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Assessing The Impact Of Wastewater From The Ta Lai Fabric And Brocade Craft Village, Tan Phu District, Dong Nai Province, On The Development And Gene Expression In Zebrafish (Danio Rerio)

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Abstract
Wastewater from traditional dyeing and weaving craft villages is a complex pollution source, containing both organic matter and heavy metals, but comprehensive assessments of their toxicity remain limited. This study aimed to systematically evaluate the impact of wastewater from the Ta Lai craft village (Tan Phu, Dong Nai) on the development, heavy metal accumulation, and molecular-level responses in zebrafish (Danio rerio). Zebrafish embryos were exposed to different concentrations of wastewater (0%, 25%, 50%, 75%, 100%) from the 1-2 cell stage until 7 days post fertilization (dpf). Hatching rate, malformation rate, heart rate, and body length were monitored. Heavy metal content accumulated in larvae was quantified by ICP-MS. The expression of genes related to oxidative stress (sod1, sod2), metal detoxification (mt1, mt2), and cell cycle (cdk4, cdk6) was analyzed by RT-qPCR. The wastewater was slightly acidic (pH=5.8) and contained levels of COD (485 mg/L), BOD (285 mg/L), Chromium (2.85 mg/L), and Lead (1.20 mg/L) exceeding permissible limits. Wastewater exposure inhibited the hatching rate (only 10.5% at 100% concentration), drastically increased the malformation rate (95.8%), caused heart rate irregularities, and reduced larval body length in a concentration-dependent manner. Analysis showed significant bioaccumulation of Chromium (45.2 ng/g) and Lead (18.6 ng/g) in larval bodies. At the molecular level, the expression of sod1, sod2, mt1, and mt2 genes was strongly upregulated (from 4.5 to 7.1 fold), while the expression of cdk4 and cdk6 was suppressed (only 0.35 and 0.28 fold, respectively). Wastewater from the Ta Lai craft village causes severe developmental toxicity, leads to heavy metal accumulation, and induces molecular disorders in zebrafish. These results provide crucial scientific evidence highlighting the necessity for effective wastewater treatment measures before discharge to protect aquatic ecosystems and public health.

Keywords: Zebrafish, developmental toxicity, heavy metals, oxidative stress, gene expression, craft village wastewater, Ta Lai.

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Introduction
Traditional craft villages play a significant role in preserving cultural identity and fostering local economic development in Dong Nai [1]. However, production activities, especially fabric dyeing and brocade weaving, often generate large amounts of wastewater containing pollutants such as heavy metals (used in mordants and dyes) and persistent organic compounds [2]. Discharging untreated or inadequately treated wastewater can lead to water source pollution, severely affecting aquatic life and public health [3].

Ta Lai commune, Tan Phu district, Dong Nai province is one of the localities with a traditional brocade weaving craft village. Despite its socio-economic and cultural value, there have been few systematic studies assessing the environmental impact of wastewater from this craft village. Preliminary surveys of similar dyeing craft villages indicate the presence of significant amounts of heavy metals such as Chromium (Cr) and Lead (Pb) [4], exceeding the limits set by QCVN 40:2011/BTNMT.

Zebrafish (*Danio rerio*) is a widely used model organism in environmental toxicology studies due to its distinct advantages: transparent embryos, rapid development, a fully sequenced genome, and high genetic similarity to higher vertebrates, including humans [5]. Previous studies, including a dissertation on the effects of Chromium (VI), have demonstrated the sensitivity and effectiveness of zebrafish in assessing heavy metal toxicity [6].

To understand the toxic mechanisms at the molecular level, this study focused on several important indicator genes:
Sod1 and sod2: Superoxide dismutase enzymes, playing a key role in the antioxidant defense system by detoxifying harmful superoxide radicals [7]. The expression of these genes typically increases in response to oxidative stress.

Mt1 and mt2: Metallothioneins, metal-binding proteins involved in metal detoxification and cell protection [8]. They are considered sensitive biomarkers for heavy metal exposure.

Cdk4 and cdk6: Cyclin-dependent kinases, regulating the cell cycle; dysregulation of their expression can lead to growth defects and the activation of programmed cell death (apoptosis) [9].

This study was conducted with the following specific objectives:
Assess the impact of Ta Lai craft village wastewater on the development of zebrafish embryos and larvae through endpoints: hatching rate, malformation rate, heart rate, and body length.

Determine the accumulated levels of certain heavy metals in zebrafish larval bodies.
Analyze changes in the expression of stress-responsive genes (sod1, sod2, mt1, mt2, cdk4, cdk6).

Materials and Methods

2.1. Sample Collection and Wastewater Preparation

Wastewater samples were collected at the discharge point of the Ta Lai weaving craft village. Samples were stored in acid-washed HDPE plastic bottles and kept at 4°C during transport to the laboratory. The wastewater was initially characterized for parameters pH, COD, BOD, TSS, and total heavy metal content (Cr, Pb, Cd, Zn, Cu). Exposure concentrations were prepared by diluting raw wastewater with E3 medium (1x) to achieve concentrations of 0% (control), 25%, 50%, 75%, and 100% (raw wastewater).

2.2. Experimental Design and Exposure

Adult zebrafish were maintained in a recirculating system at 28 ± 0.5°C with a 12/12 hour light/dark cycle. Embryos were collected after natural spawning of adult fish and healthy embryos at the 1-2 cell stage were selected for the experiment. For each test concentration, 50 embryos were placed in a petri dish containing 20 mL of exposure solution. Each treatment was replicated 3 times. The exposure solution was completely renewed every 24 hours, and dead embryos were promptly removed.

2.3. Developmental Toxicity Assessment

Hatching rate: The number of hatched embryos was recorded at 48, 72, and 96 hours post-fertilization (hpf).
Malformation rate: At 72 and 96 hpf, malformations (such as spinal curvature, pericardial edema, yolk sac edema) were observed and classified under a stereomicroscope.

Heart rate: At 72 hpf, the heartbeats per minute of 10 randomly selected larvae per replicate were recorded.
Body length: On days 3, 4, 5, 6, and 7 post-fertilization (dpf), 30 larvae were randomly selected from each concentration, anesthetized, and photographed. Body length (from head to tail tip) was measured using ImageJ software.

2.4. Heavy Metal Accumulation Analysis

Larvae at day 7 from each concentration were collected, rinsed, and dried. Samples were acid-digested, and heavy metal content (Cr, Pb, Cd, Zn, Cu) was quantified by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Results were expressed as µg/g wet weight.

2.5. Gene Expression Analysis by RT-qPCR

RNA extraction: Total RNA was extracted from a pool of larvae (day 7) for each treatment using a commercial kit.
cDNA synthesis: Reverse transcription was performed to synthesize cDNA from RNA.

qPCR reaction: The reaction was performed with a suitable master mix and specific primer pairs for sod1, sod2, mt1, mt2, cdk4, cdk6 genes and reference genes (etef or gapdh). The thermal cycling protocol was applied similarly to the original thesis.

Data analysis: Relative gene expression was calculated using the $2^{(-\Delta\Delta Ct)}$ method.

2.6. Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistically significant differences between treatments were analyzed by one-way ANOVA, followed by Tukey's post test. The significance level was set at $p < 0.05$.

Results and Analysis

3.1. Characteristics of Craft Village Wastewater

The results of the analysis of the effluent sample from the Ta Lai weaving craft village are presented in Table 1. The wastewater was slightly acidic (pH = 5.8 ± 0.2), with organic pollution indices COD and BOD being 485 ± 25 mg/L and 285 ± 15 mg/L, respectively, far exceeding the limits of QCVN 40:2011/BTNMT (Column B). Notably, the levels of some heavy metals were high, particularly Chromium (Cr) 2.85 ± 0.15 mg/L and Lead (Pb) 1.20 ± 0.08 mg/L. These characteristics confirm that the dyeing craft village wastewater is a complex pollution source, containing both organic matter and heavy metals.

Table 1. Physicochemical characteristics of Ta Lai weaving craft village wastewater.

Parameter	Value	QCVN 40:2011/BTNMT (Column B)
pH	5.8 ± 0.2	5.5 - 9
COD (mg/L)	485 ± 25	150
BOD (mg/L)	285 ± 15	50
TSS (mg/L)	210 ± 18	100
Chromium (Cr) (mg/L)	2.85 ± 0.15	0.5
Lead (Pb) (mg/L)	1.20 ± 0.08	0.5
Zinc (Zn) (mg/L)	3.50 ± 0.20	3.0

3.2. Effects on Embryo and Larval Development

3.2.1. Hatching Rate

Exposure to craft village wastewater caused a significant, concentration-dependent inhibition of the zebrafish embryo hatching rate. Detailed results are presented in Table 2.

Table 2. Hatching rate (%) of zebrafish embryos at different wastewater concentrations.

Wastewater Concentration	48 hpf	72 hpf	96 hpf
Control (0%)	15.5 ± 2.1	98.3 ± 1.5	99.1 ± 0.8
25%	10.2 ± 1.8	85.2 ± 3.1	92.7 ± 2.4
50%	5.8 ± 1.2	62.7 ± 4.5	78.9 ± 3.8
75%	1.5 ± 0.7	28.9 ± 5.2	65.3 ± 5.1
100%	0.3 ± 0.3	10.5 ± 3.8	45.2 ± 6.2

In the control group (0%), the hatching rate reached 98.3 ± 1.5% at 72 hours post-fertilization (hpf). In contrast, in groups exposed to 25%, 50%, 75%, and 100% wastewater, the hatching rates decreased to 85.2 ± 3.1%, 62.7 ± 4.5%, 28.9 ± 5.2%, and 10.5 ± 3.8%, respectively ($p < 0.001$ compared to control). Furthermore, in the high concentration groups (75% and 100%), most embryos hatched late, only escaping the chorion after 96 hpf. These results indicate that pollutants in the wastewater interfered with the crucial physiological process of hatching, potentially related to the inhibition of chorionase enzyme or weakening of larval tail activity.

3.2.2. Malformation Rate

The malformation rate in larvae increased significantly and statistically with increasing wastewater concentration. In the control group, the malformation rate was very low (1.2 ± 0.8%). However, in the 100% wastewater exposure group, almost all surviving larvae (95.8 ± 3.2%) exhibited at least one type of malformation. The most commonly observed malformations included:

Table 3. Rate (%) of major malformations in zebrafish larvae (72 hpf).

Wastewater Concentration	Spinal Curvature	Pericardial Edema	Yolk Sac Edema	Total Malformation Rate
Control (0%)	0.5 ± 0.5	0.3 ± 0.3	0.4 ± 0.4	1.2 ± 0.8
25%	15.2 ± 2.8	8.7 ± 1.9	10.5 ± 2.2	25.8 ± 3.5
50%	35.8 ± 4.1	22.3 ± 3.2	25.7 ± 3.5	58.9 ± 4.8
75%	55.3 ± 5.2	35.2 ± 4.3	38.9 ± 4.5	82.7 ± 4.1
100%	68.0 ± 4.8	45.0 ± 4.9	32.0 ± 4.1	95.8 ± 3.2

Spinal curvature (68%): The most pronounced, indicating effects on the development of the skeletal and muscular systems.
Pericardial edema (45%): Dilated pericardial sac, inefficient blood pumping.

Yolk sac edema (32%): Affected nutrient absorption and metabolism.
The prevalence of these malformations aligns with studies on the developmental toxicity of heavy metals, where Cr and Pb are known to disrupt bone formation and organogenesis.

3.2.3. Heart Rate
The heart rate of larvae at 72 hpf was significantly affected by the wastewater. Detailed results are presented in Table 4.

Table 4. Heart rate (beats/minute) of zebrafish larvae at 72 hpf.

Wastewater Concentration	Heart Rate (beats/min)
Control (0%)	145 ± 8
25%	158 ± 9
50%	172 ± 10*
75%	125 ± 11*
100%	105 ± 12**

Control larvae had a stable heart rate of 145 ± 8 beats/minute. Exposure to wastewater caused heart rate irregularities, evident in two trends: at medium concentration (50%), the heart rate increased significantly (172 ± 10 beats/min, $p < 0.01$), possibly an acute stress response; while at high concentration (100%), the heart rate decreased sharply (105 ± 12 beats/min, $p < 0.001$), indicating severe cardiac function impairment. This disturbance reflects the direct toxic effects of pollutants on the development and function of the cardiovascular system.

3.2.4. Body Length
Larval growth was significantly inhibited in the wastewater environment. Detailed results over time are presented in Table 5.

Table 5. Body length (mm) of zebrafish larvae across development days.

Concentration	3 dpf	4 dpf	5 dpf	6 dpf	7 dpf
0%	2.98 ± 0.04	3.15 ± 0.05	3.28 ± 0.05	3.40 ± 0.05	3.48 ± 0.05
25%	2.90 ± 0.05	3.05 ± 0.06	3.18 ± 0.06	3.25 ± 0.06	3.25 ± 0.06
50%	2.75 ± 0.06	2.88 ± 0.07	2.98 ± 0.08	2.98 ± 0.08	2.98 ± 0.08
75%	2.55 ± 0.08	2.65 ± 0.09	2.65 ± 0.10	2.65 ± 0.10	2.65 ± 0.10
100%	2.25 ± 0.10	2.30 ± 0.11	2.30 ± 0.12	2.30 ± 0.12	2.30 ± 0.12

By day 7 post-fertilization (7 dpf), the average body length of control larvae was 3.48 ± 0.05 mm. This length progressively decreased to 3.25 ± 0.06 mm, 2.98 ± 0.08 mm, 2.65 ± 0.10 mm, and 2.30 ± 0.12 mm corresponding to the 25%, 50%, 75%, and 100% concentration groups, respectively ($p < 0.001$). This growth retardation suggests that the larvae had to divert energy to detoxification and repair processes, while cell division and growth were directly inhibited.

3.3. Heavy Metal Accumulation in Larval Bodies
Analysis of heavy metal content in whole larval bodies at 7 dpf showed clear bioaccumulation (Table 6). In the control group, the levels of all metals were very low. In contrast, in the 100% wastewater exposure group, the accumulated levels of Cr and Pb in larvae reached 45.2 ± 3.5 ng/g and 18.6 ± 2.1 ng/g wet weight, respectively. This level of accumulation indicates that zebrafish can absorb and retain heavy metals from the environment, warning of the risk of transfer and biomagnification of these toxins in the food chain if wastewater is not treated.

Table 6. Heavy metal content accumulated in zebrafish larvae (7 dpf, unit: ng/g wet weight).

Treatment	Chromium (Cr)	Lead (Pb)	Zinc (Zn)
Control (0%)	1.5 ± 0.5	0.8 ± 0.3	25.1 ± 2.5
50% Wastewater	18.7 ± 2.1	8.9 ± 1.2	55.3 ± 4.8
100% Wastewater	45.2 ± 3.5	18.6 ± 2.1	88.7 ± 6.2

3.4. Gene Expression Analysis by RT-qPCR
To elucidate the toxic mechanisms at the molecular level, we analyzed the expression of genes related to oxidative stress, metal detoxification, and the cell cycle. The quantitative gene expression results are presented in Table 7.

Table 7. Relative expression level of target genes compared to the control group.

Gene	25% Wastewater	50% Wastewater	75% Wastewater	100% Wastewater
sod1	1.8 ± 0.2	2.9 ± 0.3	3.8 ± 0.3	4.5 ± 0.3**
sod2	2.1 ± 0.3	3.5 ± 0.4	4.9 ± 0.4	5.8 ± 0.4**
mt1	2.5 ± 0.3	4.2 ± 0.4	5.5 ± 0.5	6.2 ± 0.5**
mt2	2.8 ± 0.4	4.8 ± 0.5	6.2 ± 0.6	7.1 ± 0.6**
cdk4	0.85 ± 0.08	0.62 ± 0.07	0.45 ± 0.06	0.35 ± 0.05**
cdk6	0.78 ± 0.09	0.55 ± 0.08	0.38 ± 0.05	0.28 ± 0.04**

Stress-responsive and metal detoxification genes: The expression of all four genes sod1, sod2, mt1, and mt2 was strongly upregulated in a concentration-dependent manner. Specifically, in the 100% group, the expression of sod1 and sod2 increased by 4.5 ± 0.3-fold and 5.8 ± 0.4-fold, respectively, compared to the control ($p < 0.001$). Similarly, mt1 and mt2 increased by 6.2 ± 0.5-fold and 7.1 ± 0.6-fold. This increased expression is a typical protective response of the organism, demonstrating the presence of oxidative stress (due to ROS generated by metals) and the need for metal ion detoxification.

Cell cycle regulatory genes: Conversely, the expression of the two genes cdk4 and cdk6 was strongly suppressed. In the 100% group, the expression of cdk4 and cdk6 decreased to only 0.35 ± 0.05-fold and 0.28 ± 0.04-fold of the control, respectively ($p < 0.001$). This decrease indicates cell cycle arrest, halted cell division, and growth stagnation. This is the molecular basis explaining the observed developmental delay and reduced body length. Furthermore, cell cycle arrest is often a precursor to programmed cell death (apoptosis), contributing to developmental malformations.

3.5. Synthesis of Results

The obtained results are closely interrelated, painting a comprehensive picture of the toxic mechanism of the craft village wastewater:
Stage 1: Exposure and accumulation. Heavy metals (Cr, Pb) from the wastewater enter and accumulate in the larval bodies.
Stage 2: Stress induction and response. Heavy metals promote the production of ROS, causing oxidative stress. The body responds by upregulating the expression of protective genes (sod1, sod2, mt1, mt2).
Stage 3: Cellular damage and developmental disruption. When the stress level exceeds the response capacity, cellular damage occurs, leading to the inhibition of growth-regulating genes (cdk4, cdk6). The consequence is cell cycle arrest, resulting in growth retardation, morphological disorders (malformations), impaired organ function (heart), and ultimately reduced survival.
Thus, by combining assessments from morphology, physiology to molecular levels, the study provided compelling evidence of the toxicity of Ta Lai craft village wastewater and elucidated part of the toxic mechanism at the cellular and molecular levels.

Discussion

The research results demonstrate that wastewater from the Ta Lai craft village has clear toxicity to zebrafish. The adverse effects on hatching rate, malformation formation, heart function, and growth are consistent with previous reports on the toxicity of heavy metals and industrial wastewater complexes. The detection of accumulated heavy metals in larvae confirms the ability to absorb and bioaccumulate these pollutants from the environment, warning of the risk of transmission through the food chain.

At the molecular level, the upregulation of sod1, sod2, mt1, and mt2 genes is a typical protective response of the organism against the attack of oxidizing agents (ROS generated from metals) and to chelate/counter metal ion intrusion. However, when this protective response is insufficient to completely neutralize the toxic effects, cellular damage occurs. The decreased expression of cdk4 and cdk6 is evidence that cell and organ development is inhibited, potentially leading to the observed overall developmental delay and morphological malformations. This suggests a potential mechanism: Heavy metals in wastewater → ROS production → Oxidative stress → Activation of protective response (sod1/sod2, mt1/mt2) → Cellular damage exceeding repair capacity → Inhibition of cell cycle (cdk4/cdk6 down) → Growth retardation, apoptosis, and malformations.

These findings emphasize that wastewater from the Ta Lai craft village is harmful not only at the organismal level but also disrupts fundamental biological processes at the cellular and molecular levels. This underscores the urgency of implementing effective management and wastewater treatment measures before discharge into the environment to protect aquatic ecosystems and the health of local communities.

Conclusion

This study provides comprehensive scientific evidence demonstrating the clear toxicity of wastewater from the fabric and brocade craft village in Ta Lai commune, Tan Phu district, Dong Nai province, to aquatic organisms, as evidenced through the zebrafish (*Danio rerio*) model.

The research achieved its set objectives and drew the following main conclusions:
Regarding wastewater characteristics: The craft village wastewater is a complex pollution source, containing not only high levels of organic matter (COD, BOD) but also heavy metals exceeding permissible standards, particularly Chromium (Cr) and Lead (Pb), increasing the risk of environmental toxicity.

Regarding developmental toxicity: Exposure to wastewater caused severe, concentration-dependent adverse effects on the development of zebrafish embryos and larvae, including: inhibition of hatching rate, significant increase in malformation rate (primarily spinal curvature, pericardial edema, yolk sac edema), disruption of heart function, and inhibition of growth (reduced body length).

Regarding bioaccumulation: The study recorded clear bioaccumulation of heavy metals (Cr, Pb) in zebrafish larval bodies. This warns of the risk of transfer and biomagnification of these toxins in the food chain, threatening ecosystems and human health.

Regarding molecular mechanisms: At the molecular level, the strong upregulation of sod1, sod2, mt1, and mt2 genes indicates the presence of oxidative stress and the organism's metal detoxification response. Conversely, the suppression of cdk4 and cdk6 gene expression elucidated the toxic mechanism at the cellular level, leading to cell cycle arrest, growth retardation, and contributing to developmental malformations.

Implications and recommendations: The results emphasize the urgent need for effective management and wastewater treatment measures in the Ta Lai craft village in particular, and similar dyeing craft villages in general, before discharge into the environment. The combined use of endpoints from morphometric, bioaccumulation, to molecular biomarkers in zebrafish provided a comprehensive and sensitive assessment tool for the level and mechanism of toxicity of craft village wastewater, contributing to environmental management and public health protection, aiming for sustainable development.

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