Cyto and genotoxicity induced by exposure to textile production effluents on *Astyanax lacustris* (Pisces: Characidae) can be reduced after treatment through a vertical flow constructed wetland system

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Textile effluents possess a complex composition that includes physical, chemical, and biological elements, and they may exhibit mutagenic, genotoxic, and carcinogenic properties. A portion of the dyes utilized in the textile industry is lost during the dyeing process, ultimately contaminating aquatic environments when released into bodies of water. Thus, the objective of this study was to assess the cyto/genotoxic effects of textile production effluents on *Astyanax lacustris* specimens both before and after treatment with a vertical flow constructed wetland system (VFCW). We examined 60 fish (divided into five treatment conditions, with four fish per condition, in triplicate). The fish were exposed to the contaminants for a duration of seven days. The analysis methods employed included biomarker assessment, the micronucleus (MN) test, cellular morphological changes (CMC) analysis, and the comet assay. Fish exposed to 5% v/v of raw textile effluent and the effluents that underwent VFCW treatment exhibited significant damage in comparison to the control groups. The bioremediation of the dye carried out by the VFCW system proved to be efficient, as confirmed by physicochemical analysis. We conclude that the biodegradation of textile production effluents can successfully be achieved through the use of VFCW.

Keywords: Cellular morphological changes, Comet assay, Micronucleus test, Phytoremediation, Sustainable method.

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Os efluentes têxteis possuem uma composição complexa que inclui elementos físicos, químicos e biológicos, e podem apresentar propriedades mutagênicas, genotóxicas e carcinogênicas. Uma parte dos corantes utilizados na indústria têxtil é perdida durante o processo de tingimento, contaminando ambientes aquáticos quando liberados em corpos d'água. Assim, o objetivo deste estudo foi avaliar os efeitos cito/genotóxicos dos efluentes da produção têxtil em espécimes de *Astyanax lacustris* antes e depois do tratamento com um sistema de wetland construído de fluxo vertical (WCFV). Examinamos 60 peixes (divididos em cinco condições de tratamento, com quatro peixes por condição, em triplicata). Os peixes foram expostos aos contaminantes por um período de sete dias. Os métodos de análise empregados incluíram avaliação de biomarcadores, teste de micronúcleo (MN), análise de alterações morfológicas celulares (AMC) e ensaio cometa. Peixes expostos a 5% v/v de efluente têxtil bruto e os efluentes que passaram por tratamento WCFV exibiram danos significativos em comparação ao grupo controle. A biorremediação do efluente realizada pelo sistema WCFV mostrou-se eficiente, conforme comprovado por análises físico-químicas. Concluímos que a biodegradação de efluentes de produção têxtil pode ser alcançada com sucesso por meio do uso de WCFV.

Palavras-chave: Alterações morfológicas celulares, Ensaio cometa, Fitorremediação, Método sustentável, Teste de micronúcleo.

INTRODUCTION

The indiscriminate disposal of contaminated waste into the environment is a regrettable reality that has resulted in irreparable consequences for all living organisms. Among the industries contributing to environmental degradation is the textile sector, which not only consumes significant amounts of water during the dyeing process but also releases a portion of the dyes into aquatic systems (Yanto, Tachibana, 2014). These dyes are noted for their toxicity and vibrant colors, which adversely affect ecosystems due to their complex composition and challenging degradation (Kumar, Gunasundari, 2018).

Furthermore, according to Chandanshive *et al*. (2017), textile effluents contain metals, which further amplify their toxicity. Azo dyes, the most prevalent group used in the textile industry, are distinguished by their characteristic azo bonds linking aromatic rings (Popli, Patel, 2015). These dyes adversely affect the concentration of total organic carbon, as well as biological and chemical oxygen demand (Saratale *et al*., 2011), and are recognized as carcinogenic and/or mutagenic to humans, animals, and plants (Almeida *et al*., 2019).

Various biotechnological methods have been proposed to reduce the harmful effects of chemical contaminants in aquatic environments, primarily by eliminating these pollutants. Among these methods, phytoremediation has garnered considerable attention due to its natural selectivity and resistance manifested by certain plant species in response to specific xenobiotic agents (Pires *et al*., 2003). This approach can mitigate or remediate the residual impacts of various contaminants in soil or water, either by completely eliminating them or by reducing their toxicity (Krutz *et al*., 2009).

In the context of treating contaminated wastewater, a technology known as Constructed Wetlands (CW) has gained significant recognition. It is regarded as a comprehensive and effective alternative for phytoremediation, as its mechanisms involve a combination of physical processes (such as filtration and sedimentation), chemical actions (including adsorption and precipitation), and biological processes (like biodegradation and phytoremediation). This multi-faceted approach effectively meets current needs for the pre-treatment of effluents before they are discharged into water bodies (Hassan *et al*., 2021; Kiflay *et al*., 2021). Furthermore, the CW consist of ponds or shallow artificial channels with a filtering bed that accommodates aquatic plants, which play a vital role in attenuating the various contaminants present in the effluent (Dotro *et al*., 2017).

The CW biotechnology is an environmentally friendly and sustainable method for remediating polluted water with varying characteristics. It offers several advantages over conventional treatment techniques, including lower installation and operational costs, simplicity in process, and effective removal of pollutants such as total suspended solids, organic loads, and soluble nutrients (Sezerino *et al*., 2018).

There are various configurations of constructed wetlands designed specifically for treating industrial effluents. The selection of substrate material for the treatment bed occurs in the project's initial phase, with options including conventional materials like gravel and sand, as well as alternative materials such as gravel, slag, or bamboo that can be repurposed. Furthermore, the classification of CW configurations is generally based on the direction of effluent flow within each treatment unit (either horizontal or vertical), the types of plants used and their arrangement (either floating or submerged), and the system's hydrological regime (surface or subsurface water) (Khan, Faisal, 2018; Sezerino *et al*., 2018).

Among the various plants suitable for phytoremediation in CW, macrophytes are the most commonly employed due to their high tolerance for the toxicity of various contaminants and their capacity for purification (Guittonny-Philippe *et al*., 2015). In Brazil, the native plant species most frequently utilized for CW establishment belong to the Typhaceae and Poaceae families, notably including *Typha* spp. and *Cynodon* spp. Additionally, *Eleocharis* spp. and *Zizaniopsis* spp. are also considered, though to a lesser extent (Sezerino *et al*., 2015; Machado *et al*., 2017).

Fish serve as crucial biological models in ecotoxicological research (Bu, 2013). Specimens of *Astyanax lacustris* (Lütken, 1875) have demonstrated sensitivity to various chemical contaminants found in polluted waters, often exposed to mutagens or carcinogens that can induce alterations in the DNA molecule (Tincani *et al*., 2019). Therefore, measuring biomarkers in fish can provide essential data on the environmental damage inflicted by toxic agents, such as textile effluents, and assist in monitoring aquatic ecosystems. Several biotechnological methods capable of detecting and verifying genetic alterations include the comet assay, the micronucleus test, and the analysis of cellular morphological changes in erythrocytes. The present study aims to evaluate the cyto/genotoxic effects of textile production effluents on *A. lacustris*, both before and after treatment using a vertical flow constructed wetland system (VFCW). The assays employed include biomarker analysis, the micronucleus (MN) test, cellular morphological change (CMC) assessment, the comet assay, and the measurement of the primary physicochemical characteristics of both raw and treated effluents.

MATERIAL AND METHODS

Textile effluent. The textile effluent was collected from the stabilization ponds of a knitting factory in Campo Mourão, Paraná, Brazil. After collection, the effluent was transported in a plastic container to the laboratory and kept at room temperature.

Experimental design. Adult individuals (males and females) of *A. lacustris*, with a mean weight of 9±12 g and length of 8±12 cm, were obtained from a local breeding facility. The fish were acclimatized in aquaria with dechlorinated water at room temperature, constant aeration, a natural photoperiod (12:12 h light/dark cycle), and fed with specific small fish feed once daily (Basic Alcon® Fish Food, Camboriú/SC, Brazil) for 10 days in the Sectorial Vivarium for Fish Keeping and Experimentation at the Universidade Estadual de Maringá (UEM) in Maringá, Paraná State, Brazil.

Five groups/conditions were tested, with four fish per group, and all experiments were performed in triplicate; in total, 60 fish were used in the study. The number of individuals per aquarium was established according to the body mass/water volume ratio, which should not exceed 0.5–2 g/L (CONCEA Guide, annex I, fish). The control group (Group 1) fish were maintained in dechlorinated water; Group 2 – raw textile effluent at 5% v/v concentration (*i.e.*, 500 ml effluent diluted to 10 L with dechlorinated water); Group 3 and 4 – the effluent treated by phytoremediation (*Typha domingensis*) through the VFCW system, used at concentrations of 25% v/v and 50% v/v (*i.e.*, 2.5 L and 5 L of the treated effluent diluted in 10 L of dechlorinated water, respectively); Group 5 – with 100% v/v of the textile effluent treated by phytoremediation through the VFCW system. In all cases, the total aquarium volume was 10 L.

The percentages of textile effluent used were defined based on preliminary tests, in which it was observed that concentrations higher than 5% of the raw effluent caused mortality in *A. lacustris*. The concentrations of textile effluent at 25%, 50%, and 100% were subjected to treatment through the VFCW system to evaluate its efficiency in mitigating contaminants. Additionally, the study aimed to assess the potential cyto/genotoxic effects of the treated effluent.

Dissection and sampling. After 7 days of exposure, the fish were removed from the aquarium (one at a time) and bathed in an anesthetic solution: clove oil, 5 ml, diluted with ethyl alcohol, 20 ml, as per Inoue *et al*. (2005). Then, 1 ml of this solution was added per L of water. The animal was only manipulated after it failed to respond to physical stimulus, denoting death from an anesthetic overdose (Ferraro *et al*., 2004). After euthanasia, blood was collected to analyze micronucleus tests, cellular morphological changes, and comet assay in erythrocytes, as described below. Finally, the animals were fixed in commercial absolute alcohol, recorded with number and origin, and stored in glass vials from the UEM/NUPELIA laboratory. Voucher specimens were deposited in the Fish Collection of the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (NUPELIA), Universidade Estadual de Maringá, municipality of Maringá, Paraná State, Brazil, as *Astyanax lacustris* (NUP 25442).

Construction of experimental built wetland treatment units with the vertical flow (VFCW). The making of the VFCW units followed some suggestions from the manual by Sezerino *et al.* (2018). This study made them from two cylindrical high-density polyethylene (HDPE) containers (0.80 m in height x 0.55 m in diameter). The filter mass of the first stage was composed of 0.2m of crushed stone (25–50 mm), 0.15m of crushed stone (19–25 mm), and 0.45 m of crushed stone (7–9.5 mm) (gravel). The second stage consisted of 0.2 m of gravel (19–25 mm), 0.15 m of gravel (7–9.5 mm), and 0.45 m of sand (1.2–2 mm). The adduction system consisted of 25 mm diameter polyvinyl chloride pipes and connections. The effluent was drained through an adductor system with perforations of 8.0 mm in diameter distributed along its entire extremity. The drainage pipe was positioned horizontally at the bottom of the bed, extending across the whole diameter of the units. The system contains a faucet for outputting the treated effluent at the bottom of the reservoir, installed 10 cm from the bottom (Fig. 1).

The beds were populated with *Typha domingensis* at 16 plants per square meter density. The propagules were collected manually in a naturally flooded area located on a rural property in the municipality of Cianorte at the beginning of December 2021. The collection was carried out so that the rhizomes were preserved, carefully transported, and transplanted in the experimental units. The residence time of the effluent in the treatment system was 96 h, 48 h in the first stage, and 48 h in the second stage.

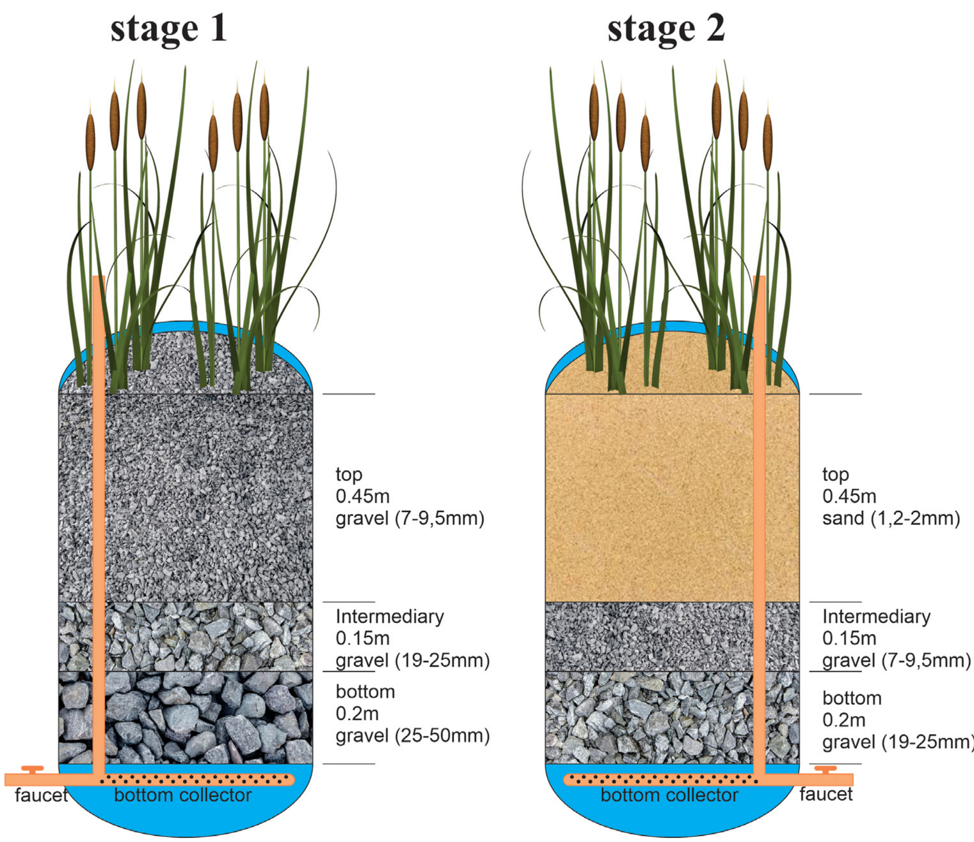

FIGURE 1 | Scheme of an experimental vertical flow constructed wetland system (VFCW) unit.

Physical-chemical analysis of the effluent. The physical-chemical analysis was conducted on the raw effluent sourced directly from the textile industry, as well as on the same raw effluent (at 100% concentration) after treatment by the VFCW system. The raw and treated effluent were stored in a thermal box, kept at ± 5ºC, and taken to the laboratory for physical-chemical characterization. The analyses were conducted in the Departamento de Engenharia Civil, Laboratório de Qualidade da Água e Controle da Poluição – Saneamento Ambiental of the UEM. Six physicochemical parameters of this textile effluent – pH, apparent color, BOD, COD, total suspended solids, and electrical conductivity were determined using standard methods (Rice *et al*., 2012). The physical-chemical parameters were defined according to CONAMA Resolution Nº 430/2011.

Micronucleus test and cellular morphological changes in erythrocytes. The Micronucleus (MN) and cellular morphological changes (CMC) in the erythrocytes test were performed based on the description by Hooftman, Raat (1982). After the anesthesia of the animals, blood was extracted from the caudal vein with a heparinized syringe. A sample (approximately 10 ìL) was dripped onto a sanitized glass slide, and the smear was performed with the aid of another slide. The dripped slide was kept at room temperature, drying for at least 12 h. Afterward, they were fixed with immersion in absolute ethanol for 20 min. Staining was performed for 10 min with 5% Giemsa solution diluted in phosphate buffer (pH 6.8). Then, the slides were washed in distilled water, left to dry naturally, and kept in closed boxes until analysis under microscopy. The slides were analyzed by optical microscopy under 1000 ´ magnification. The MN count and the study of CMC were performed on 2,000 red blood cells per fish.

Comet assay. The comet assay was conducted according to Speit, Hartmann (1999) with modifications according to Ferraro *et al.* (2004). A sample (approximately 10 ìL) of blood was collected from the tail vein of each fish with a heparinized syringe and diluted in fetal calf serum, 1 ml. Slides were prepared for microscopy using this cell suspension, 10 ìL, added to low-melting-point agarose at 37EC, 120 ìL, followed by incubation in lysis solution (1 ml of Triton X-100, 10 mL of DMSO, and 89 ml of stock lysis solution consisting of 2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10, 2.5 M NaOH and 1% N-lauryl sarcosinate). The slides were then dark for 1 h at 8EC.

After lysis, slides were placed in the electrophoresis tank, covered in buffer solution (0.3 N NaOH, 1 mM EDTA, pH > 13) in a cold environment at 8EC for 20 min to unwind the DNA. Electrophoresis was performed for 20 min at 25 V, 300 mA, field strength 1V/cm; slides were neutralized (0.4 M Tris) for 10 min, fixed in absolute ethanol for 10 min, and then stained with ethidium bromide (2 ìg/ml), and covered with coverslips for analysis, after drying for approximately 12 h. Analyses were performed blind to test conditions under an epifluorescence microscope, with image capture at 400´ magnification, counting 100 nucleoids per fish. DNA damage was visually classified into four classes based on DNA migration: 0 (no apparent damage), 1 (slight damage), 2 (medium damage), 3 (large damage), and 4 (maximum damage).

Statistical analysis. Biomarkers (MN test, CMC assay, and comet assay) were analyzed using the Kruskal-Wallis test, a nonparametric analysis of variance, with Dunn's post hoc test for multiple comparisons, performed in GraphPad Prism v. 5. The level of significance for all studies was $p < 0.05$.

RESULTS

MN test and CMC in erythrocytes. In a detailed analysis of 120,000 erythrocytes from 60 specimens of *A. lacustris*, MN were found in 54 erythrocytes. Furthermore, a total of 3,826 CMC were recorded (Tab. 1; Fig. 2). The specimens exposed to a concentration of 5% v/v of raw textile effluent and the 50% v/v and 100% v/v concentrations of VFCW-treated textile effluents showed a significantly higher frequency of CMC compared to the control ($p < 0.05$ in Tab. 1).

On the other hand, there was no significant difference in CMC in the comparison between 25% v/v of textile effluent treated by VFCW and the control ($p > 0.05$ in Tab. 1). Furthermore, there were no significant differences in MN across all groups when compared to the control ($p > 0.05$ in Tab. 1).

Comet assay. The comet assay demonstrated higher levels of DNA damage (Fig. 3) recorded in the 5% v/v concentration of raw textile effluent compared to the control ($p < 0.05$ in Tab. 2). There was no significant difference in the effluent treated by VFCW at a concentration of 25% v/v when compared with the control ($p > 0.05$ in Tab. 2). On the other hand, the concentrations of 50% v/v and 100% v/v of effluent treated by VFCW differed significantly from the control ($p < 0.05$ in Tab. 2).

Physical-chemical analysis of the effluent. The results of the physical-chemical analysis of the effluent are summarized in Tab. 3. The physical-chemical analyses conducted on both the raw and VFCW-treated textile effluent (coming directly from the textile industry, both without dilution) demonstrated a significant reduction across all evaluated parameters, notably in apparent color, which decreased by 99.29% of

TABLE 1 | Absolute numbers and percentage for alteration in *Astyanax lacustris* erythrocytes. For each fish, 2000 cells were analyzed. *Significantly different to control group ($p < 0.05$); MN = micronuclei; CMC = cellular morphological changes.

Group	MN	% MN	Mean ± standard deviation of MNnumbers	CMC	%CMC	Mean ± standard deviation of CMC numbers
Control	0	0%	0.0 ±0.0	212	0.88%	17.66 ± 5.61
Raw textile effluent at 5% v/v concentration	33	0.14%	2.75±1.65*	2046	8.53%	170.5 ± 31.88*
Effluent treated by VFCW at 25% v/v concentration	1	0.004%	0.08±2.88	276	1.15%	23.08 ± 9.41
Effluent treated by VFCW at 50% v/v concentration	10	0.042%	0.8±1.02	474	1.98%	39.5± 9.60*
Effluent treated by VFCW at 100% v/v concentration	14	0.058%	1.16±1.46	814	3.39%	67.8 ± 11.51*

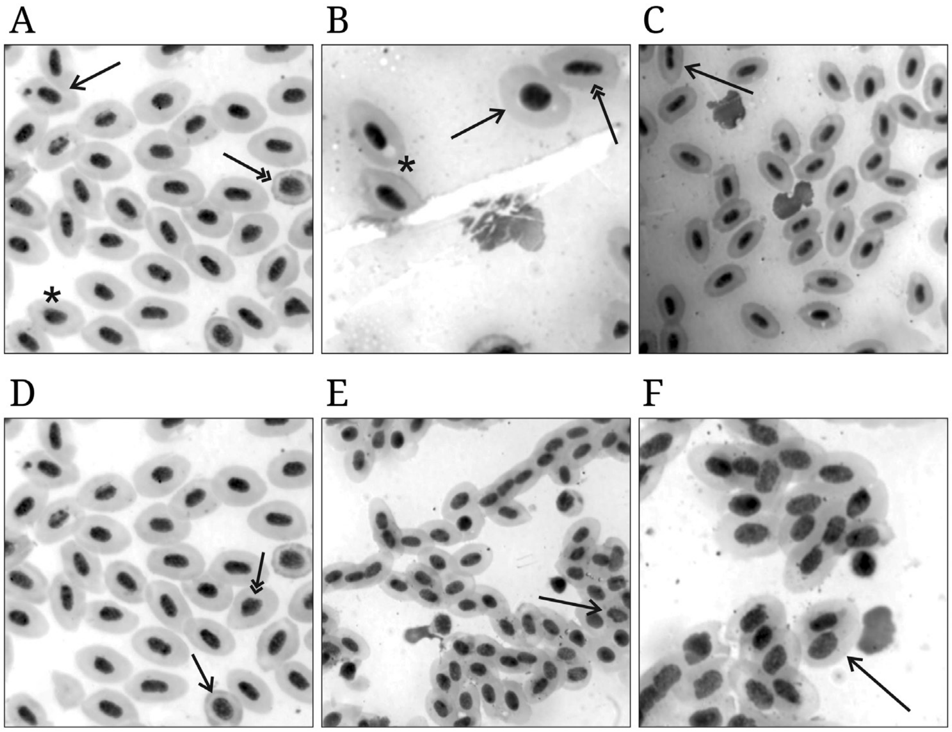

FIGURE 2 | Erythrocytes of *Astyanax lacustris* from the control group and after exposure to raw and treated textile effluent using a vertical flow constructed wetland system (VFCW). (**A**) Normal erythrocytes (asterisk), Micronucleus (single arrow), Crenate erythrocyte (double arrow), (**B**) Cytoplasmic vacuole (asterisk), Immature erythrocyte (single arrow), Elliptocyte (double arrow), (**C**) Nuclear constriction (single arrow), (**D**) and nuclear fragmentation (single arrow), Notched (double arrow), (**E**) Blebbed (single arrow), (**F**) Binucleated (single arrow). Magnification: 1000X.

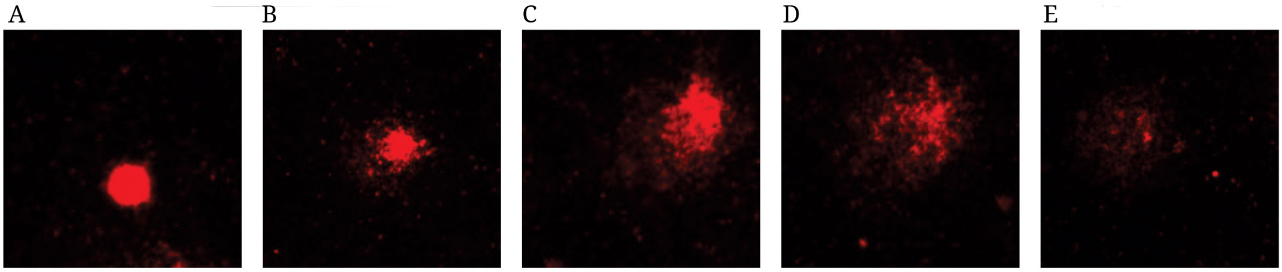

Figure 3 | Damage to erythrocytes from *Astyanax lacustris* specimens from the control group and following exposure to raw and treated textile effluent using a vertical flow constructed wetland system (VFCW) was measured by the comet assay (Magnification 400´). **A**) normal nucleus, **B**) damage 1, **C**) damage 2, **D**) damage 3, **E**) damage 4.

TABLE 2 | Damage recorded in the DNA of erythrocytes of *Astyanax lacustris* specimens, following exposure to raw and VFCW-treated textile effluent. One hundred nucleoids from each fish were analyzed. *Significantly different to control group ($p < 0.05$).

Groups	Specimens	Damage classes					Scores	Mean and standard error of scores
		0	1	2	3	4		
Control	1	88	9	3	0	0	15	13.33 ± 1.92
	2	95	3	2	0	0	7	
	3	81	12	7	0	0	26	
	4	90	7	3	0	0	13	
	5	92	8	0	0	0	8	
	6	89	11	1	0	0	13	
	7	77	19	4	0	0	27	
	8	92	5	3	0	0	11	
	9	94	4	2	0	0	8	
	10	91	7	2	0	0	11	
	11	92	8	0	0	0	8	
	12	92	7	3	0	0	13	
Raw textile effluent at 5% v/v concentration	13	27	38	33	2	0	110	129.91 ± 3.09*
	14	27	32	28	13	0	127	
	15	21	39	27	10	3	135	
	16	29	33	26	12	0	121	
	17	25	36	31	6	2	124	
	18	24	34	21	18	3	142	
	19	16	40	34	8	2	140	
	20	23	37	28	12	0	129	
	21	23	32	41	2	2	128	
	22	29	36	19	16	0	122	
	23	24	25	31	17	3	150	
	24	23	38	24	15	0	131	
Effluent treated by VFCW at 25% v/v concentration	25	78	16	6	0	0	28	32.08 ± 4.44
	26	67	20	9	4	0	50	
	27	73	14	10	3	0	43	
	28	69	12	15	4	0	54	
	29	76	20	4	0	0	28	
	30	82	9	6	3	0	30	
	31	85	8	5	2	0	24	
	32	66	25	5	4	0	47	
	33	78	7	10	3	2	44	
	34	91	5	3	1	0	14	
	35	93	7	0	0	0	7	
	36	87	10	3	0	0	16	
Effluent treated by VFCW at 50% v/v concentration	37	65	21	11	3	0	52	56.0 ± 4.81*
	38	59	24	13	4	0	62	
	39	53	34	10	3	0	63	
	40	59	19	17	4	1	69	
	41	68	28	4	0	0	36	
	42	62	22	5	6	5	70	
	43	55	42	3	0	0	48	
	44	71	24	5	0	0	34	
	45	76	13	7	4	0	39	
	46	78	12	5	2	3	40	
	47	47	35	12	4	2	79	
	48	55	23	12	7	3	80	
Effluent treated by VFCW at 100% v/v concentration	49	39	29	24	8	0	101	69.5 ± 7.85*
	50	57	22	13	8	0	72	
	51	50	29	12	9	0	80	
	52	47	28	14	10	1	90	
	53	69	13	8	0	10	69	
	54	82	13	5	0	0	23	
	55	71	23	6	0	0	35	
	56	34	39	12	8	7	115	
	57	67	21	2	5	4	56	
	58	58	34	3	5	0	55	
	59	67	21	8	4	0	49	
	60	35	52	6	3	4	89	

TABLE 3 | Values of physicochemical parameter rs before and after treatment of textile effluent treated by vertical flow constructed wetland system (VFCW).

Assay	Raw textile effluent	VFCW-treated textile effluent
Electric conductivity (µS/cm)	3090	1973
Apparent color (AC)	983.4	6.9
Biochemical oxygen demand (BOD) (mg L^{-1})	96.59	< 0.64
Chemical oxygen demand (COD) (mg L^{-1})	253.51	< 11.5
pH	7.25	7.25
Total dissolved solids (mg L^{-1})	1764	1453

the treated textile effluent in comparasion with raw textile effluent. The Biochemical Oxygen Demand (BOD) also showed a remarkable reduction of 99.33%. Additionally, the Chemical Oxygen Demand (COD), a crucial parameter for assessing water pollution, as it accounts for the oxygen required to degrade both biodegradable organic matter and non-biodegradable organic materials derived from chemical processes, exhibited a reduction of 95.46%. Additionally, there was a decrease in electrical conductivity by 36.14% and total dissolved solids by 17.6%.

DISCUSSION

Our data indicated considerable damage to the erythrocytes of *A. lacustris* both before and after treatment with the VFCW system. While the use of phytoremediation reduced these damages, it did not completely eliminate them. Similar changes have been documented in studies involving fish exposed to textile effluents, which are known for their toxic, mutagenic, or carcinogenic properties (Çavaş, Ergene-Gözükara, 2003; Hemachandra, Pathiratne, 2016; Parmar, Barot, 2016; Silva *et al*., 2023).

The VFCW system, particularly at a concentration of 25% v/v, demonstrated effectiveness, as statistical analyses for MN test, CMC, and the comet assay did not show significant differences compared to the control. Furthermore, during the tests, the exposure of specimens to wastewater treated with VFCW at 25%, 50%, and 100% v/v did not lead to fish mortality. This finding highlights that, despite the observed cellular alterations, the system contributed to the detoxification of the effluent. It not only reduced toxicity but also mitigated potential harmful metabolites, avoiding any toxic effects.

In alignment with our findings, Hussain *et al.* (2018) conducted tests with both vegetated and non-vegetated VFCWs, utilizing a consortium of plants and endophytic bacteria. Their study evaluated the toxicity of treated textile effluents by VFCWs in fish from 12 h to 72 h, utilizing wastewater collected from the equalization tank at Interloop Limited in Khurrianwala, Faisalabad, Pakistan. The findings confirmed the effectiveness of the treatment, with even more promising results obtained through the use of a plant consortium.

In the present study, exposures to raw textile effluent concentrations exceeding 5% v/v resulted in fish mortality, consistent with the findings of Zhang *et al*. (2012), who evaluated acute toxicity in *Danio rerio* using water samples collected during textile industry processing. Notably, the maximum non-lethal dose was also identified as 5% v/v of the raw effluent. While the cell damage observed from effluent treated by the VFCW system was significantly lower than that from raw textile effluent at the 5% v/v concentration, statistical analysis of the comet assay revealed substantial differences in DNA damage between the control and the 50% v/v and 100% v/v concentrations of effluent treated by the VFCW system. Furthermore, comparisons between the raw effluent at 5% v/v and the treated effluents showed significant differences, indicating that the treatments were statistically distinct in both the control and raw effluent groups (Tab. 3). This can be attributed to the high sensitivity of the comet assay, which has been documented in several studies; even low concentrations of contaminants can elicit a genotoxic response (Mitchelmore, Chipman, 1998; Bücker *et al*., 2006; Frenzilli *et al*., 2009), demonstrating concentration-dependent damage (Zhang *et al*., 2012).

The expressive decline of the BOD observed in the present study is closely linked to the presence of biodegradable organic matter, which necessitates oxygen for its degradation (Menezes *et al*., 2009). Elevated BOD levels in raw textile effluent can be detrimental to aquatic environments, as they may lead to the complete depletion of dissolved oxygen, ultimately threatening various forms of aquatic life at the discharge site (Matos *et al*., 2014).

Research utilizing constructed wetlands for the treatment of textile effluents has yielded promising results. For instance, Ong *et al.* (2009b) reported that a constructed wetland featuring an ascending flow and populated with *Phragmites australis* achieved an impressive 86% reduction in COD and a 96% efficiency rate in removing the azo dye Acid Orange 7. Similarly, Ong *et al*. (2009a) demonstrated that combining aerobic and anaerobic processes in constructed wetlands resulted in a 96% reduction of NH4-N and aromatic amines, thereby enhancing treatment performance for textile effluents. In Tanzania, Mbuligwe (2005) conducted a comparative analysis of wastewater treatment rich in dyes using vegetated wetland systems with various plant species, achieving average reductions exceeding 70% across several parameters, with the coco yam plant exhibiting the best performance.

In a prototype system featuring built-up-flow wetlands designed for treating effluent from the textile industry, Chandanshive *et al*. (2017) conducted tests using *Typha angustifolia*, *Paspalum scrobiculatum*, and their co-plantation (consortium). The study found that the *in situ* treatment of effluent by *T. angustifolia*, *P. scrobiculatum*, and the consortium resulted in the removal of color and metals — such as arsenic, cadmium, chromium, and lead — by up to 77% following phytoremediation. *T. angustifolia* and *P. scrobiculatum* demonstrated a removal efficiency of up to 80% for Congo Red dye (100 mg L-1), while the consortium achieved a remarkable 94% discoloration. Similarly, research by Shehzadi *et al.* (2014) utilized the *Typha domingensis* plant in vertical flow constructed wetlands, highlighting its potential to degrade textile effluent. The introduction of endophytic bacteria significantly enhanced the degradation process, leading to a reduction in values of BOD, COD, and apparent color compared to non-inoculated plants. Collectively, these studies, along with our own, affirm the effectiveness of CW systems in treatment applications, contingent on the level of effluent contamination. These findings emphasize the necessity for further investigations aimed at mitigating cyto/genotoxic damage to *A. lacustris* specimens through consortia of plants and endophytic bacteria, as well as between different plant species, to achieve even greater results.

Numerous studies have demonstrated the effectiveness of CW systems in treating textile effluents and various other pollutants. According to Domingos *et al*. (2011), the CW systems are capable of efficiently treating a wide range of effluents, including those from textiles, dairy production, swine farms, septic tank dewatering, sanitary landfill leachate, inorganic industrial discharges, and tannery operations, among others. In their research, Domingos and his colleagues examined the performance of vertical flow constructed wetlands in treating effluents from a chemical and fertilizer industry in Australia, with a focus on removing nitrogen and metals. They observed impressive results, achieving a 90% removal rate of ammonia.

The effectiveness of CW systems in eliminating commonly used pharmaceuticals globally has been well-documented, with removal efficiencies exceeding 90% (Ranieri *et al*., 2011; Ávila *et al*., 2014; Chen *et al*., 2016; Santos *et al*., 2019). Brazil research on CW targets the treatment of domestic sewage, focusing on the removal of carbonaceous organic matter, as indicated by COD and total suspended solids, as well as essential nutrients like nitrogen (N), phosphorus (P), and potassium (K) (Machado *et al*., 2017; Pelissari *et al*., 2018; Santos *et al*., 2018; Melo Júnior *et al*., 2019; Mazucato *et al*., 2020).

In this study, the biological evaluation using fish erythrocyte biomarkers proved to be effective for environmental biomonitoring. Similarly, the VFCW system demonstrated positive outcomes concerning the physicochemical parameters of the treated effluent and did not show lethal toxicity to the specimens. However, the cyto/genotoxic damage observed in samples of *A. lacustris* indicates that the VFCW system warrants further investigation and optimization regarding the use of aquatic macrophytes, their co-planting, and the integration of microorganisms. This approach may enhance the efficiency of wastewater treatment. Further research is essential to identify key species of plants and bacteria and to explore their combined application on a larger scale for improved degradation. Additionally, various types of constructed wetlands should be tested to assess their efficacy in conjunction with different plants, microorganisms, and a range of contaminants.

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AUTHORS' CONTRIBUTION

Josiane Rodrigues Rocha da Silva: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Writing-original draft.

Cleiton Campos da Silva: Data curation, Methodology, Visualization.

Layon Zafra-Lemos: Data curation, Software.

Ana Luiza de Brito Portela-Castro: Validation, Visualization, Writing-review and editing.

Carlos Alexandre Fernandes: Project administration, Supervision, Validation, Visualization, Writing-review and editing.

ETHICAL STATEMENT

The Ethics Committee approved all procedures for using Animals in Research (CEUA - UEM), license Nº 3062160921.

DATA AVAILABILITY STATEMENT.

The authors confirm that the data supporting the findings of this study are available within the article.

COMPETING INTERESTS

The authors declare no competing interests.

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