



Effects of ultrafine bubble water on acute metal toxicity in the non-biting midge *Polypedilum nubifer*

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ABSTRACT

This study investigated the acute toxicity of cadmium (Cd), copper (Cu), lead (Pb), aluminium (Al), and zinc (Zn) to first-instar *Polypedilum nubifer* larvae and examined the influence of ultrafine bubble (UFB) water on metal toxicity. Larvae were exposed to a range of metal concentrations in control water (dechlorinated tap water) and in UFB treated water, while physicochemical parameters such as particle size, zeta potential, dissolved oxygen, pH, and temperature were characterized. Acute toxicity was assessed over 48 hours following OECD Guideline 235, and LC₅₀ values were estimated using probit analysis. Pb showed the highest acute toxicity in control water (LC₅₀ = 0.84 mg/L), followed by Cu (LC₅₀ = 0.92 mg/L), whereas Cd, Al, and Zn showed low toxicity, with LC₅₀ values exceeding the maximum tested concentrations. Exposure in UFB water increased survival rates of larvae exposed to Pb and Cu, raising LC₅₀ values to 1.06 mg/L and 1.33 mg/L, respectively. Physicochemical characterization indicated the presence of nanoscale bubbles with an average diameter of 172 nm and negative zeta potential of -12 mV, which likely reduced the bioavailability of dissolved metal ions. These findings demonstrate that UFB water may decrease the acute toxicity of Pb and Cu to *P. nubifer*, potentially by reducing free metal ions and mitigating oxidative stress. Overall, the results establish first-instar *P. nubifer* as a sensitive bioindicator of metal toxicity and demonstrate that UFB technology can modify metal bioavailability in freshwater ecosystems.

Keywords: acute, toxicity, metal, midge, ultrafine bubble.

INTRODUCTION

Metal contamination in freshwater ecosystems has remained a critical issue in recent years. Rapid industrialization, including mining, manufacturing, and agricultural expansion, has emerged as a major pollution threat to aquatic ecosystems (Saravanan et al., 2024). The toxicity of metals such as lead (Pb) and cadmium (Cd) is known to have adverse impacts on aquatic biota, such as physiological problems and metabolic disruption (Zhang and Reynolds, 2019). While certain metals, such as copper (Cu), aluminium (Al) and zinc (Zn), are essential for organisms, but they become toxic when present in excessive

concentrations (Mebane et al., 2020). Elevated concentrations of these metals can induce oxidative stress, for example through the catalytic generation of reactive oxygen species (ROS), ultimately leading to cellular damage and impaired organismal performance (Zhang et al., 2025). Therefore, determining the toxic thresholds of these metals is important for ecological risk assessment and biomonitoring.

Metal toxicity in aquatic environments is strongly influenced by water chemistry and physicochemical processes that control metal speciation and bioavailability. In recent years, ultrafine bubble (UFB) has emerged as a novel approach for environmental remediation (Atkinson et al.,

2019; Meetiayagoda et al., 2026; Temesgen and Han, 2023). Ultrafine bubbles, with diameters less than 1 μm , have several unique physicochemical characteristics compared to macro- and micro-bubbles, including prolonged stability in the liquid phase, high gas mass transfer efficiency, and a large interfacial area (Agarwal et al., 2011; Yasui et al., 2018). Furthermore, UFB typically possess a negatively charged surface, which prevents coalescence and allows them to persist in water for extended periods, ranging from days to months (Ohgaki et al., 2010). These characteristics suggest that UFBs can modify the chemical environment of aqueous systems, potentially altering metal speciation, mobility, and bioavailability. However, the extent to which UFBs influence metal toxicity to aquatic organisms remains poorly understood.

Previous studies have demonstrated that UFB may influence metal speciation and mobility by altering soluble metal ions and promoting precipitation (Liu et al., 2021; Huang, Nhung, Dod-biba, et al., 2023). These changes could modify metal bioavailability and, consequently, the acute responses of aquatic organisms. Although the general ecological effects of UFBs have been explored, their specific impact on metal toxicity in benthic invertebrates remains largely unknown. Benthic invertebrates, particularly Chironomidae (non-biting midges), are widely used as bioindicators in freshwater ecotoxicology due to their ecological relevance, widespread distribution, and sensitivity to pollutants (Raunio et al., 2011). In particular, *Polypedilum nubifer* larvae represents a suitable model for investigating how UFB conditions might modulate metal toxicity at the organismal level. We hypothesize that UFB-treated water alters metal bioavailability, resulting in modified acute toxicity responses in *P. nubifer* larvae.

The present study aimed to evaluate the acute toxicity of Cd, Cu, Pb, Al, and Zn to *P. nubifer*

larvae and to assess how UFB water modifies these responses. By comparing conventional and UFB-treated water conditions, we seek to determine whether UFB can alter metal bioavailability and toxicity, thereby providing mechanistic insights into the potential role of UFB technology in modulating contaminant effects in freshwater ecosystems.

MATERIALS AND METHODS

Chemicals

Metal stock solutions (CdCl_2 , $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan) (Figure 1). Standard metal stock solutions for the exposures were prepared by dissolving these compounds in distilled water and were stored at 4 °C.

Test organisms

Polypedilum nubifer cultures were maintained in aerated plastic aquaria containing dechlorinated tap water and a 1–2 cm substrate layer of natural sand (particle size <1 mm; GEX Aqua sand). The cultures were kept at a controlled temperature of 20 ± 2 °C with a 16:8 h light: dark photoperiod. Larvae were fed finely ground fish flakes (Tetra-Min®, Tetra Werke, Melle, Germany) three times per week, and the rearing medium was renewed weekly to ensure water quality. Egg masses were collected from the sediment surface and transferred to separate aquaria for hatching (Figure 2). The newly hatched larvae were reared under these identical conditions until they reached the required developmental stage for experimentation.

Ultrafine bubble

Ultrafine bubbles (UFBs) were produced using a UP0290M-1 generator (1.4 L/min, 2 MPa, 250 W; Maruyama MFG. Co., Inc., Tokyo, Japan) using ambient air as the gas source (Figure 3a). UFB water was generated by operating the system for 10 min prior to each experiment to ensure stable bubble formation (Meetiayagoda et al., 2026). The presence of UFBs was visually confirmed using laser light scattering, which revealed a clearly visible Tyndall effect in UFB water compared with control water (Figure 3b). The zeta potential and UFB particle size were



Figure 1. Metal stock solutions for the acute toxicity test

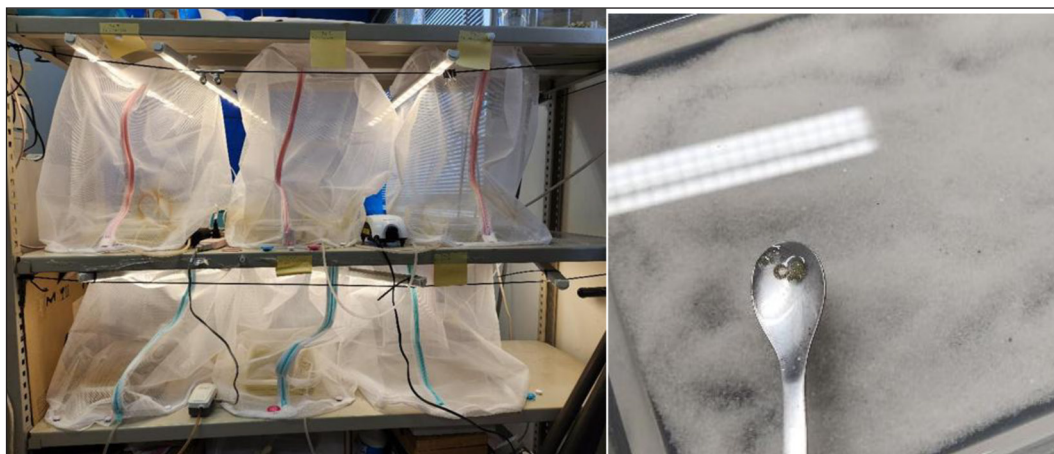


Figure 2. Culture conditions of *Polypedilum nubifer* and representative egg masses used for toxicity testing

analyzed using an ELSZ-2000 system (Otsuka Electronics, Hirakata, Japan) (Figure 3c). Water quality parameters, including dissolved oxygen (DO) concentration and temperature, were monitored using an HQ30d meter (Hach, USA), while pH was measured with a Mettler Toledo pH meter (Switzerland) (Figure 3d).

Acute toxicity test

Acute toxicity assays were performed under static conditions in accordance with OECD

Guideline 235 (OECD, 2011), utilizing UFB water, with dechlorinated tap water serving as the control. The tested concentration ranges were 0.05, 0.125, 0.25, 0.5, 1, and 2 mg/L for Cd; 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/L for Cu; 0.125, 0.25, 0.5, 0.75, 1, 1.25, 1.5, and 2 mg/L for Pb; and 0.25, 0.5, 1, 2, 5, and 10 mg/L for Al and Zn (Figure 4a). Each treatment group consisted of four replicates, with five first-instar larvae per replicate. The larvae were kept in 45-mm glass crystallizing dishes containing 100 mL of the respective test solution and were not fed during

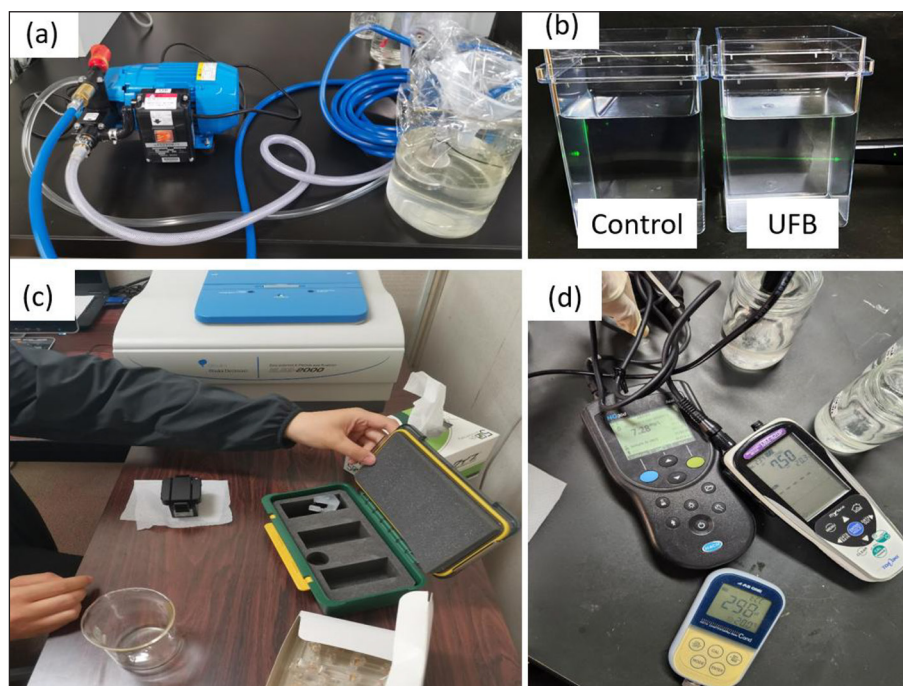


Figure 3. Experimental setup for UFB preparation and characterization: (a) UFB water generation system, (b) laser light scattering comparison between UFB and control water, (c) measurement of particle size distribution and zeta potential, and (d) monitoring of water quality parameters

the experiment. Conditions were maintained at 20 ± 1 °C with a 16:8 h light:dark photoperiod. After 48 hours, mortality was quantified by checking for immobilization following mechanical stimulation under stereo microscope (Figure 4b).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 10 (GraphPad Software Inc.; USA). The survival rates were expressed as percentages, and the data were reported as mean \pm standard deviation (SD). To determine the appropriate statistical approach, we first evaluated the dataset for normality and variance equality using the Shapiro–Wilk and Levene’s tests, respectively. Because the data failed to meet these parametric assumptions, non-parametric Mann–Whitney U test was used to evaluate the differences between UFB water and the control group across the various metal exposures. The 48-h LC_{50} were estimated according to Finney’s probit analysis method. Significant differences between treatments were set at $p < 0.05$.

RESULTS AND DISCUSSION

Physicochemical properties of control and UFB water

The physicochemical properties of UFB and control water used in the toxicity experiments are presented in Table 1. The temperatures were similar between treatments, with the control

water recorded at 20.63 ± 0.31 °C and UFB water at 20.73 ± 0.12 °C. The pH values were 7.83 ± 0.02 and 7.78 ± 0.48 , respectively. Electrical conductivity was higher in the control water (293 ± 3.46 $\mu\text{S}/\text{cm}$) compared to the UFB water (223.33 ± 3.79 $\mu\text{S}/\text{cm}$). Additionally, DO was slightly higher in the UFB water (8.18 ± 0.22 mg/L) than in the control water (7.99 ± 0.04 mg/L).

Measurement using a particle size analyzer also showed the presence of nanoscale bubbles in UFB water, with a mean particle diameter of 172.17 ± 30.8 nm. In contrast, no detectable nano-sized bubbles were observed in the control water. Zeta potential measurements revealed that the UFB water exhibited a negative surface charge of -12 ± 3.92 mV, whereas the control water showed a zeta potential of 0.48 ± 0.24 mV. The negative zeta potential arises from the adsorption of hydroxide ions (OH^-) at the gas–liquid interface, which subsequently attracts counter-ions, forming an electrical double-layer structure (Takahashi, 2005). This unique property of UFB generates repulsive forces that prevent bubble coalescence and, under favorable conditions, contributes to the stability and persistence of UFB in water (Ushikubo et al., 2010).

The measured physicochemical parameters showed only minor differences between control and UFB treatments during the exposure period (Table 2). Dissolved oxygen and conductivity tended to be slightly lower in UFB water, whereas pH and temperature minimal fluctuations among all metal treatments. Importantly, all measured values remained within the acceptable ranges recommended by OECD guidelines for acute toxicity

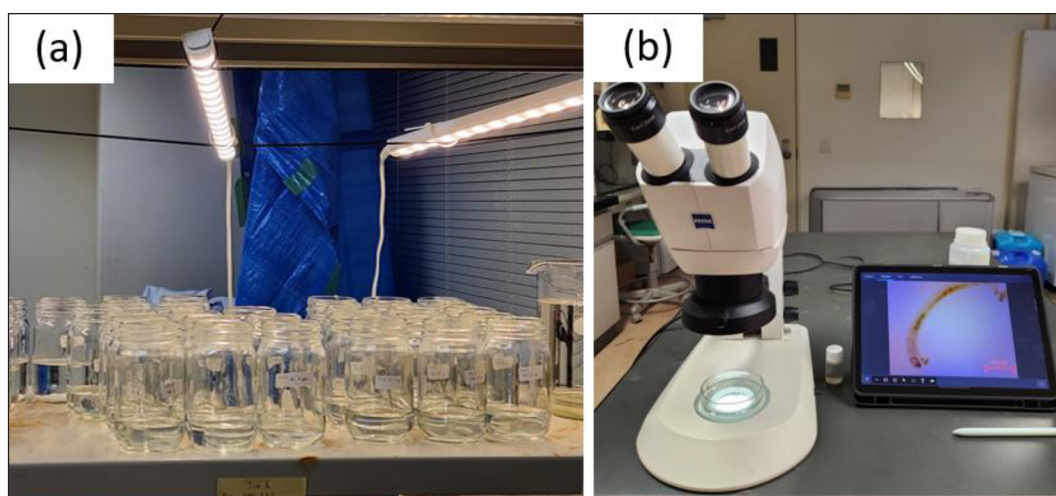


Figure 4. Acute toxicity testing of metals on *Polypedilum nubifer*: (a) experimental exposure setup, (b) assessment of larval survival and mortality

Table 1. Physicochemical properties of control and UFB water

Parameter	Control	UFB
Particle size (nm)	0	172.17±30.8
Zeta potential (mV)	0.48±0.24	-12±3.92
Dissolved oxygen (mg/L)	7.99±0.04	8.18±0.22
pH	7.83±0.02	7.78±0.48
Temperature (°C)	20.63±0.31	20.73±0.12
Conductivity (µS/cm)	293±3.46	223.33±3.79

Table 2. Physicochemical properties of the test water during the acute toxicity experiments with metals

Metal	DO (mg/L)		pH		Temperature (°C)		Conductivity (µS/cm)	
	Control	UFB	Control	UFB	Control	UFB	Control	UFB
Al	8.18±0.08	7.77±0.35	6.70±0.07	6.73±0.07	20.28±0.16	20.24±0.15	224.29±2.36	217.43±0.79
Cd	8.11±0.09	7.67±0.17	7.60±0.05	7.52±0.09	20.20±0.11	20.20±0.15	227.88±2.30	215.75±1.49
Cu	8.03±0.17	7.65±0.16	7.54±0.09	7.56±0.11	20.25±0.16	20.27±0.12	226.67±3.43	217.22±1.09
Pb	8.17±0.10	7.56±0.26	7.52±0.11	7.54±0.08	20.24±0.13	20.27±0.17	225.44±3.32	216.67±2.29
Zn	8.10±0.15	7.55±0.21	7.54±0.09	7.52±0.06	20.21±0.10	20.24±0.14	226.71±3.04	216.29±1.38

testing with chironomid. Therefore, the observed toxicity responses can be attributed primarily to metal exposure and UFB treatment rather than to changes in basic water quality.

Acute toxicity effect of metals on *Polypedilum nubifer*

Figure 5 shows the survival rates of *P. nubifer* larvae during the 48 h acute toxicity test for Cd, Cu, Pb, Al, and Zn. Survival rates for Al and Zn remained near 100%, and Cd exposure resulted in low mortality across all tested concentrations and treatments. In contrast, *P. nubifer* showed dose-dependent mortality when exposed to Cu and Pb. After 48 h of exposure, *P. nubifer* larvae exposed to 0.1, 0.2 and 0.4 mg/L of Cu demonstrated significantly higher survival rates in UFB water compared to the control water ($p < 0.05$). The 48 h LC₅₀ values of Cd, Cu, Pb, Al, and Zn in *P. nubifer* under both control water and UFB conditions are presented in Table 3. LC₅₀ values for both Pb and Cu were higher in the UFB water compared to the control, indicating that the presence of UFBs reduced the toxicity of these metals. Specifically, the LC₅₀ for Cu increased from 0.92 mg/L (95% CI: 0.67–1.27 mg/L) in the control to 1.33 mg/L (95% CI: 1.11–1.67 mg/L) in the UFB water. Similarly, the Pb LC₅₀ increased from 0.84 mg/L (95% CI:

0.75–0.93 mg/L) in control to 1.06 mg/L (95% CI: 0.99–1.13 mg/L) in UFB water. For Cd, Zn, and Al, the LC₅₀ values exceeded the maximum tested concentrations of 2 mg/L (Cd) and 10 mg/L (Zn and Al) under both water conditions, indicating relatively low acute toxicity within the tested ranges. Consequently, the LC₅₀ values for these three metals were reported as greater than the maximum tested concentrations.

Interestingly, acute mortality was not observed during the Cd exposure within the tested concentration range. This result is consistent with previous findings indicating that chironomids may tolerate relatively high short-term Cd exposure. As presented in Table 4, Watts and Pascoe (2000) reported Cd LC₅₀ values ranging from 2.62 to 9.34 mg/L in *Chironomus riparius* and *Chironomus tentans*, whereas Shuhaimi-Othman et al., (2011) documented a much lower LC₅₀ (0.13 mg/L) in fourth-instar *Chironomus javanus*. Chironomids are generally considered relatively tolerant to Cd due to physiological mechanisms, such as calcium-regulated ion transport and the induction of metal-binding proteins, which can reduce intracellular Cd toxicity (Gillis and Wood, 2008; Leonard et al., 2018).

Moreover, the results from this study demonstrated that Pb was the most toxic metal to *P. nubifer*. Our observed LC₅₀ value for Pb was higher

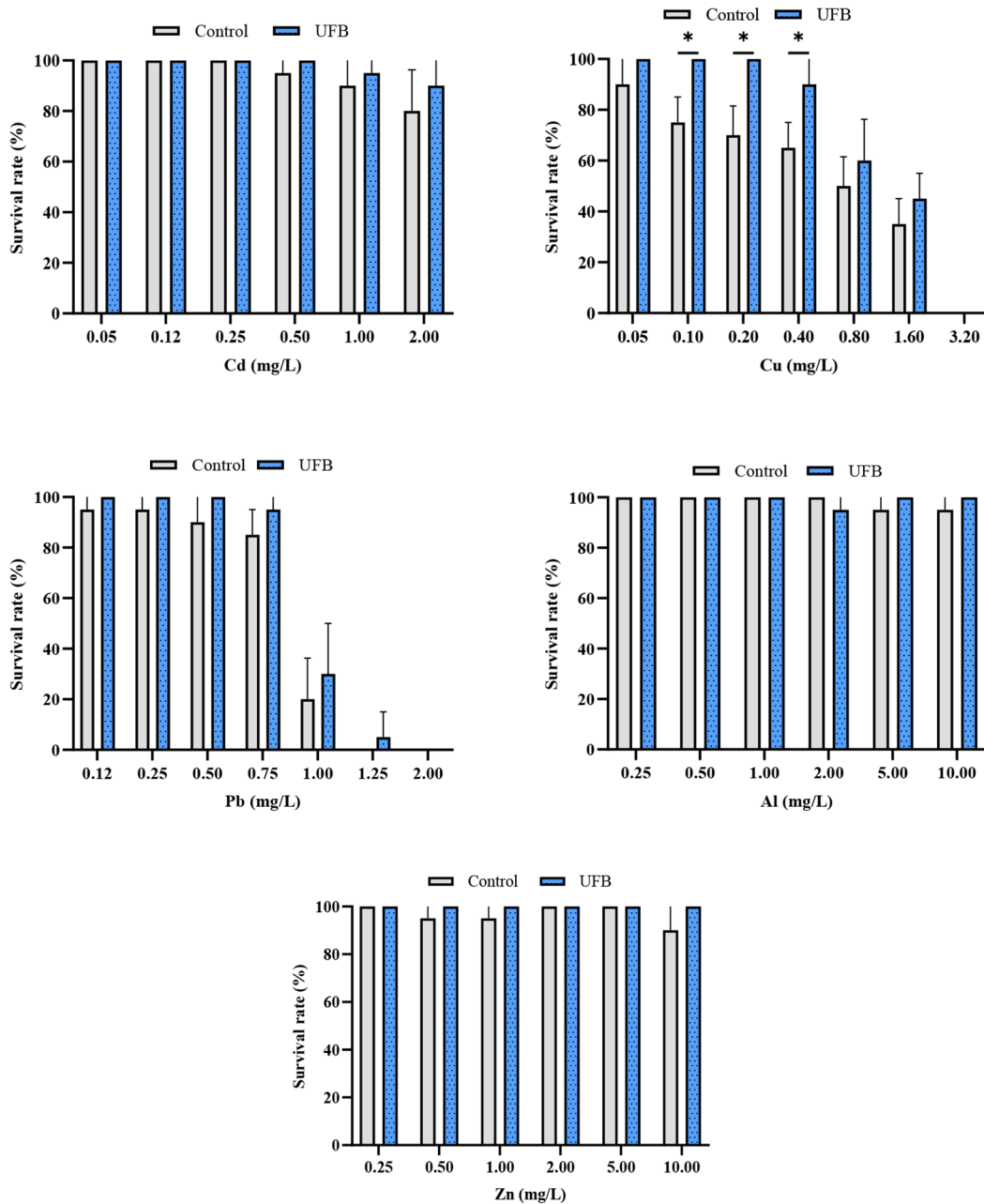


Figure 5. Survival rate of *P. nubifer* during acute toxicity test of Cd, Cu, Pb, Al, and Zn under control and UFB water. *asterisk indicates significantly different values between control and the UFB water ($n = 4$; Mann–Whitney U test; $p < 0.05$)

than those reported by B echard et al., (2008), who found a 24 h LC_{50} of 0.61 mg/L for first-instar *C. riparius*, and by Shuhaimi-Othman et al., (2011), who reported a 48 h LC_{50} of 0.13 mg/L for fourth-instar *C. javanus*. In contrast, other studies (Table 3) have documented much higher LC_{50} values, ranging from 6 to 100 mg/L, across different instar stages in other chironomid species, including *C. javanus*, *C. kiiensis*, and *C. riparius* (Ebau et al., 2012; Im et al., 2019). The high toxicity of Pb to aquatic invertebrates might be associated with

enzyme inhibition and the induction of oxidative stress through the generation of reactive oxygen species (ROS), ultimately leading to cellular damage and mortality.

For copper, the LC_{50} observed in the control water of this study (0.927 mg/L) is comparable to previously reported values. Dornfeld et al., (2009) reported a 48 h LC_{50} of approximately 1.0 mg/L for first-instar *C. riparius*, while Peck et al., (2002) reported a value of 0.9 mg/L for *C. crassiforceps* at pH 6. However, the present LC_{50} is higher than

Table 3. Acute toxicity (LC₅₀) 48 h of Cd, Cu, Pb, Al, and Zn for *Polypedilum nubifer* under control and UFB water

Metals	Control (mg/L) (95% confidence interval)	UFB (mg/L) (95% confidence interval)
Cd	>2	>2
Cu	0.92 (0.67–1.27)	1.33 (1.11–1.67)
Pb	0.84 (0.75–0.93)	1.06 (0.99–1.13)
Al	>10	>10
Zn	>10	>10

that reported for fourth instar *C. javanus* (0.46 mg/L) (Shuhaimi-Othman et al., 2011). Compared to Pb, Cu toxicity may be moderated by physiological regulation mechanisms because Cu is an

essential trace element that organisms can actively regulate within a certain range. Nevertheless, elevated concentrations or prolonged exposure to Cu can still lead to severe toxic and lethal effects on aquatic organisms (Joachim et al., 2017).

The LC₅₀ values for the acute toxicity tests of Al and Zn to *P. nubifer* were higher than 10 mg/L. Previous studies have already reported 48 h Zn LC₅₀ values greater than 25 mg/L in first-instar *C. riparius* (Bécharde et al., 2008), while Khangarot and Ray (1989) documented a 48 h LC₅₀ of 11.8 mg/L for third-instar *C. tentans*. Meanwhile, limited research has been conducted on the LC₅₀ of Al in chironomids, Shuhaimi-Othman et al., (2011) reported a 48 h LC₅₀ of 10.53 mg/L in fourth instar *C. javanus*. Zinc is an essential micronutrient involved in numerous enzymatic processes,

Table 4. Comparison of acute toxicity values of Cd, Cu, Pb, Al and Zn for chironomid

Metal	Species	Instar/age	Duration (h)	LC ₅₀ (95% CI) mg/L	Reference
Cd	<i>Chironomus riparius</i>	4 th	48	>100	(Im et al., 2019)
	<i>Chironomus riparius</i>	1 st	24	9.38	(Bécharde et al., 2008)
	<i>Chironomus kiiensis</i>	1 st	48	44.95 (21.68–942.33)	(Ebau et al., 2012)
	<i>Chironomus riparius</i>	3 rd –4 th	48	1106 (1001–1211)	(Gillis and Wood, 2008)
	<i>Chironomus riparius</i>	3 rd	48	465.14 (323.23–603.22)	(Lee et al., 2006)
	<i>Chironomus riparius</i>	2 nd	48	2.62 (1.8–3.8)	(Watts and Pascoe, 2000)
	<i>Chironomus tentans</i>	2 nd	48	9.34 (3.4–25.3)	(Watts and Pascoe, 2000)
	<i>Chironomus javanus</i>	4 th	48	0.13 (0.05–0.24)	(Shuhaimi-Othman et al., 2011)
	<i>Polypedilum nubifer</i>	1 st	48	>2	This study
Cu	<i>Chironomus riparius</i>	1 st	24	2.09 (1.57–2.96)	(Bécharde et al., 2008)
	<i>Chironomus tentans</i>	Not reported	48	31.8 (25–38.6)	(Warrin et al., 2009)
	<i>Chironomus riparius</i>	1 st	48	1 (0.61–1.6)	(Dornfeld et al., 2009)
	<i>Chironomus crassiforceps</i>	3 rd	48	0.9 (0.84–0.95) at pH 6	(Peck et al., 2002)
	<i>Chironomus tentans</i>	10–11 Day	48	1.1365	(Mastin and Rodgers, 2000)
	<i>Chironomus javanus</i>	4 th	48	0.46 (0.24–0.80)	(Shuhaimi-Othman et al., 2011)
	<i>Polypedilum nubifer</i>	1 st	48	0.92 (0.67–1.27)	This study
Pb	<i>Chironomus javanus</i>	1 st	48	6.96 (4.38–9.69)	(Ebau et al., 2012)
	<i>Chironomus riparius</i>	4 th	48	>100	(Im et al., 2019)
	<i>Chironomus riparius</i>	1 st	24	0.61 (0.26–1.15)	(Bécharde et al., 2008)
	<i>Chironomus kiiensis</i>	1 st	48	66.21 (53.33–88.53)	(Ebau et al., 2012)
	<i>Chironomus javanus</i>	1 st	48	50.27 (37.44–75.76)	(Ebau et al., 2012)
	<i>Chironomus javanus</i>	4 th	48	6.53 (3.86–25.72)	(Shuhaimi-Othman et al., 2011)
	<i>Polypedilum nubifer</i>	1 st	48	0.84 (0.75–0.93)	This study
Al	<i>Chironomus javanus</i>	4 th	48	10.53	(Shuhaimi-Othman et al., 2011)
	<i>Polypedilum nubifer</i>	1 st	48	>10	This study
Zn	<i>Chironomus riparius</i>	1 st	24	>25	(Bécharde et al., 2008)
	<i>Chironomus tentans</i>	3 rd	48	11.80 (8.27–15.23)	(Khangarot and Ray, 1989)
	<i>Chironomus javanus</i>	4 th	48	8.71	(Shuhaimi-Othman et al., 2011)
	<i>Polypedilum nubifer</i>	1 st	48	>10	This study

and aquatic organisms generally possess efficient homeostatic regulation mechanisms that reduce acute toxicity unless concentrations are extremely high (Muyssen et al., 2002; Rainbow and Luoma, 2011). Likewise, aluminium toxicity is largely influenced by water chemistry, particularly pH, and is typically lower near neutral conditions, where less bioavailable forms of aluminium are more common (Rodriguez et al., 2019). These results suggest either relatively high intrinsic tolerance of *P. nubifer* under the tested conditions or that acute mortality may not represent the most sensitive endpoint for these metals. Longer exposure durations or sub-lethal endpoints may be required to detect biologically meaningful effects.

Effects and mechanisms of UFB on metals toxicity in *Polypedilum nubifer*

The application of UFB revealed the reduced acute toxicity of Pb and Cu to *P. nubifer* larvae compared to the control water. This reduction is likely attributable to specific physicochemical interactions occurring in the UFB-modified water, as shown by the particles size and negative zeta potential. Ultrafine bubbles typically possess a negatively charged surface, which enables electrostatic attraction of positively charged metal cations such as Pb^{2+} , Cu^{2+} , and Cd^{2+} (Hewage et al., 2021). Adsorption of these cations onto bubble surfaces and the promotion of metal complex formation may decrease the free metals ions from the bulk solution, thereby reducing their interactions with biological membranes and inhibit their uptake by the larvae (Guo et al., 2025; Singh et al., 2021). Supporting this mechanism, Huang, Nhung, Wu, et al., (2023) demonstrated that adsorption of Cd^{2+} onto nanobubbles in nutrient solutions reduced Cd bioavailability and accumulation in rice seedlings. Similar adsorption-driven reductions in metal uptake may occur in aquatic invertebrates, where toxicity is strongly correlated with the activity of free metal ions.

Furthermore, metal exposure commonly induces the overproduction of reactive oxygen species (ROS), leading to oxidative stress and cellular damage. Ultrafine bubbles may also influence organisms by upregulating antioxidant enzymes, which helps mitigate the excessive oxidative stress induced by pollutants. By strengthening this cellular defense system, UFB indirectly assist organisms in clearing excess ROS. Previous research reported that hydrogen nanobubbles

mitigated Cu-induced oxidative toxicity in *Daphnia magna* and *Chlorella* (Fan et al., 2020; Liu et al., 2021) by reducing ROS levels. Although the present study did not directly quantify oxidative stress biomarkers, similar antioxidant-related mechanisms may contribute to the reduced Pb and Cu toxicity observed under UFB conditions.

CONCLUSIONS

This study provides new evidence that UFB water can influence the toxicity of dissolved metals to aquatic invertebrates. The results demonstrate that UFB treatment reduced the acute toxicity of Pb and Cu to first-instar *P. nubifer*, suggesting that UFB-induced physicochemical changes in water can alter metal bioavailability and potentially by the enhancement of antioxidant defenses, mitigating reactive oxygen species-induced oxidative stress. While oxidative stress biomarkers were not directly measured in this study, previous literature supports the plausibility of this mechanism. The findings support the hypothesis that electrostatic interactions between negatively charged bubble surfaces and dissolved metal ions may decrease the biologically available metal fraction and thereby mitigate toxicity.

Importantly, this study establishes first-instar *P. nubifer* as a sensitive and practical model organism for evaluating metal toxicity under modified water conditions. By linking UFB physicochemical properties with biological responses, the present work fills an important knowledge gap regarding the ecotoxicological implications of UFB technology.

Nevertheless, the mechanisms underlying the toxicity-reducing effects of UFB require further investigation, particularly with respect to oxidative stress biomarkers, metal speciation, and long-term or chronic exposure scenarios. Future studies integrating chemical speciation analysis and physiological biomarkers will be essential to fully elucidate the role of UFB in aquatic environmental management and water treatment applications.

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