toxic to aquatic organisms even after treatment processes. However, the toxicity of effluent from textile wastewater treatment, particularly in wetland treatment systems, is seldom evaluated, and the quality of treated effluent may remain questionable, even if high removal efficiency is achieved. This study aimed to assess the acute toxicity of treated textile wastewater from wetland treatment systems (with and without bacterial augmentation) using whole effluent toxicity testing *D. magna* bioassays. The research identified the lethal impacts of effluent from constructed wetlands (CWs) on *D. magna*, even at low concentrations of pollutants. This methodology will contribute to a more thorough ecological risk assessment of treated textile wastewater, providing a better understanding of the acute toxicity levels associated with its discharge into surface waters.

MATERIALS AND METHODS

Wetland reactor construction

Triplicated constructed wetland treatments consisted of untreated wastewater (K), constructed wetland (C1), and constructed wetland with the addition of consortium bacteria (C2). A CW reactor was made using a 55 L plastic box container and contained medium gravel, fine gravel, sands, sterilised soils, as well as 12 groups of *Vetiveria zizanioides* plant (Fig. 1). Three strains of identified bacteria (Fig. 3), *Bacillus cereus* (NR_115714), *Bacillus spizizenii* (NR_112686), and *Stenotrophomonas malthopilia* (NR_040804) from the previous report were used in this study (Fajri et al., 2024). *Vetiveria zizanioides* plants were planted in each CW reactor and acclimatized for 3.5 months before treatments. The concentrations of textile wastewater were prepared for 25% and 50% from artificial dyes (naphthol dye-based colours), and 100% wastewater was collected from the effluent of the woven home industry in Jepara District, Central Java Province (Fig. 2). Constructed wetlands treatments were run for two months in a greenhouse with three batches of textile concentrations: batch 1 for 25% concentration from 0–14 days, batch 2 for 50% concentration from 14–28 days, and batch 3 for 100% concentration from 28–56 days.

Daphnia magna bioassays

The toxicity assessments of treated textile wastewater in different concentrations were done using living aquatic organisms of *Daphnia magna* obtained from a local breeder. A month of *Daphnia magna* was cultured in a laboratory in a 22.5 L aquarium containing aerated tap water (Fig. 4). The culturing process was conducted for over three generations, and the water condition was maintained under DO ≥ 3 mg/L, pH neutral (7–8), and room temperature 25±3 °C. *D. magna* were fed with yeast diluted in tap water every single day. The mature *D. magna* with eggs were placed into a new reactor, and newly born larvae (< 24 h) were available for the LC_{50} toxicity test refered to USEPA (2002).

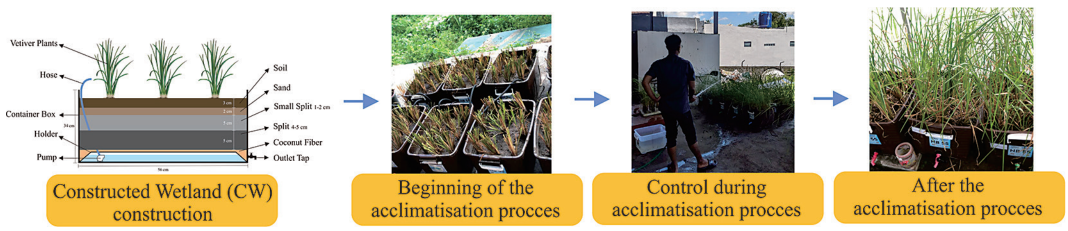

Figure 1. Construction and acclimatisation processes of CW reactors

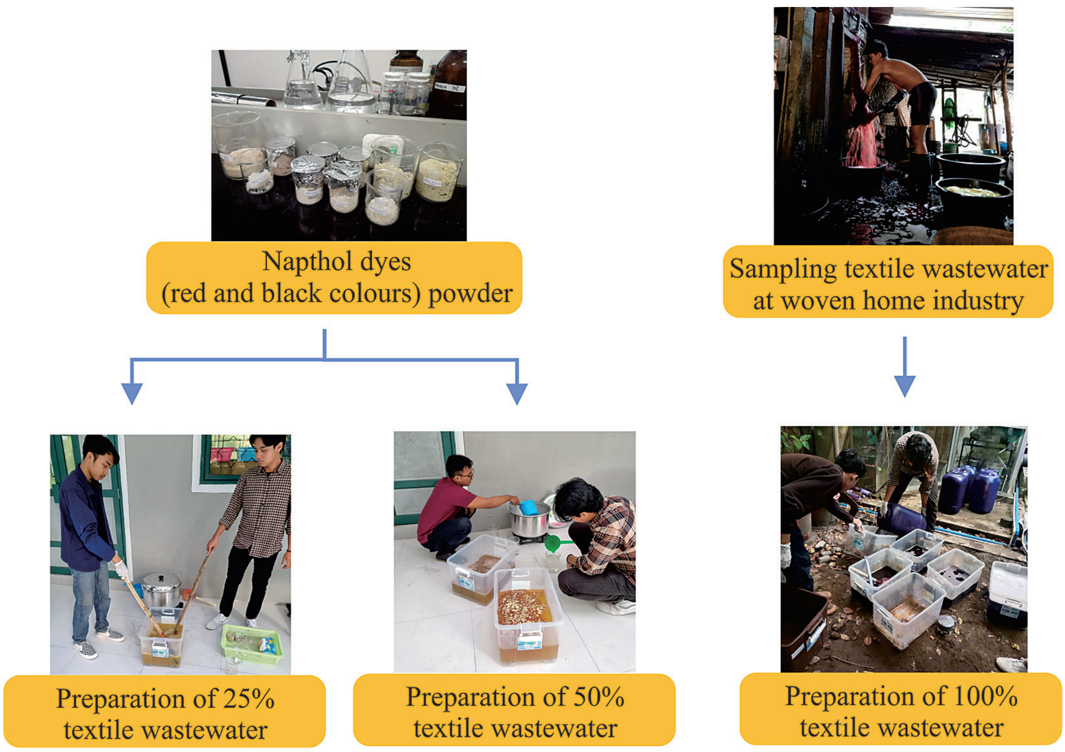

Figure 2. Textile wastewater preparation before running the CW treatment

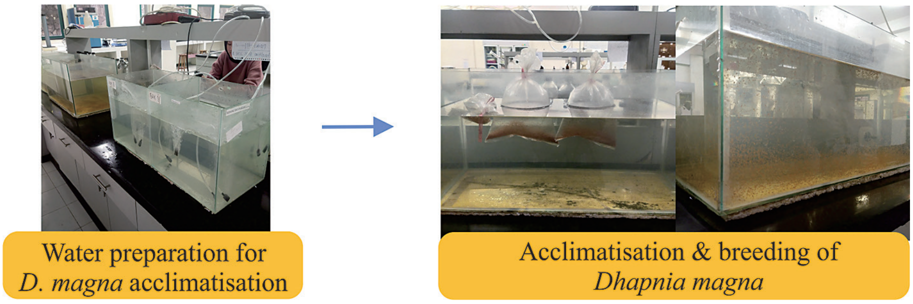

Figure 3. Re-culture selected strain consortium bacteria

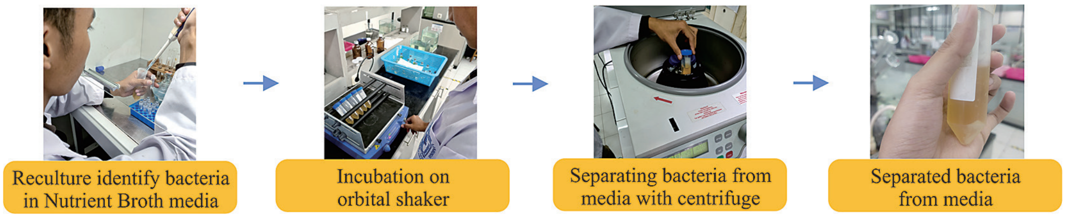

Figure 4. Acclimatisation and breeding of *Daphnia magna* processes prior to WET analysis

Sampling and water quality analysis

Composite samples were collected from effluents of three of the same treatments at the end of each batch concentration. Prior to toxicity assessment, composite treated wastewater samples were measured for physicochemical parameters, including chemical oxygen demand (COD), ammonia, colour, dissolved oxygen (DO), pH, water temperature (Tw) and heavy Pb and Cd heavy metals (Fig. 5). The COD, ammonia, colour and heavy metals were measured by following the instructions of the Indonesian National Standards for Water and Wastewater Quality (BSILHK), which referred to the APHA. Meanwhile, the physical parameters of DO, pH, and Tw were measured

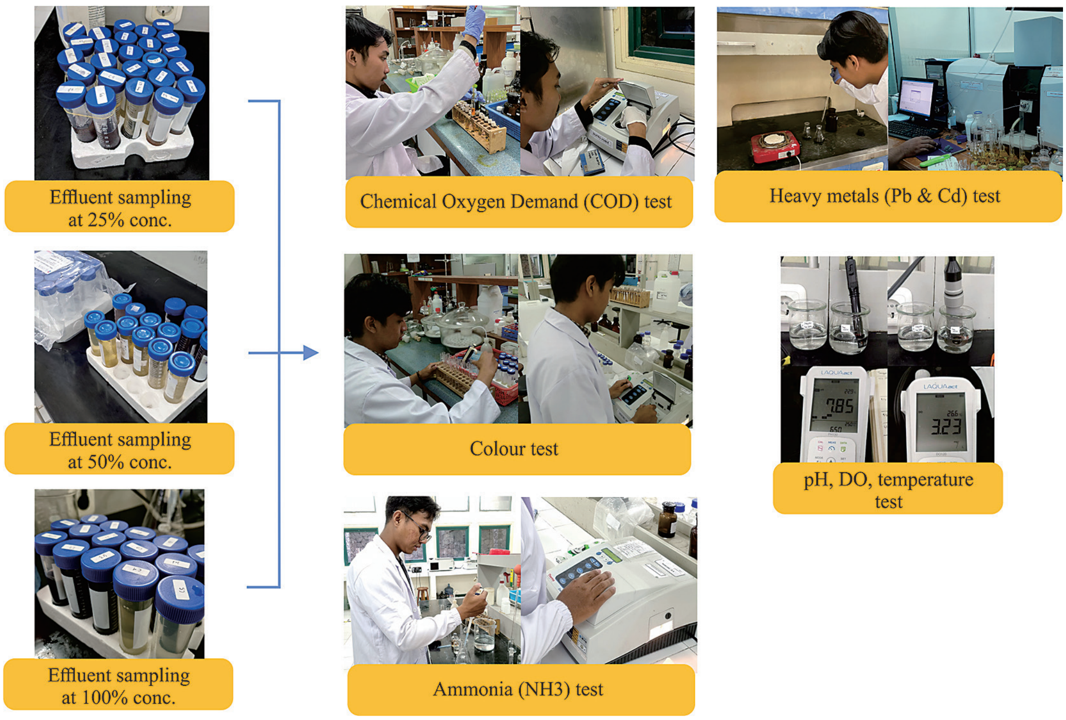

Figure 5. Sampling effluents and water quality analysis after CW treatment

using a portable probe meter produced by Horiba LAQUAact PD110 and PH110 Water Quality Meter. A detailed list of methods for analysing the wastewater quality was provided in Supplement A.

Whole effluent toxicity test

A WET test was conducted, referred to the United States Environmental Protection Agency (US EPA, 2000) method to assess acute toxicity and calculate LC_{50}, representing the effluent concentration that causes 50% mortality (Fig. 6). Toxicity was assessed by exposing *D. magna* as bioassay at different effluent concentrations for 96 hours, and lethal concentration (LC_{50}) was identified when 50% mortality of bioassays animal. The toxicity assessment was done by preparing three replicates of a 100 mL composite sample in a 200 mL glass bottle containing 10 larvae of *D. magna* (Bownik et al., 2015). The toxicity assessment was measured using two steps of range test concentrations: finding test and definitive test. A preliminary range-finding test was conducted using dilution variations of 0% (control), 6.25%, 12.5%, 25%, 50%, and 100%. Mortality was monitored daily for 96 h, and the results were acceptable when the animals in the control remained 90% alive. The subsequent concentrations of the definitive test were conducted when 50% bioassay mortality was confirmed at one concentration of the range-finding test. The new range concentrations of the definitive test were determined using Equation 1 by multiplying the R-value with the lowest concentration.

$$R = \left(\frac{a}{b}\right)^{\frac{1}{n-1}} \qquad (1)$$

R is constant, *a* is the highest concentration with mortality of 50%, *b* is the lowest concentration with mortality close to 50%, and *n* is the number of concentrations (Rohmah et al., 2018). The LC_{50} was estimated using dose-response analysis with the probit method (US EPA, 2002). This analysis involved transforming mortality rates using a probit transformation, converting effluent concentrations to $\log_{10}$, and calculating LC_{50} from linear regression parameters. Additionally, the toxic unit-acute (TUa) was calculated to categorize the acute toxicity level using Equation 2 (EPA, 2000).

$$TUa = \frac{100}{LC_{50}} \qquad (2)$$

Morphological and physiological analysis

The morphological and physiological analysis of *D. magna* followed a modified method of Bownik et al. (2015) and Li et al. (2023). The experiment involved 10 organisms in a 100 mL effluent sample and observed after 96 h exposure (Fig. 7). The samples analysed were taken from the highest concentration in definitive test result for each effluent batch (25%, 50%, and 100% loading concentration) and compared to the unexposed control (0%). Physiological parameters, including heart rate, thoracic limb movement, and physical damage of *D. magna* by dye absorption in body tissue, were examined under an Olympus BX53 microscope at 40x magnification. Observations were conducted in three replicates and recorded for 1 min. The recorded videos were analysed using a media player application. The number of beats or movements was counted manually in 10 seconds and multiplied by 4 to estimate beats per minute for heart rate and times per minute for thoracic movements.

Statistical analysis

One-way variance (ANOVA) statistical analysis with the Tukey post-hoc test was conducted using SPSS 15 to determine the significant difference ($p < 0.05$) data in effluent quality; in addition, morphology and physiological analysis were

Figure 6. WET experiment on various concentrations of effluent CW treatment

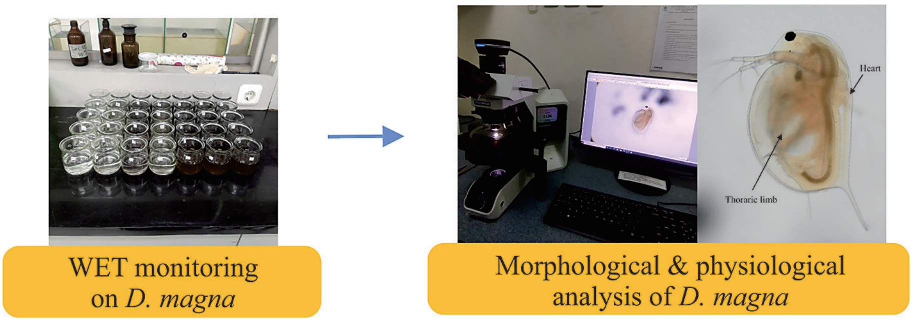

Figure 7. Analysis of D. magna morphology and physiology during WET monitoring

carried out. The statistical analysis compared the alteration of heart rate and thoracic limb movement of *D. magna* after effluent exposure to textile wastewater.

RESULTS AND DISCUSSION

Textile effluent characteristics

The physicochemical characteristics of the textile effluent are shown in Table 1. Concentrations of COD from untreated wastewater (K) and treated water from reactors of C1 and C2 exhibited high levels (ranging from 1082.10 to 3588.14 mg/L) as well as exceeded the standard limit at all loading concentrations. No significant differences in COD concentrations were observed between the treated and untreated wastewater ($p > 0.05$), even though the treated wastewater showed better quality than untreated wastewater (K). These results may indicate that wetland treatments can reduce COD, compared to any treatment. The COD parameter reflects the oxygen required to decompose chemical pollutants in a water body. When COD levels are exceptionally high, oxygen can be entirely depleted, creating uninhabitable conditions for aquatic organisms (Durotoye et al., 2018).

The colour level of untreated wastewater (K) was significantly higher than the treated effluent ($p < 0.05$) and exceeded the standard limit. The treated effluent from reactor C1 and C2 remained within the permitted limit for all loading concentrations, except for reactor C2 at 100% loading concentration, which slightly exceeded the standard. These results were similar to other reports that wetland treatments perform well in reducing colour (Kadam et al., 2018; Rasool et al., 2023). The performance of wetland treatments is raised by combining the endophytic bacteria to reduce the plant stresses and improve plant growth under extreme conditions (Goud et al., 2020). The physical parameters from all reactors, mainly DO, Tw, and pH, are shown in the standard levels for the aquatic live organisms (Table 1). The DO concentrations met the survival standard for *D. magna* (> 3 mg/L) across all samples ($p > 0.05$), except in the untreated wastewater (K) at 100% loading concentration, which recorded a low concentration of 1.16 mg/L ($p < 0.05$). The low dissolved oxygen (DO) levels of the effluent may be affected due to the high organic load, which correlates with the elevated content of total solids. These significant amounts of organic matter demand large quantities of oxygen for oxidation and decomposition, potentially threatening the survival of aquatic organisms (Adeogun & Chukwuka, 2011). In turn, the pH values were constantly neutral, ranging from 7.87 to 9.12, showing an upward trend with higher loading concentrations of textile wastewater ($p < 0.05$). The water temperature fluctuated from 24.67 to 26.90 °C ($p < 0.05$) and remained within a safe range for *D. magna* survival (Adamczuk, 2020; Müller et al., 2018).

The heavy metals (Pb and Cd) remained in textile wastewater even after treatment using wetland systems and bacterial inoculation, with no significant differences among the various treatment methods ($p > 0.05$). The concentration of Pb ranged from 0.36 to 2.21 mg/L, with the highest level obtained by untreated wastewater at 50% loading concentration. Cd concentrations ranged from 0.05 to 0.25 mg/L, and some samples from treated effluent had a higher concentration than the untreated effluent. These indicate that acute toxicity can still be a concern, even after treatment. Heavy metals, defined as chemical elements with substantial atomic weight and density, are recognised as some of the most hazardous inorganic pollutants due to their toxicity, even at low concentrations (Ali et al., 2019). These findings

Table 1. The physicochemical characteristics of textile effluent from wetland treatments

Treatments	Parameters							
	COD (mg/L)	Ammonia (mg/L)	Color (Pt-Co)	DO (mg/L)	pH	Tw (°C)	Pb (mg/L)	Cd (mg/L)
25% Wastewater Effluent								
K	2563.18^a ± 284.71	5.86^a ± 0.29	1592.17^b ± 83.03	4.08^a ± 0.12	7.92^d ± 0.06	25.13^c ± 0.03	0.95^a ± 0.44	0.21^a ± 0.02
C1	3588.14^a ± 512.48	2.04^a ± 0.02	117.27^d ± 1.29	4.58^a ± 0.06	7.87^d ± 0.04	25.20^b ± 0.00	1.49^a ± 0.34	0.25^a ± 0.10
C2	2406.40^a ± 783.15	4.20^a ± 0.17	114.68^d ± 0.00	4.23^a ± 0.23	7.95^d ± 0.18	25.93^b ± 0.42	1.50^a ± 0.15	0.23^a ± 0.05
50% Wastewater Effluent								
K	1366.91^b ± 227.87	2.74^a ± 0.02	2114.84^b ± 80.60	4.50^a ± 0.09	8.56^b ± 0.06	24.83^c ± 0.03	2.21^a ± 1.95	0.10^a ± 0.06
C1	1117.78^b ± 533.93	1.64^a ± 0.19	115.98^d ± 12.91	4.37^a ± 0.04	8.11^d ± 0.04	26.90^a ± 0.00	1.21^a ± 0.10	0.06^a ± 0.02
C2	1509.36^b ± 113.88	2.89^a ± 0.02	118.56^d ± 2.24	4.34^a ± 0.05	8.28^c ± 0.06	26.87^a ± 0.03	1.91^a ± 0.95	0.13^a ± 0.04
100% Wastewater Effluent								
K	1447.48^b ± 90.53	7.65^a ± 0.93	7283.03^a ± 806.01	1.16^b ± 0.59	8.83^a ± 0.04	24.67^c ± 0.03	0.45^a ± 0.03	0.06^a ± 0.01
C1	1082.10^b ± 80.95	3.82^a ± 2.70	119.85^d ± 32.96	4.73^a ± 0.06	8.83^a ± 0.11	24.85^c ± 0.05	0.39^a ± 0.05	0.06^a ± 0.00
C2	1124.80^b ± 73.97	4.69^a ± 2.07	530.40^c ± 192.79	4.17^a ± 0.04	9.12^a ± 0.07	25.23^b ± 0.03	0.36^a ± 0.00	0.05^a ± 0.01
Effluent standards	150	8	200	NG	6–9	NG	NG	NG

Note: each value is mean ± the standard error of three replicates. Values in the same column followed by the same letter indicate no significant difference. The data were analysed using ANOVA and Tukey post-hoc tests. *Effluent standards for textile wastewater in accordance with the Regulation of the Minister of Environment and Forestry of Indonesia (PermenLHK No. P.16/2019). NG means Not Given. K means untreated wastewater, while C1 and C2 are wetland treatments without and with consortium endophyte bacteria, respectively.

suggest that the risk of acute toxicity can persist despite treatment efforts.

Acute toxicity assessment of textile effluent using Daphnia magna bioassay

The average mortality rate and acute toxicity (LC_{50}) of effluent-wetland treatments at various concentration loadings are displayed in Figure 8 and Table 2, respectively. The number of *D. magna* deaths increased alongside the length of exposure and the concentration levels of pollutants in textile wastewater. Untreated textile wastewater demonstrated a high mortality rate for all range concentrations from 0–100% after a 96-hour exposure period at different effluent concentrations. Probit analysis of the 96-h LC_{50} of untreated wastewater (K) exhibited acute lethal toxicity compared to those exposed to the treated wastewater (wetland reactor C1 and C2), which resulted in the LC_{50} of *D. manga* range at confirmation test concentrations of 1.52% to 6.48%. Additionally, the TUa results for the untreated wastewater were 65.62 and 15.43, which are classified as high acute toxicity, even at the lowest 25% loading concentration of textile effluent. This finding aligns with previous studies showing that the acute toxicity assessment using *D. magna* of raw effluent from various textile industry processes has TUa values > 1, which are classified as acute to very high acute toxicity (Methneni et al., 2021; Villegas-Navarro et al., 2001).

In comparison, the treated wastewater from vegetation only (C1) and vegetation-augmented endophyte bacteria (C2) showed similar outcomes, such as a low mortality rate of *D. magna* for all loading concentrations. Moreover, the effluent from reactor C2 had a lower mortality rate, ranging from 0–20%, while reactor C1 had a mortality rate ranging from 3.33–23.33%. Meanwhile, the untreated wastewater at 50% loading concentration falls under slight acute toxicity due to lower COD, ammonia, and Cd values compared to the concentrations at 25% and 100%.

In contrast, following the phytoremediation process and bacterial inoculation (reactor C1 and C2 samples, respectively), no measurable acute

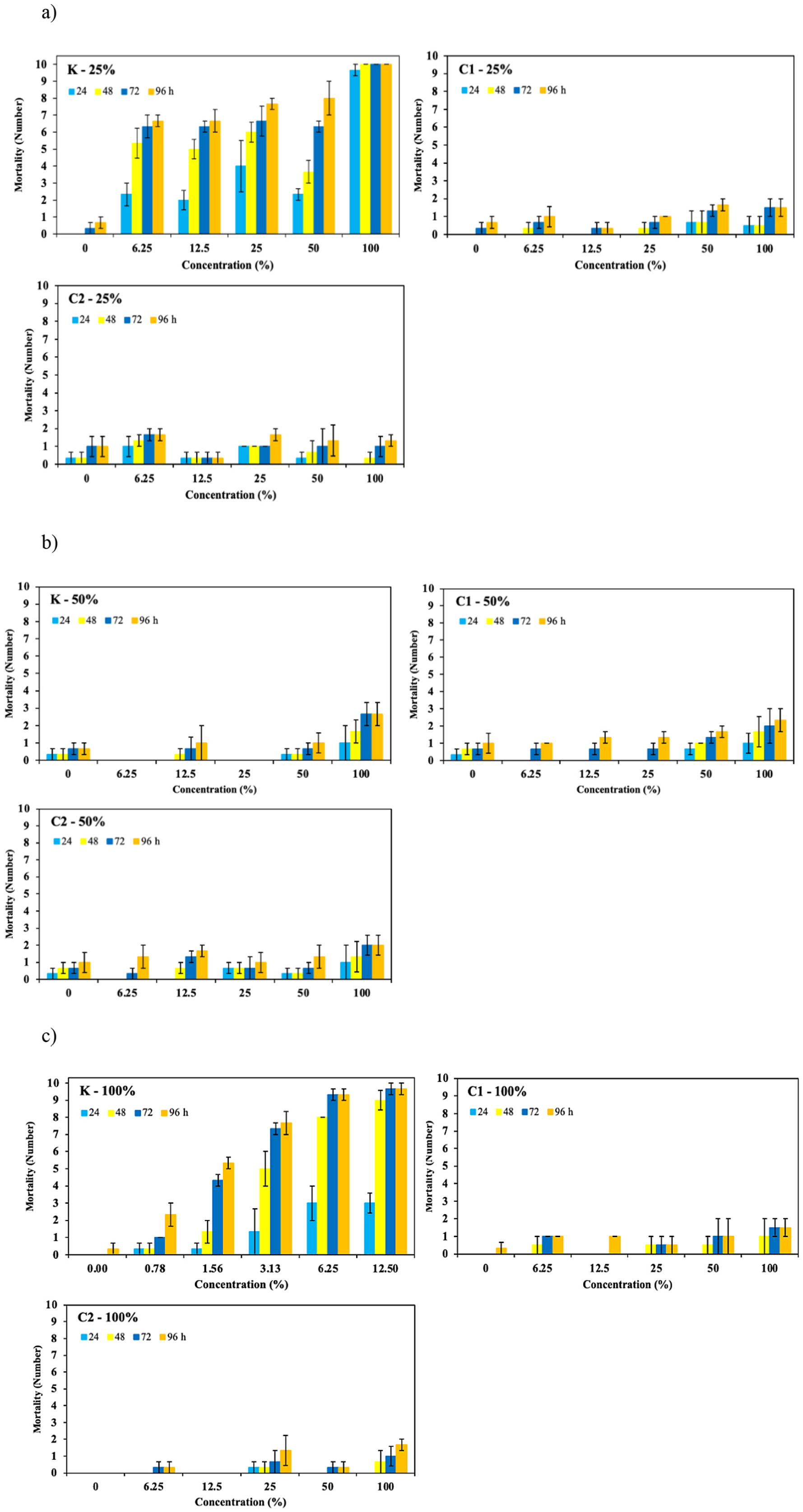

Figure 8. Average mortality rate of *D. magna* for 96 h exposure at various dilutions of textile effluent: (a) effluent of 25% loading concentration, (b) effluent of 50% loading concentration, (c) effluent of 100% loading concentration. Each value is the mean of three replicates, and the error bar represents the standard error. K means untreated wastewater, while C1 and C2 are wetland treatments without and with consortium endophyte bacteria, respectively

Table 2. Assessment of acute contaminants of (LC_{50}) of textile effluent at 96 h

Treatments	R^2	LC_{50} (%)	TUa	Category
25% Wastewater Effluent				
K	0.646	6.48	15.43	High acute toxicity
C1	0.378	13915.68	0.01	No acute toxicity
C2	0.025	5.27×10^{10}	1.90×10^{-9}	No acute toxicity
50% Wastewater Effluent				
K	0.407	200.00	0.50	Slight acute toxicity
C1	0.925	7888.00	0.01	No acute toxicity
C2	0.135	2.63×10^{9}	3.80×10^{-8}	No acute toxicity
100% Wastewater Effluent				
K	0.989	1.52	65.62	High acute toxicity
C1	0.125	3.77×10^{9}	2.66×10^{-8}	No acute toxicity
C2	0.231	511.03	0.20	No acute toxicity

Note: K means untreated wastewater, while C1 and C2 are wetland treatment without and with consortium endophyte bacteria, respectively.

toxicity was detected in the effluent samples, with results showing LC_{50} > 100% and TUa < 1. This demonstrates the effectiveness of combining phytoremediation with bacterial treatment for textile wastewater at all stages of loading concentrations. This outcome was also observed in a study by de Alkimin et al. (2020) involving a *D. magna* bioassay, which showed no acute toxicity in the textile effluent treated using a phytoremediation system. Similarly, Hussain et al. (2018a) reported the effectiveness of constructed wetlands with vegetation only and in combination with bacteria, in reducing effluent toxicity level for *Labeo rohita*, with untreated effluent being highly toxic and the combination treatment proving non-toxic results. This indicates that improved treatment efficacy led to substantial enhancement in effluent quality, attributable to the wetland as well as bacteria's ability to absorb and reduce the concentration of chemicals that contributed to high COD values in the effluent. In turn, the research by Verma (2011) found that treated textile effluents exhibited TUa classifications ranging from non-toxic to highly toxic in *D. magna* bioassays, with the majority still classified as toxic. Similarly, Rohmah et al. (2018) also reported that treated textile effluents remained toxic, with LC_{50} values ranging from 2.72 to 88.40% and TUa values between 1.13 to 36.78.

Acute toxicity assessments are conducted to understand the biological impacts of hazardous chemical substances in textile effluents. Textile dyes can decompose into intermediate substances in the environment, posing potential toxicity risks to organisms. The breakdown of azo dye bonds leads to the formation of aromatic amines, which are known to have carcinogenic and mutagenic properties (Dutta et al., 2024). This underscores the need for toxicity assessments of the by-products formed during the degradation of textile dyes. Many textile dyes have reported acute toxicity levels, and some textile effluents can cause lethal and sublethal effects on various aquatic organisms (Garcia et al., 2021). Even textile effluents treated in wastewater treatment plants and other treatment technologies can still lead to lethal and sublethal effects, potentially causing long-term impacts on aquatic populations (Castro et al., 2019; Dehghani et al., 2016; Liang et al., 2017).

Morphological and physiological evaluation

The morphology of *D. magna* after the exposure to different concentrations of textile effluent from various treatments is displayed in Figure 9. Significant differences were observed among the unexposed control, untreated, and treated effluent samples. The unexposed control exhibited normal morphology with no visible deformities, and its body was free of black masses indicated as absorption of dyes textile wastewater. In contrast, the *D. magna* exposed to untreated wastewater (K reactor) caused the most severe morphological alterations, including body deformities and widespread black masses in the gut and thoracic appendages, indicating the ingestion of dye substances. The organisms exposed to untreated wastewater (K)

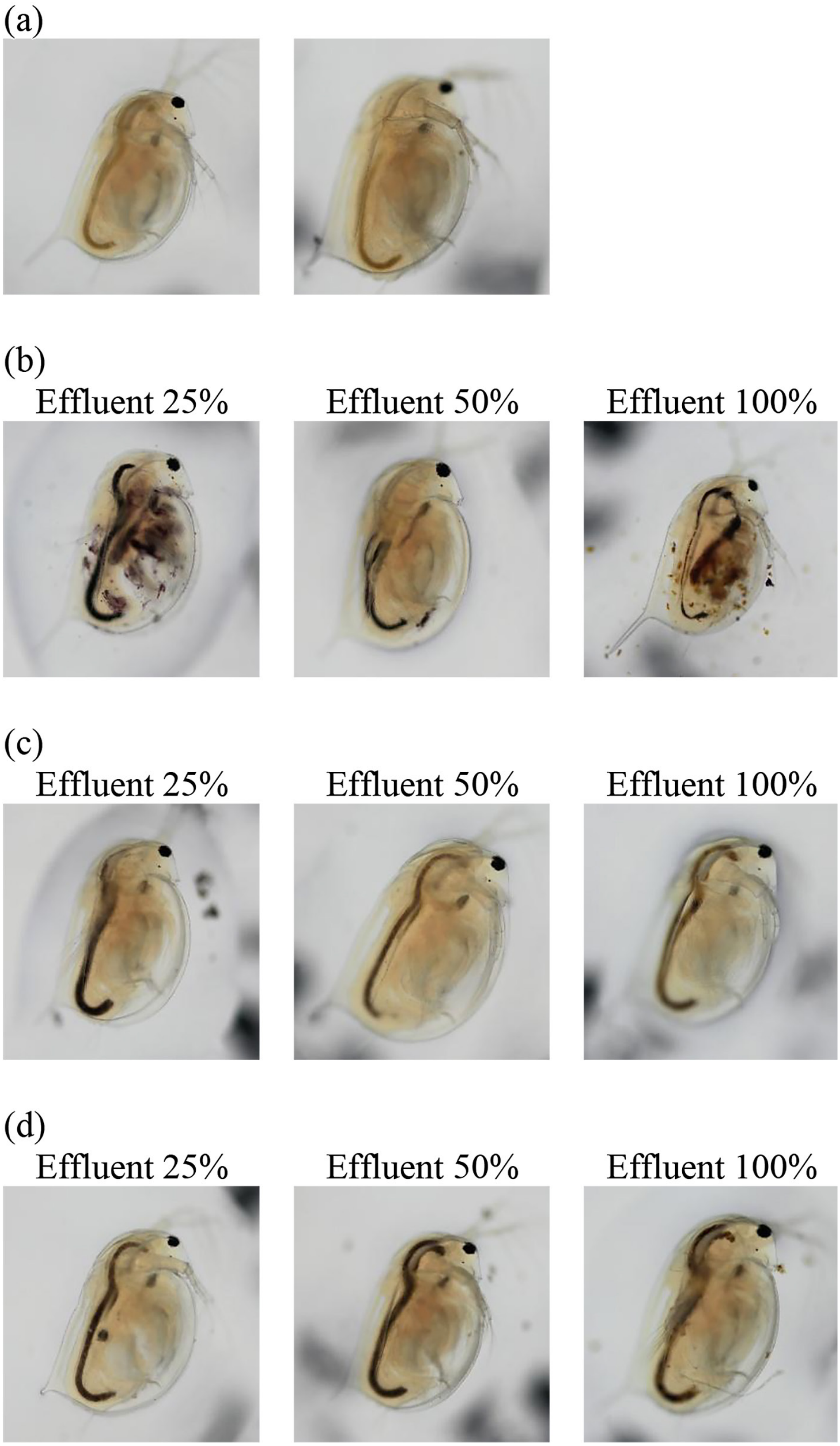

Figure 9. Morphology of *D. magna* under a microscope at 40x magnification after the exposure to effluent from CWs treating textile wastewater, (a) unexposed control, (b) effluent untreated wastewater (K), (c) effluent CW (C1), (d) effluentt CW with consotrium endophyt bacteria (C2)

Figure 10. Physical damage observed in *D. magna* under a microscope at 40x magnification after the exposure to untreated wastewater

Table 3. Physiological analysis of *D. magna* exposed after CWs treatments of textile wastewater

Samples	Heart rate (BPM)			Thoracic limb movement (Times/min)		
	25%	50%	100%	25%	50%	100%
Unexposed control	$624^a \pm 3.5$	$624^a \pm 3.5$	$624^a \pm 3.5$	$342^a \pm 40.8$	$342^a \pm 40.8$	$342^a \pm 40.8$
K	No live	$352^b \pm 64.1$	$156.7^c \pm 21.7$	No live	$42^b \pm 39$	$48^b \pm 12.5$
C1	$594^a \pm 6.9$	$606^a \pm 18$	$618^a \pm 35.2$	$352^a \pm 31.4$	$362^a \pm 20.3$	$304^a \pm 17.1$
C2	$622^a \pm 10.6$	$614^a \pm 25.5$	$594^a \pm 31.7$	$282^a \pm 13.9$	$354^a \pm 21.6$	$276^a \pm 81.9$

Note: each value is mean ± the standard error of three replicates. Values within each parameter followed by the same letter indicate no significant difference. The data were analysed with ANOVA and Tukey post-hoc test. K means untreated wastewater, while C1 and C2 mean wetland treatment without and with consortium endophyte bacteria, respectively.

exhibited notable physical damage, including carapace deformities and loss of thoracic limbs (Fig. 10). Similar morphological impairments in *Daphnia magna* were reported by Li et al. (2023), where the exposure to dye compounds led to body damage. The exposure to treated effluent also resulted in black masses in the gut systems but did not impact mortality. This suggests that treated textile wastewater from wetlands treatment systems could reduce mortality impact in the short exposure. Therefore, further research on sublethal and chronic toxicity studies of treated textile wastewater in long exposure still remains unclear, and it can exhibit a better understanding of their effect on the environment (Table 3).

Figure 4 shows that untreated textile effluent significantly affects the physiological state of *D. magna* (reduced heart rate and impaired limb movement). The treatment with constructed wetland (C1) and bacterial augmentation (C2) reduced the toxicity level shown by heart rate and limb movement closer to unexposed control values. This suggests that the biological treatment involving bacterial augmentation improves the effluent quality of treated textile wastewater and reduces harmful impacts on aquatic organisms.

Acute toxicity effect and ecological impact of textile effluent on several aquatic organisms

Understanding the impacts of textile effluents on aquatic life is crucial for assessing potential ecological risks. Previous studies have documented the adverse effects of these pollutants on aquatic organisms (Table 4). According to Garcia et al. (2020), sublethal and reproductive impacts on *D. similis* were observed following 21 d exposure to textile effluent. The results indicated that untreated effluents significantly affected *D. similis*, decreasing reproduction rates and dye accumulation within their eggs and filtering systems, resulting in egg malformations. Conversely, the exposure to effluent treated with electron beam irradiation (EBI) showed reduced reproduction rates compared to the control. Still, it showed no egg malformations, suggesting that the treatment partially mitigated the harmful effects through treatment.

Table 4. Acute toxicity of various textile effluents and dyes in aquatic bioassays

Samples	Treatments	Organism species	Acute toxicity value	References
Textile effluent	Untreated wastewater	*D. magna*	EC_{50} (48 h): 18.2%	(Castro et al., 2019)
	Wastewater treatment plant	*D. magna*	EC_{50} (24 h): 68.4%	
Acid 4092 dye	UV/ZnO	*D. magna*	LC_{50} (96 h): 91.55 mg/L	(Dehghani et al., 2016)
Textile effluent	Untreated wastewater	*D. magna*	EC_{50} (24 h): 44.8%	(Methneni et al., 2021)
	Wastewater treatment plant	*D. magna*	EC_{50} (24 h): 52.8%	
Textile effluent (dyeing process)	Untreated wastewater	*D. magna*	LC_{50} (48 h): 19.5%	(Villegas-Navarro et al., 2001)
Textile effluent	Untreated wastewater	*D. magna*	EC_{50} (48 h): 53.82%	(de Alkimin et al., 2020)
	Phytoremediation with *L. minor*	*D. magna*	Not toxic	
Textile effluent	Wastewater treatment plant	*D. magna*	EC_{50} (48 h): 75.9%	(Verma, 2011)
Textile effluent	Wastewater treatment plant	*D. magna*	LC_{50} (96 h): 6.36%	(Rohmah et al., 2018)
Textile effluent containing reactive red 239 dye	Untreated wastewater	*D. similis*	EC_{50} (48 h): 6.31%	(Garcia et al., 2020)
	Electron beam irradiation (EBI)	*D. similis*	EC_{50} (48 h): 27.56%	
Textile effluent (cotton dyeing)	Untreated wastewater	*D. similis*	EC_{50} (48 h): 9.28%	(Garcia et al., 2021)
	Untreated wastewater	*B. glabrata*	LC_{50} (24 h): 10.78%	
Textile effluent from traditional weaving industry	Untreated wastewater	*C. carpio L.*	LC_{50} (96 h): 2.3%	(Nuha et al., 2016)
Textile effluent	Untreated wastewater	*C. batrachus L.*	LC_{50} (96 h): 16.59%	(Makwana, 2020)
Acid black 1	Untreated wastewater	*R. subcapitata*	ErC_{50} (72 h): 54.20 mg/L	(Croce et al., 2017)
Textile effluent from traditional weaving industry	Untreated wastewater	*D. magna*	LC_{50} (96 h): 1.52%	Present study
	Constructed wetland using *V. zizanioides*	*D. magna*	Not toxic	Present study
	Constructed wetland using *V. zizanioides* and endophytic bacteria	*D. magna*	Not toxic	Present study

The studies examining effluents from Indonesia's traditional weaving and batik industries have substantially impacted *Cyprinus carpio L.* In one case, untreated weaving industry effluent was found to have a 96-h LC_{50} value of 2.3%, indicating high acute toxicity (Nuha et al., 2016). Additionally, even after phytoremediation treatment with hyacinth plants, batik industry effluent exhibited sub-lethal effects (Susilo et al., 2021). Both studies observed symptoms in the fish, including behavioural changes, such as altered movement, loss of balance, ramjet ventilation, increased mucus secretion, and morphological colour changes. Similar findings have been reported for the *Clarias batrachus (L.)* exposed to textile effluents, where acute toxicity led to symptoms including hyperactivity, restlessness, surfacing behaviour, and loss of buoyancy (Makwana, 2020). The exposure to textile pollutants impacted microalgae such as *Raphidocelis subcapitata*, *Chlorella vulgaris*, and *Spirulina platensis*, disrupting growth and metabolic functions and contributing to trophic level disruptions and ecological imbalance (Croce et al., 2017; Sharma et al., 2021). The presence of textile effluent can alter the abundance of aquatic life in water ecosystems. Research on a Mediterranean river demonstrated that diatoms, macroinvertebrates, and fish diversity indices were the most effective measures for detecting ecological disturbances (Colin et al., 2016). These findings underscore the importance of comprehensive monitoring programs incorporating biological indicators to better assess the long-term impacts of textile pollution and suggest next steps for further research or policy.

CONCLUSIONS

Textile effluents are often marked by high levels of chemical pollutants, contributing to toxicity for living organisms in aquatic environments. The treated textile wastewater from constructed wetlands maintained high concentrations in chemical parameters, including COD, colour, and ammonia, which emphasise the necessity for assessments of toxicity levels to evaluate the impact of effluent. Untreated wastewater (K) posed LC_{50} 1.52–200% and TUa 0.50–65.62, classified as slight to high acute toxicity for 25%, 50%, and 100% concentration loadings of textile wastewater compared to other treatments. The treatment of vegetated wastewater (C1) and vegetated with consortium endophytic bacteria (C2) exhibited LC_{50} > 100% and TUa < 1, indicating non-acute toxicity for those three concentration loadings. The physiological damage of D. magna was confirmed at 100% concentration with the indicator of low heart rate, limb movement, and adsorption of dye substances. This toxicity assessment exhibited helpful treatment to reduce the physicochemical and heavy metal compounds in textile wastewater and reduce the acute risk in the harmless effluent to aquatic organisms. This approach is essential for ensuring a safer aquatic environment and encouraging sustainable practices in the future.

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REFERENCES

1. Adamczuk, M. (2020). Population dynamics and life history traits of Daphnia magna across thermal regimes of environments. *Science of the Total Environment*, *723*, 4–11. https://doi.org/10.1016/j.scitotenv.2020.137963
2. Adeogun, A. O., & Chukwuka, A. V. (2011). Effect of textile factory effluent on otolith and somatic growth parameters in Clarias gariepinus. *The Zoologist*, *9*, 70–77. https://www.ajol.info/index.php/tzool/article/view/73130
3. Akeredolu, O. E., Adegboro, A. O., Adebanjo, O. G., & Dada, O. (2023). Assessing the effectiveness of textile wastewater treatment through fish acute toxicity studies and water quality evaluation. *Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control*, *15*(2), 141–151. https://doi.org/10.21608/eajbsf.2023.353956
4. Alderete, B. L., da Silva, J., Godoi, R., da Silva, F. R., Taffarel, S. R., da Silva, L. P., Garcia, A. L. H., Júnior, H. M., de Amorim, H. L. N., & Picada, J. N. (2021). Evaluation of toxicity and mutagenicity of a synthetic effluent containing azo dye after Advanced Oxidation Process treatment. *Chemosphere*, *263*. https://doi.org/10.1016/j.chemosphere.2020.128291
5. Ali, H., Khan, E., & Ilahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry*, *2019*(Cd). https://doi.org/10.1155/2019/6730305
6. Berradi, M., Hsissou, R., Khudhair, M., Assouag, M., Cherkaoui, O., El Bachiri, A., & El Harfi, A. (2019). Textile finishing dyes and their impact on aquatic environs. *Heliyon*, *5*(11). https://doi.org/10.1016/j.heliyon.2019.e02711
7. Bownik, A. (2017). Daphnia swimming behaviour as a biomarker in toxicity assessment: A review. *Science of the Total Environment*, *601–602*, 194–205. https://doi.org/10.1016/j.scitotenv.2017.05.199
8. Bownik, A., Stępniewska, Z., & Skowroński, T. (2015). Effects of ectoine on behavioural, physiological and biochemical parameters of Daphnia magna. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, *168*, 2–10. https://doi.org/10.1016/j.cbpc.2014.11.001
9. Carney Almroth, B., Cartine, J., Jönander, C., Karlsson, M., Langlois, J., Lindström, M., Lundin, J., Melander, N., Pesqueda, A., Rahmqvist, I., Renaux, J., Roos, J., Spilsbury, F., Svalin, J., Vestlund, H., Zhao, L., Asker, N., Ašmonaitė, G., Birgersson, L., … Sturve, J. (2021). Assessing the effects of textile leachates in fish using multiple testing methods: From gene expression to behavior. *Ecotoxicology and Environmental Safety*, *207*(July 2020). https://doi.org/10.1016/j.ecoenv.2020.111523
10. Castro, A. M., Nogueira, V., Lopes, I., Rocha-santos, T., & Pereira, R. (2019). Evaluation of the Potential Toxicity of Effluents from the Textile Industry before and after Treatment. *Applied Sciences*. https://www.mdpi.com/2076-3417/9/18/3804
11. Choudhury, A. K. R. (2014). *Environmental Impacts of the Textile Industry and Its Assessment Through Life Cycle Assessment*. https://doi.

org/10.1007/978-981-287-110-7_1

12. Colin, N., Maceda-Veiga, A., Flor-Arnau, N., Mora, J., Fortuño, P., Vieira, C., Prat, N., Cambra, J., & de Sostoa, A. (2016). Ecological impact and recovery of a Mediterranean river after receiving the effluent from a textile dyeing industry. *Ecotoxicology and Environmental Safety*, *132*, 295–303. https://doi.org/10.1016/j.ecoenv.2016.06.017
13. Croce, R., Cinà, F., Lombardo, A., Crispeyn, G., Cappelli, C. I., Vian, M., Maiorana, S., Benfenati, E., & Baderna, D. (2017). Aquatic toxicity of several textile dye formulations: Acute and chronic assays with *Daphnia magna* and *Raphidocelis subcapitata*. *Ecotoxicology and Environmental Safety*, *144*(January), 79–87. https://doi.org/10.1016/j.ecoenv.2017.05.046
14. de Alkimin, G. D., Paisio, C., Agostini, E., & Nunes, B. (2020). Phytoremediation processes of domestic and textile effluents: evaluation of the efficacy and toxicological effects in Lemna minor and Daphnia magna. *Environmental Science and Pollution Research*, *27*(4), 4423–4441. https://doi.org/10.1007/s11356-019-07098-3
15. Dehghani, M. H., Mahdavi, P., Tyagi, I., Agarwal, S., & Gupta, V. K. (2016). Investigating the toxicity of acid dyes from textile effluent under UV/ZNO process using Daphnia magna. *Desalination and Water Treatment*, *57*(51), 24359–24367. https://doi.org/10.1080/19443994.2016.1141327
16. Durotoye, T. O., Adeyemi, A. A., Omole, D. O., & Onakunle, O. (2018). Impact assessment of wastewater discharge from a textile industry in Lagos, Nigeria. *Cogent Engineering*, *5*(1), 1–11. https://doi.org/10.1080/23311916.2018.1531687
17. Dutta, S., Adhikary, S., Bhattacharya, S., Roy, D., Chatterjee, S., Chakraborty, A., Banerjee, D., Ganguly, A., Nanda, S., & Rajak, P. (2024). Contamination of textile dyes in aquatic environment: Adverse impacts on aquatic ecosystem and human health, and its management using bioremediation. *Journal of Environmental Management*, *353*(October 2023), 120103. https://doi.org/10.1016/j.jenvman.2024.120103
18. Environmental and Forestry Instrument Standardization Agency (BSILHK), "Indonesian National Standard for Water and Wastewater Quality (SNI)." [Online]. Available: https://sispk-v2.bsn.go.id/sni/index.
19. EPA. (2000). Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the Nat. *Text*, *June*.
20. Fajri, J. A., Nurmiyanto, A., Sa'adah, N. N., Sagita, N. D., Nuryana, I., Rahayu, A., & Lathifah, A. N. (2024). Selection of endophyte and indigenous bacteria degrading textile wastewater in floating treatment wetland. *International Journal of Environmental Science and Technology*, *0123456789*. https://doi.org/10.1007/s13762-024-05654-0
21. Fajri, J. A., Nurmiyanto, A., Sa`adah, N. N., Nuryana, I., Anfaresi, S. L. N., & Lathifah, A. N. (2024). Effectiveness of endophytes bacteria in enhancing floating treatment wetland to treat textile wastewater. *Journal of Ecological Engineering*, *25*(3), 12–24. https://doi.org/10.12911/22998993/177593
22. Garcia, V. S. G., de Freitas Tallarico, L., Rosa, J. M., Suzuki, C. F., Roubicek, D. A., Nakano, E., & Borrely, S. I. (2021). Multiple adverse effects of textile effluents and reactive Red 239 dye to aquatic organisms. *Environmental Science and Pollution Research*, *28*(44), 63202–63214. https://doi.org/10.1007/s11356-021-15115-7
23. Garcia, V. S. G., Rosa, J. M., & Borrely, S. I. (2020). Toxicity and color reduction of a textile effluent containing reactive red 239 dye by electron beam irradiation. *Radiation Physics and Chemistry*, *172*(September 2019), 108765. https://doi.org/10.1016/j.radphyschem.2020.108765
24. Gómez, N., Sierra, M. V., Cortelezzi, A., & Rodrigues Capítulo, A. (2008). Effects of discharges from the textile industry on the biotic integrity of benthic assemblages. *Ecotoxicology and Environmental Safety*, *69*(3), 472–479. https://doi.org/10.1016/j.ecoenv.2007.03.007
25. Goud, B. S., Cha, H. L., Koyyada, G., & Kim, J. H. (2020). Augmented Biodegradation of Textile Azo Dye Effluents by Plant Endophytes: A Sustainable, Eco-Friendly Alternative. In *Current Microbiology* 77(11), 3240–3255. Springer. https://doi.org/10.1007/s00284-020-02202-0
26. Gyllström, M., & Hansson, L. A. (2004). Dormancy in freshwater zooplankton: Induction, termination and the importance of benthic-pelagic coupling. *Aquatic Sciences*, *66*(3), 274–295. https://doi.org/10.1007/s00027-004-0712-y
27. Hussain, Z., Arslan, M., Malik, M. H., Mohsin, M., Iqbal, S., & Afzal, M. (2018a). Integrated perspectives on the use of bacterial endophytes in horizontal flow constructed wetlands for the treatment of liquid textile effluent: Phytoremediation advances in the field. *Journal of Environmental Management*, *224*(May), 387–395. https://doi.org/10.1016/j.jenvman.2018.07.057
28. Hussain, Z., Arslan, M., Malik, M. H., Mohsin, M., Iqbal, S., & Afzal, M. (2018b). Treatment of the textile industry effluent in a pilot-scale vertical flow constructed wetland system augmented with bacterial endophytes. *Science of the Total Environment*, *645*, 966–973. https://doi.org/10.1016/j.scitotenv.2018.07.163.
29. Jin, J., & Kusui, T. (2019). Sewage effluent toxicity

assessment: Comparison between whole effluent toxicity (WET) and the Japanese pollutant release and transfer register (PRTR) data. *Journal of Water and Environment Technology*, *17*(4), 218–230. https://doi.org/10.2965/jwet.18-028

30. Kadam, S. K., Chandanshive, V. V., Rane, N. R., Patil, S. M., Gholave, A. R., Khandare, R. V., Bhosale, A. R., Jeon, B. H., & Govindwar, S. P. (2018). Phytobeds with Fimbristylis dichotoma and Ammannia baccifera for treatment of real textile effluent: An in situ treatment, anatomical studies and toxicity evaluation. *Environmental Research*, *160*(June 2017), 1–11. https://doi.org/10.1016/j.envres.2017.09.009
31. Kakka, A. I., Herlekar, M. D., & Awale, S. (2021). Comparative toxicity study of chemical pesticide and biopesticide by Daphnia Bioassay. *Nature Environment and Pollution Technology*, *20*(2), 695–701. https://doi.org/10.46488/NEPT.2021.v20i02.028
32. Lach, C. E., Pauli, C. S., Coan, A. S., Simionatto, E. L., & Koslowski, L. A. D. (2022). Investigating the process of electrocoagulation in the removal of azo dye from synthetic textile effluents and the effects of acute toxicity on Daphnia magna test organisms. *Journal of Water Process Engineering*, *45*(December 2021). https://doi.org/10.1016/j.jwpe.2021.102485
33. Li, S., Cui, Y., Wen, M., & Ji, G. (2023). Toxic Effects of Methylene Blue on the Growth, Reproduction and Physiology of Daphnia magna. *Toxics*, *11*(7). https://doi.org/10.3390/toxics11070594
34. Liang, J., Ning, X. an, Kong, M., Liu, D., Wang, G., Cai, H., Sun, J., Zhang, Y., Lu, X., & Yuan, Y. (2017). Elimination and ecotoxicity evaluation of phthalic acid esters from textile-dyeing wastewater. *Environmental Pollution*, *231*, 115–122. https://doi.org/10.1016/j.envpol.2017.08.006
35. Makwana, S. (2020). Acute toxicity and haematological studies of textile based industrial effluent of Pali city on a freshwater fish clarias batrachus (L.). *Nature Environment and Pollution Technology*, *19*(5), 1995–2003. https://doi.org/10.46488/NEPT.2020.v19i05.025
36. Marshall, R. (2016). *Whole Effluent Toxicity Testing Guidance and Test Review Criteria* (Issue December). Washington State Department of Ecology.
37. Methneni, N., Morales-González, J. A., Jaziri, A., Mansour, H. Ben, & Fernandez-Serrano, M. (2021). Persistent organic and inorganic pollutants in the effluents from the textile dyeing industries: Ecotoxicology appraisal via a battery of biotests. *Environmental Research*, *196*(February). https://doi.org/10.1016/j.envres.2021.110956
38. Müller, M. F., Colomer, J., & Serra, T. (2018). Temperature-driven response reversibility and short-term quasi-acclimation of Daphnia magna. *PLoS ONE*, *13*(12), 1–14. https://doi.org/10.1371/journal.pone.0209705
39. Nuha, A. U., B, F. P. M. H., & Mubarok, I. (2016). Toksisitas letal akut limbah cair tenun troso terhadap ikan mas (Cyprinus carpio L.). *Life Science*, *5*(1), 1–8.
40. Parmar, A. I., & Shah, A. I. (2021). Acute toxicity, behavioural response and haematological alterations of Catla catla exposed to Reactive Red 120 textile dye. *Indian Journal of Experimental Biology*, *59*(April), 275–279. https://doi.org/10.56042/ijeb.v59i04.48236
41. Ramadhani, R. D., & Subandi, S. (2015). Keberadaan Dan Perkembangan Tenun Troso Jepara. *Ornamen Jurnal Kriya Seni ISI Surakarta*, *12*(1), 117–130. https://jurnal.isi-ska.ac.id/index.php/ornamen/article/view/1623
42. Rasool, T., Ishtiaq Ali, M., Asif Jamal, Huang, Z., & Urynowicz, M. (2023). Performance evaluation and microbial profiling of integrated vertical flow constructed wetland (IVFCW) for simultaneous treatment of domestic and pulp and paper industry waste water. *Saudi Journal of Biological Sciences*, *30*(12). https://doi.org/10.1016/j.sjbs.2023.103850
43. Rohmah, N., Roosmini, D., & Septiono, M. A. (2018). Study of whole effluent acute toxicity test (Daphnia magna) as an evaluation of Ministry of Environment and Forestry Decree No. 3 in 2014 concerning industrial performance rank in environmental management. *MATEC Web of Conferences*, *147*(3), 1–10. https://doi.org/10.1051/matecconf/201814708005
44. Sharma, J., Sharma, S., & Soni, V. (2021). Classification and impact of synthetic textile dyes on Aquatic Flora: A review. *Regional Studies in Marine Science*, *45*. https://doi.org/10.1016/j.rsma.2021.101802
45. Shehzadi, M., Fatima, K., Imran, A., Mirza, M. S., Khan, Q. M., & Afzal, M. (2016). Ecology of bacterial endophytes associated with wetland plants growing in textile effluent for pollutant-degradation and plant growth-promotion potentials. *Plant Biosystems*, *150*(6), 1261–1270. https://doi.org/10.1080/11263504.2015.1022238
46. Susilo, H., Oktavia, S., & Roudotussaadah, R. (2021). Phytoremediation of batik industry wastewater using water hyacinth plant as a medium for maintaining Cyprinus Carpio L. *Biological Environment and Pollution*, *1*(1), 11–18. https://doi.org/10.31763/bioenvipo.v1i1.382
47. Tara, N., Arslan, M., Hussain, Z., Iqbal, M., Khan, Q. M., & Afzal, M. (2019). On-site performance of floating treatment wetland macrocosms augmented with dye-degrading bacteria for the remediation of textile industry wastewater. *Journal of Cleaner Production*, *217*, 541–548. https://doi.org/10.1016/j.jclepro.2019.01.258
48. US EPA. (2000). Method Guidance and

Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136). *Office of Water*, *July*, 60.

49. US EPA. (2002). Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms: Fifth Edition. *Epa/821/R02/012*, *October*, 266. http://www.epa.gov/waterscience/WET/disk1/ctm.pdf
50. Verma, Y. (2011). Toxicity assessment of dye containing industrial effluents by acute toxicity test using Daphnia magna. *Toxicology and Industrial Health*, *27*(1), 41–49. https://doi.org/10.1177/0748233710380218
51. Villegas-Navarro, A., Ramírez-M., Y., Salvador-S. B., M. S., & Gallardo, J. M. (2001). Determination of wastewater LC50 of the different process stages of the textile industry. *Ecotoxicology and Environmental Safety*, *48*(1), 56–61. https://doi.org/10.1006/eesa.2000.1986
52. Vymazal, J. (2010). Constructed wetlands for wastewater treatment. *Water (Switzerland)*, *2*(3), 530–549. https://doi.org/10.3390/w2030530
53. Wei, F., Shahid, M. J., Alnusairi, G. S. H., Afzal, M., Khan, A., El-Esawi, M. A., Abbas, Z., Wei, K., Zaheer, I. E., Rizwan, M., & Ali, S. (2020). Implementation of floating treatment wetlands for textile wastewater management: A review. *Sustainability (Switzerland)*, *12*(14), 1–29. https://doi.org/10.3390/su12145801
54. Zhang, W., Liu, W., Zhang, J., Zhao, H., Zhang, Y., Quan, X., & Jin, Y. (2012). Characterisation of acute toxicity, genotoxicity and oxidative stress posed by textile effluent on zebrafish. *Journal of Environmental Sciences (China)*, *24*(11), 2019–2027. https://doi.org/10.1016/S1001-0742(11)61030-9