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COMBINATION IMPACT OF pH AND TEMPERATURE ON THE TOXICITY OF LEAD ON ZOOPLANKTON IN THE CONTEXT OF GLOBAL WARMING

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Abstract. Heavy metal pollution in Hanoi's urban lakes has become one of the significant environmental issues in the last decades. Research on the toxicity of Pb is necessary due to the extent of its harm to aquatic ecosystems. The toxicity of Pb to aquatic organisms (EC50 values) depends on different environmental conditions. The study was conducted to evaluate the effect of temperature on Pb's toxicity on the test organism *Moina Dubia.* *Moina Dubia* belonging to the Cladocera group (Cladocera), an indigenous creature collected at Lake Hanoi. *Moina Dubia* was cultured under laboratory conditions and has been used to conduct EC50 determination for Pb. *Moina Dubia* grew and developed well in the laboratory and was suitable as a test organism in toxicological studies. A survey from 24 to 28 ^{o}C water temperature conditions showed that the EC50 value decreases from 1402 to 775 ug/l. The combination of temperature and pH strongly affects the toxicity of Pb on *Moina Dubia.* The apparent impact of water temperature and on the toxicity of Pb showed that global warming might gradually lead to potential biodegradation in freshwater ecology.

Keywords: metal toxicity, macro-zooplankton, *Moina Dubia*, urban lakes.

Classification numbers: 3.1.2, 3.4.2, 3.6.1.

1. INTRODUCTION

Trace metals (Hg, Ni, Pb, Cd, Pt, Cu) widely distributed in fresh ecosystem mostly come from untreated domestic and industrial wastewater [1]. These metals imply species bio-diversity of many aquatic organisms, including invertebrates [2] and fauna [3]. Among metals, lead (Pb) is a non-essential and widespread metal in aquatic ecosystems. Pb is used in lead-acid battery manufacturing, lead bullets, painting, plumbing, printing, lead mining and refining, smelting. The adverse effects of Pb to aquatic organisms have been observed extensively, such as the reduced survival and impairments of reproductions in *Daphnia Magna* [4], changes in the morphology in fathead minnow (*Pimephales promelas*) [5], and lifespan of *Daphnia pulex* [6].

Toxicology of the environmental pollutants is a dependency of water characteristics, such as pH, hardness and dissolved organic matter (DOM) [7, 8, 9]. These factors mostly affect the

circumstances around metals and their bioavailability. Among those factors, pH is the primary factor influencing the metal toxicology. pH can affect the distribution of metal species [9] and the exchangeable sites [8], which can inhibit the pathway of metal entering the organism. Contamination of rivers and lakes by drainage from untreated wastewater and industrial wastewater would change the water body; therefore, a study about the effect of pH on the metal Pb toxicology needs to be considered in the toxicology studies.

In the context of global warming associate with the raising temperature (currently 0.60 degrees C) [10], the temperature can speed up the metabolism [11] and decrease the oxygen content in water [12], which can make the aerobic metabolism more stressful. As predicted, 2016 - 2035, the global average temperature will increase by 0.3 ÷ 0.7 ^{o}C [13]. In the whole country Viet Nam, according to the Ministry of Natural Resource and Environment scenarios, the average temperature during the period of 1958 - 2014 increased by about 0.6 ^{o}C; in the period (1985 - 2014), the temperature increased by 0.42 ^{o}C [14]. The rising in temperature and metal pollutants in the water body may cause biodiversity reduction, especially zooplankton, a critical species in the food chain of lakes and rivers in Viet Nam. One urgent question is how temperature and pH can affect the metal toxicology on zooplankton, which is the leading cause of biodiversity reduction.

In Viet Nam, although a study of the toxicity of heavy metal on aquatic organisms published by many authors [15, 16, 17], the study about the integration effect of multiple stressors on the aquatic organism is still limited. This study was conducted to determine the integration effect of temperature and pH on the acute toxicity of lead to *Moina Dubia* in different water temperatures in climate change scenarios. Before that, we surveyed the abundance of macrozooplankton of urban lake to see the effect of water characteristic effect on macrozooplankton species composition in natural conditions.

2. MATERIALS AND METHODS

2.1. Sampling location

Our study conducted zooplankton sampling and analysis in the two urban lakes, Truc Bach lake and Bay Mau lake, which has a function to maintain biodiversity in the freshwater body also drainage water, preventing the flooding for the urban area but suffered the untreated wastewater influence. Bay Mau Lake is a freshwater lake located in Thong Nhat Park in Hai Ba Trung District in Hanoi. The surface area of the lake is about 28 hectares. Sampling coordination was 21,011465°N; 105,843251°E. Truc Bach Lake is a lake in Ba Dinh District, Hanoi City, originally a part of West Lake, with 22 hectares. Sampling coordination was 59°59′N 189°59′E.

2.2. Water monitoring and sampling

The process of sampling observation and preservation was carried out following ISO 5667-4: 2006 - Water quality - Sampling - Part 1: Guidance on sampling techniques and TCVN 5994: 1995 (ISO 5667-4: 1987) - Water quality - Sampling - Guidance on sampling of natural and artificial ponds and lakes. Field measurements included water temperature (°C), pH (pH Meter DREL/2010), dissolved oxygen (WTW Oxy 330) Water temperature and pH were measured at sites. Chlorophyll-a (Chl-a) had been analyzed according to standard methods (SMWW 10200 H). The verification of Pb concentration in water was verified by ICKP MS Elan 9000 (detection limit = 0.1 µg Pb L^{-1}). Monitoring had been performed twice a month, from July

2019 to May 2020. Each sample had been taken three replicates and mixed to make a combined sample.

2.3. Zooplankton collecting and identification

The macrozooplankton samples were collected randomly. Samples were collected at the surface layer at each location, about 0.5 m below the water surface in the morning hour (7-9 AM). Samples were collected using a plankton net (200 µm) to obtain the macrozooplankton. The samples were preserved in dark plastic bottles with 5 % formalin in the site. The identification was conducted in the lab.

50 ml of each sample was brought in a 100 ml flat bottom glass flask. The sample was stirred in a zigzag motion; a 1 ml sub-sample was then transferred into a 1-ml Sedgwick Rafter Counting Chamber to determine the species composition and density of macrozooplankton. All the zooplankton in the counting chamber was observed and identified using standard keys [13] and counted using an inverted microscope (Nikon, Japan, model: TMS-F) with high power (× 40). The zooplankton was then identified up to the genus Qualitative Analysis of Zooplankton in Different Types level and enumerated by the identification key [18]. Quantitative analysis of carried out by mean of zooplankton number recorded and expressed in different types of the pond like culture, household and unused pond numerically per liter of water.

2.4. *Moina Dubia* laboratory culture

Moina Dubia is a small crustacean (0.3 - 0.7 mm) belonging to the Cladocera group. *M. dubia* normally live in high density in shallow ponds, lakes, ditches, streams, and swamps and is an indigenous aquatic organism in all lakes in Ha Noi. *Moina Dubia* had been selected as the target organism for the lead toxicity test in this study.

Moina Dubia was collected from Truc Bach Lake, a small urban and highly eutrophic lake in Ha Noi and was transferred to the laboratory. *M. Dubia* were isolated using a Paster pipette and then were cultured at the laboratory condition. The temperature and pH were kept stable at 24 ± 2 °C and 7.5, respectively. *M. Dubia* were acclimated to the laboratory condition for one month (ca. 5 - 7 generations) before using acute toxicity tests. The acclimation period was to minimize the environmental background from the lake [19]. The culture medium was prepared based on the Basal medium following the protocol [20]. *M. Dubia* was fed ad libitum with *Chlorella Vulgaris. C. Vulgaris* was centrifuged and washed to remove culture nutrients before feeding at a density of 1×10^6 cells.mL^{-1}. In the culture, the density of *M. Dubia* was below 150 individuals/L. The culture was maintained under the ambient light and photoperiod (12 h light: 12 h dark cycle). The newborn *M. Dubia* (< 24 h old) were selected randomly from the culture for toxicity tests.

2.5. Toxicity test

To prepare the exposure solution, Lead nitrate $Pb(NO_3)_2$ (purity > 98 %, Merck, Germany) was used to make a stock solution of 1 mg Pb.L^{-1}. The exposure solutions of 0, 100, 200, 300, 500, 600, 700, 800 and 1000 µg Pb.L^{-1} were obtained by diluting the stock solution with the culture medium of *M. Dubia*. The real exposure Pb was verified by ICKP MS Elan 9000 (detection limit = 0.1 µg Pb.L^{-1}).

The experiment was conducted in six different pH levels: 7, 7.2, 7.5, 7.7, 8, 8.3. We prepared the soft water used as the water medium (pH, 7.7, total hardness: 120 $mg.L^{-1}$ as $CaCO_3$, bicarbonate and carbonate alkalinity 104 and 0.49 $mg.L^{-1}$ $CaCO_3$) by dilution of hard water with distilled water. Different pH levels were prepared by adding 1N solutions of analytical grade NaOH or HCl in the exposure containers. After adding the desired volume of Pb-stock solution to pH adjusted water medium, the test solutions were aged for 12 h. During this period, pH levels were adjusted by adding dropwise either dilute NaOH or HCl if the pH deviated from nominal by more than 0.1 units. After this period (12 h), fishes were released to the glass jars. pH was measured daily in the exposure containers.

The toxicity test was performed in different water temperatures: 24, 26 and 28 ℃. A thermostat with a temperature ranges from 0 ℃ to 60 ℃ was applied to control the temperature of solutions

The acute test was performed according to the US.EPA [18]. Briefly, the *M. Dubia* neonates (< 24 h old) from the second or third brood were used for the acute test. The acute test had ten replicates. The neonates were fed (*Chlorella Vulgaris*) *ad libitum* for 2 h before the test, but not during the exposure time. The neonates were exposed to Pb^{2+} solutions, from 0 to 1000 µg $m.L^{-1}$. Ten neonates were introduced into a flask containing 40 mL of ISO medium in each concentration, and four replicates were prepared. The acute test was conducted in the dark and checked every 24 h. The effect on neonates was confirmed by immobility observed on a microscope. The immobility data was utilized for determining median effective concentration (EC50) via EPA Probit Analysis Program. The estimation of EC50 values was done at the end of the test after 24 hours of exposed Pb.

3. RESULTS AND DISCUSIONS

3.1. Characteristics of water and zooplankton distribution in Hanoi lakes

A wide range of water temperatures from 16 to 35.4 ^oC was measured during the sampling period in the Truc Bach and Bay Mau lakes. The temperature dropped from 15.5 - 16.5 ^oC in the winter but increased to 35.4 ^oC in the summer day (Fig. 1).

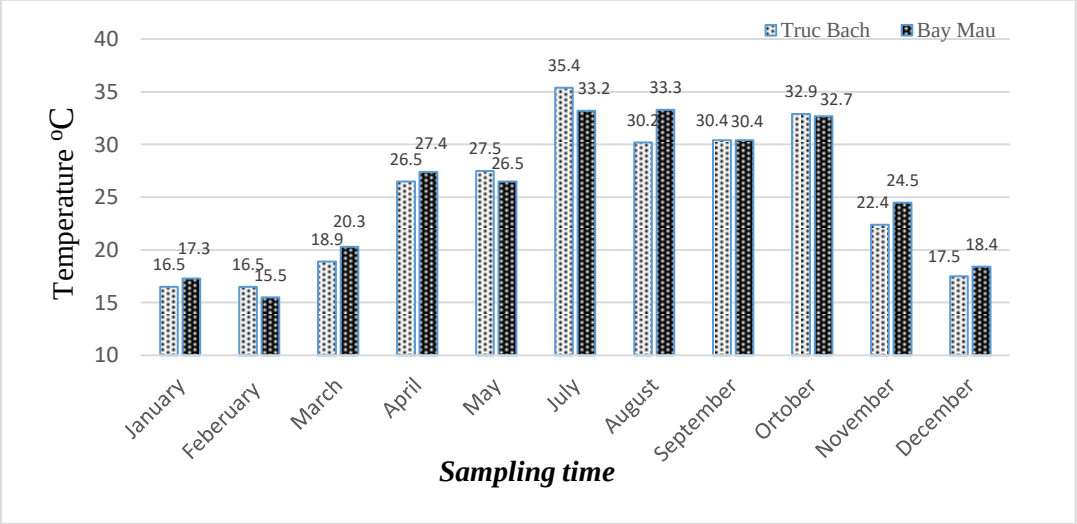

Figure 1. Water temperature variation during the sampling time.

The pH was deviated from neutral to slightly base (7.3 - 8.3). Aquatic organizations would get troubles when living in permanently alkaline environments [21]. Many factors would affect the change in the pH of aquatic bodies first. The bloom of algae, which uptake bicarbonate during the photosynthesis process, can break the H^+ balance in the water, causing the pH variation day and night [22]. This speculation was corroborated by blooming algae in the lake. Second, anthropogenic activities, including discharging untreated domestic wastewater, would also affect the lake's pH. The data on nutrients and chlorophyll-a revealed the Truc Bach and Bay Mau lakes as the eutrophic lake in agreement with a previous study [23]. The blooming of algae would significantly change water characteristics, e.g., high pH and dissolved oxygen (DO). The DO in the Truc Bach lake varied widely from 4.06 to 11.24 (mg/L and in Bay Mau lakes varied from 4.03 to 8.54 ($mg.L^{-1}$). Low oxygen (DO < 4 $mg.L^{-1}$) is usually caused by the high level of organic substances and the respiration of organisms during the night. High DO during the day was mainly the result of eutrophication [24]. Zooplankton in the Hanoi lakes was quite diverse. The standard classes of zooplankton found in Truc Bach and Bay Mau Lakes included *Moina Dubia*, *Cyclopoida*, and *Calanioda* (Fig. 2).

(a) *Moina* (b) *Cyclopoida* (c) *Calanoida*

Figure 2. Popular zooplankton in lakes of Ha Noi.

Table 1. Species composition of macrozooplankton in Truc Bach and Bay Mau lakes.

Lakes	*Moina Dubia (Individual/100l)*	*Cylopida Copepod (Individual/100l)*	*Calanoida Copepod (Individual/100l)*	*Daphnia Dubia (Individual/100l)*
Truc Bach	3034 ± 35	1169 + 55	0	0
Bay Mau	26 ± 3.2	41 ± 6	2 ± 0.5	24 ± 1.3

Densities of species were presented in Table 1. The result indicated that zooplankton density in the Truc Bach lake was much higher than those in the Bay Mau lake. We found that the zooplankton density in the lake is associated with the algae density of the lake. The chlorophyll-a concentration of Truc Bach lake was much higher than that in the Bay Mau lake. The result also showed the high-density *Moina Dubia* and *Cyclopida* in both lakes while there is a lack of *Calanoida Copepod* and *Daphnia Dubia* in Truc Bach Lake. *Calanoida Copepod* and *Daphnia Dubia* usually present in the clean water; therefore, the absence of those species indicated that Truc Bach and Bay Mau are being polluted.

Among these zooplankton classes, *Moina Dubia* was found ubiquitous in all monitoring sites and can survive in nature even at the high water temperature of 32 - 34 ^oC.

3.2. Dose-Response curve of Pb^{2+} on *Moina Dubia*

The high concentration of lead in the solution will increase the pressure of the organism's lead absorption. Because lead is similar in structure to Ca, a high concentration of lead in a solution creates a greater probability of Pb being absorbed into the body. Lead is toxic to the organism, causing a high death rate. According to observational experiments, *M. Dubia* has symptoms of paralysis active before immobilization. According to studies, Pb ingestion can affect the nervous system or kidney function.

The toxicity of Pb (II) slightly decreased with an increase in pH (Fig. 3). This well agrees with the works of other researchers [25]. When pH decreases, the toxicity of Pb(II) increases due to the increase of toxic free ions [26]. The results show the value of EC50 of lead also increases as the pH increase (Fig. 3). The phenomenon can be explained by the active form of the lead in the solutions. The unique form of lead in the water is Pb^{2+}. When pH increase closed to the alkaline condition, lead exists in the form of hydroxide complexes or other complexes, causing a decrease of flexible lead Pb^{2+}, leading to reduced toxicity on the experiment organism [26]. The effect of pH on the Pb is far more complex, and it would make relative to the species-specific [27]. When pH variation, the hydrogen ion (H^+) concentration in the water that may exert its effect either directly by altering metal uptake of *Moina Dubia* to Pb. Therefore, it would affect to the Pb toxicology [28]. The interaction between pH and Pb toxicity is likely to be masked by other related factors such as hardness and alkalinity [25], which also vary with pH.

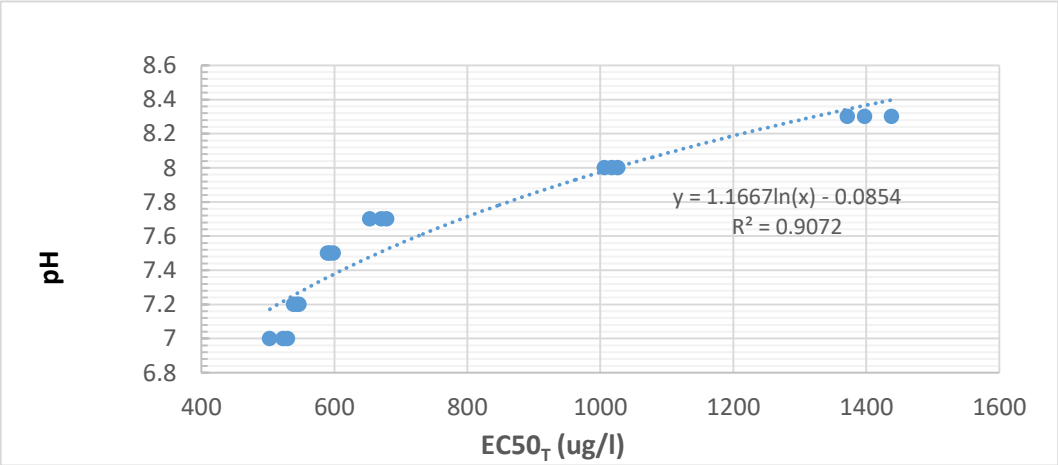

Figure 3. Impact of water pH on EC50-24h of Pb^{2+} on *Moina Dubia.*

3.3. Combination effect of temperature and pH on the acute toxicity of Pb on *Moina Dubia*

Acute toxicity tests have been performed at a temperature of 24, 26 and 28 ^oC in different pH range. The results showed an increase in water temperature caused lower EC50 values in all pH ranges (Fig. 4). That means more severe toxicity of lead on *M. Dubia* is expected to increase water bodies' temperature.

Temperature is an important environmental variable affecting the physiology and metabolism of aquatic organisms and can potentially affect the rate and pathway of the uptake and elimination of contaminants. In the condition of climate change all over the world, the average temperature in the ambient environment increases also cause the increase of the

temperature in the water column. In freshwater ecosystems, warming can induce variations in the species composition, body size, and abundance of plankton communities [28].

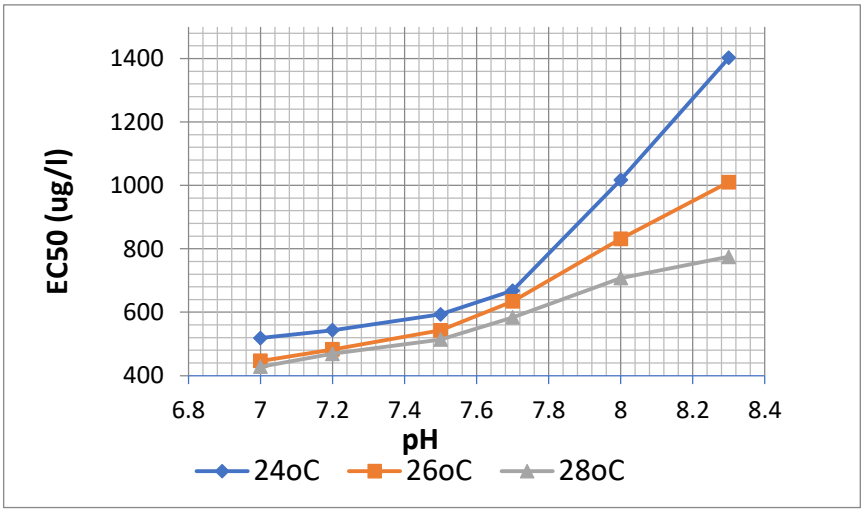

Figure 4. Impact of water temperature on EC50-24h of Pb^{2+} on *Moina Dubia.*

The effect of pH and temperature on the toxicity of other heavy metals (Cu) on cladoceran species has been mentioned in the studies of many authors and also confirmed the increased toxicity as temperatures rise (in range 20 - 25 ^{o}C) [29, 30]. The result showed that, at the same pH, EC50 of Pb conducted in 24 ^{o}C was higher than that in 26 ^{o}C and 28 ^{o}C. This result was explained by the effect of temperature on the metabolic rate of *M. Dubia*. In the higher temperature, the metabolism rate of *M. Dubia* will increase, which leads to more Pb being adsorbed into the body *M. Dubia* [28]. Effects of temperature are also more substantial on Pb-induced oxidative stress, which lead to disorder of the enzyme activities in the body of *M. Dubia* then increases the Pb toxicity [29]. As from the study, the difference in EC50 of Pb at 24 ^{o}C was smaller than at 28 ^{o}C. More specifically, at the same pH = 7, the EC50 of Pb conducted in 24 ^{o}C is approximately equal to that at 26 ^{o}C. While at the pH = 8.3, the EC50 of Pb at 24 ^{o}C was double that at 28 ^{o}C, which means Pb's toxicity accelerated when temperature increased. The temperature in the water environment can affect the toxicity, depending on the type of pollutants, the species, depending on each case's specific conditions. For acute toxicity, the duration of resistance to a lethal dose of the poison will change as the temperature changes, and the time length would depend on the species or types of toxicants. Therefore, understanding the impact of thermal stress in aquatic systems is essential as aquatic animals are subject to seasonal and daily temperature fluctuations [30]. Various cladoceran species usually play a crucial role in the food web of various freshwater systems. There were many evidences to show that climate change may influence cladoceran (especially *Daphnia* species) population dynamics, consequently altering both predator-prey interactions and the efficiency of algal biomass control in these ecosystems [30, 31].

The average temperature growth rate per decade is about 0.10 ^{o}C, according to the current climate change scenario in Viet Nam [31]. Warming of water may have significant direct and indirect effects on zooplankton biology and ecology via its influence on their life-history processes (metabolism, growth, reproduction, and DNA gene mutation) and their properties habitats [29]. Global warming, thus, may have a long-term impact causing biodiversity degradation in aquatic ecology.

4. CONCLUSIONS

The present work indicated that pH strongly affects the acute toxicity of Pb^{2+}. The toxicity of Pb^{2+} was lower when the experiment was conducted at a lower temperature (24 ^{o}C) but increases at a higher temperature (28 ^{o}C). The combined effect of temperature and pH will boot the toxicity of Pb^{2+}, which may threaten the survival of the *M. Dubia* living in the fresh ecosystem.

According to the global warming scenario, the global average temperature will increase in the coming years, leading to gradually raising of water in aquatic bodies. Temperature rise in aquatic bodies may partly contribute to the low production of zooplankton in freshwater. This finding can be an essential contribution to biodegradation in freshwater ecology due to global warming in Viet Nam. There are more studies need about the multiple stressors effect on the aquatic organisms to understanding better consequences of global warming effects.

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