DETERMINATION OF THE ACUTE TOXICITY OF NICKEL (Ni) IN WATER ENVIRONMENT TO ZEBRAFISH AT DIFFERENT pH LEVELS

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ABSTRACT

The environment has been facing numerous challenges from hazardous wastes, which discharged into water bodies, especially metals which threaten the aquatic life. Metal contents of these hazardous wastes are a threat to aquatic life. The disposal of toxic wastes, like heavy metals (Cd, Ni, Hg, Pb, As) into the water environment adversely affects the aquatic ecosystem and eventually find its way to humans through the food chain. This is the most probable root of potential diseases, affecting human health. Numerous fish species were widely applied to assess the status of aquatic ecosystems. In this study, *Danio rerio* most commonly known as the zebrafish was used as an bioindicator. This paper aims to determine the acute toxicity of nickel (Ni) at different concentrations (0.2, 0.36, 0.648, 1.166, 2.099 mg/L) to zebrafish in a water environment at different pH levels (6.2; 6.8; 7.3; 7.7). Nickel sulfate hexahydrate ($NiSO_4 \cdot 6H_2O$) was used as the Ni source for performing acute toxicity tests (after 48-h exposure [48-h LC_{50}]) with 13-day old zebrafish under the same hardness, dissolved organic carbon (DOC) and alkalinity. The study results highlighted that the nickel acute toxicity to zebrafish declined as pH increased from 7.3 to 7.7, which means the LC_{50} increased in value as 384.63 and 607.57 µg/L, respectively. The results obtained from this study was expected to bring useful information to find out the exact cause of the increasing mortality of fish.

Keywords: Acute toxicity, LC_{50}, ecological toxicology, nickel, zebrafish (*Danio rerio*).

1. INTRODUCTION

The rapid industrialization of developing countries like India and Vietnam has led to significant increases in solid and liquid waste matter being discharged into nearby natural bodies of water (rivers, lakes, seas), which causes various environmental problems including the threat to aquatic flora and fauna [1]. Heavy metals found in sewage that have been disposed by industrial establishments, in particular, adversely affect the aquatic ecosystem. These trace heavy metals find its way up the food chain thereby causing potential diseases that eventually affect human health [2]. Among all kinds of animals, fishes are the inhabitants that cannot escape from the deteriorative effects of these pollutants [3]. As such, fishes are widely used to assess the status of aquatic ecosystems and therefore act as bioindicators.

Polluted rivers, lakes and seas containing high amounts of heavy metals are considered a threat to public health most specially to the fish consuming public [4]. This has, in fact, already happened in 2016 when the sea of four central coastal provinces of Vietnam was found to be

contaminated by wastewater discharged from Ha Tinh Formosa company. Damages has been estimated to be over USD 500 million to the Vietnamese economy and has far reaching implications to various aspects of the country - societal stigma, marine environment degradation, and declining fish consumption, among others. Through bioaccumulation and non-biodegradable properties in food ingested by aquatic creatures [5], these heavy metals find its way up the food chain as humans continue sourcing food from these contaminated bodies of water. The accumulation of metal is an important tool for finding the influences of metal in an aquatic ecosystem and showing its adverse effect on aquatic organisms [6].

Determining the environmental toxicology offers a lot of applications to address practical issues. For one, results from the detections can be used by government agencies to set permissible exposure limits for effective contamination control and thus reduce further damage to the marine ecosystem. This is a fantastic way to achieve sustainable development. Combined with soil testing, water and air sampling to identify pollutants, findings can be used to gain a full understanding of the adverse results of pollution parameters to the health of native species. Vietnam, a developing country, is currently confronted with a slew of environmental problems including the degradation of of aquatic ecosystem, pollution discharged by various industries and ever growing number of vehicles, and the deterioration of human health with diseases like cancer. With these looming challenges, researches about ecological toxicology could help create reliable scientific basis in establishing appropriate discharge limits for these waste materials. Second, ecological toxicology provides evidence that determines the causes of aquatic mortality in the aquatic environment. Case in point is the recorded number in 2016 of serious incidents traced from wastewater discharged into the water environment that caused massive fish kills, which affected the economy, society and the Vietnamese public health [7]. As it is not easy to identify the exact cause of these incidents, controversies often arise in the scientific community, management agencies as well as in the public sphere. In Vietnam, most of the environmental incidents detrimentally impact aquatic life and scientific basis is thus important to determine the cause of the incident through quality control [8-9]. Indicators of toxicology such as LC_{50}, EC_{50}, NOEC, LOEC should be determined considering different substances for each type of fishes. However, studies on the levels of toxicity parameters in industrial wastewater and its effect on aquatic creatures are limited, leading to a insufficient information to fully evaluate aquatic life conditions.

Table 1. Concentrations of heavy metals in wastewater from an electroplating park in Ningbo [10]

Metal	Raw wastewater concentration (ppm)	pH
Nickel	200.93	9.8
Chromium	257.76	2.9
Copper	152.46	1.2
Cr^{6+}	111.6	2.9

A detailed look at Table 1 above, shows that wastewater from an electroplating company in Ningbo - China contains a large amount of chrome, nickel, copper, and Cr^{6+}. Metal ions can influence adversely to humans, animals, plants and the environment; therefore, it is necessary to deal with electroplating wastewater to remove these hazardous materials. It is noteworthy to mention the discharge of the especially high nickel concentration (200.93 ppm) from processing as plating wash water, plant wash water and equipment cooling and wash water comprising the principal sources of wastewater. This is alarming as nickel is a xenobiotic nonbiodegradable chemical pollutant whose toxicity is dangerous to human health [11]. One

of the aforementioned indicators, LC_{50}, is an indicator which can be applied to evaluate the toxicity of substances to aquatic organisms, in this case, the zebrafish.

Acute exposure has been tested to various types of fish, with an experimental duration of 24, 48, and 96 hours. The presence of *Danio rerio* was investigated with a negative outcome about mortality inhibition of embryonic development delay [12].

Zebrafish (*Danio rerio*) is a popular tropical freshwater fish, member of the family Cyprinidae. It has advantages for ecological toxicology research including their size, rapid maturation, and fecundity, among others. Besides, zebrafish is widely used as an indicator organism in aquatic ecosystems and other fields such as molecular biology, developmental biology, and genetics, to name a few. It is considered as an important vertebrate, which is widely used in scientific research and is one of the first cloned vertebrates (cloned frogs a decade ago). They are easily maintained in tanks and kept at a temperature of about 26 °C (at temperatures above 31 °C and below 25 °C, zebrafish will not breed and lead to abnormal growth). Males and females can be easily distinguished with the naked eye. Females' stomach swell when carrying eggs while the male is slimmer with an easily identifiable orange line between the blue lines (especially visible in the lower abdomen). Moreover, the female weight is approximately 0.65 ± 0.13 g and the male is 0.5 ± 0.1 g, fish length on average 3.8 ± 0.3 cm and 4.5 ± 0.5 of maximum size.

The fact that metals are nonbiodegradable and can accumulate in the environment make them deleterious to the aquatic organisms and consequently to a human being who consumes fish as a food source. Besides, Shabnam and Badre (2015) investigated the toxicity of dimethoate on adult, the results indicate that embryo and fingerlings of zebrafish reduced significantly in the viability and survival of fingerlings [13]. States that the toxicity of Ni was inversely related to water hardness between hardness values of 20 and 150 mg/L (as $CaCO_3$) [14]. At low hardness (21 mg/L), the 96-h LC_{50} values for Ni sulfate and Ni chloride were 0.36 and 0.40 mg Ni/L, respectively. In terms of high hardness (52 mg/L), the 96-h LC_{50} values for Ni sulfate and Ni chloride were 1.68 and 1.57 mg Ni/L, respectively. These results showed that the chemical form of Ni did not affect significantly Ni toxicity. Pyle *et al.* (2002) also found that the 96-h LC_{50} was 0.50 and 2.27 mg Ni/L at the hardness of 40 and 140 mg/L, respectively [15].

Furthermore, according to Nabinger *et al*. (2018), they conducted a test on adult zebrafish, which were exposed to $NiCl_2$ concentrations (0.025, 2.0, 5.0, and 15.0 mg/L) [16]. These results illustrate that exposure to nickel in the early life stages of zebrafish lead to morphological alterations. However, this research did not mention the hardness factor.

Thirty larvae of zebrafish (*Danio rerio*) were used, to evaluate the toxic effect of Ni 15, 20, 25, 30, 35 mg/L and the 96-h LC_{50} for larvae 31.13 mg/L, but without mentioning these parameters that impact to nickel toxicity as hardness, alkalinity and others [13].

2. MATERIALS AND METHODS

2.1. Fish culture

Zebrafishes underwent a domestication period of 2-3 months until they were able to adapt to controlled laboratory conditions. The adults which were 3 to 4 months old and had a length approx: 2.5 ± 0.2 cm were purchased from various stores, so they have a better genetic diversity. To achieve the same existent scenario, a tank (200 L) with dechlorinated tap water was used to domesticate fishes, and with the water placed was under constant mechanical and biological filtration. This ensured that the zebrafishes would grow normally [14].

The culture room was maintained at a temperature of $26\ °C \pm 1\ °C$ with a photoperiod of 12:12 hours light: dark [12]. Fish were fed two times per day with flake food, Artemia salina

or Tetramint food [14]. A recirculation system was installed where in the water flows through activated carbon to remove dissolved metabolites and the DO was maintained at 7 mg/L, pH 7.2, conductivity 552 μScm^{-1}, salinity 0.26 ppt, TDS 270 (mg/L) [18]. They were fed two times per day and excess food, as well as fecal material, was removed from the bottom of the tanks at least twice a week by siphoning.

Zebrafishes were observed for signs of disease, stress, physical damage, and mortality. Dead and abnormal specimens were removed as soon as it was discovered. A daily record of feeding, behavioral observations, and mortality was maintained. It took some time to achieve and maintain the mortality rate of 5-10% during the first 48 hours in a holding tank, because some of zebrafishes refused artificial food, leading to starvation and death.

After about two to three months, zebrafishes were domesticated with the following controlled conditions: temperature 26 °C ± 1 °C , pH 7.2, DO 6 ± 1 mg/L, TDS 60 mg/L, EC 200 mg/L, alkalinity of 60 mg/L, hardness of 45 mg/L, NO_2^- 0.003 mg/L, NH_4^+ 0.14 mg/L, NO_3^- 16.04 mg/L, SO_4^{2-} 2.26 mg/L, Cl^- 10.2, DOC 3.5 mg/L. Male and female were paired, separated, and placed into monitoring chambers to spawn.

2.2. Toxicity tests

The study aimed to evaluate nickel toxicity in a water environment for zebrafish at different pH values like 6.2, 6.8, 7.3 and 7.7, respectively. Zebrafish were inspected at intervals of 24 and 48 hours for exposure. The 48-h static-renewal tests were conducted according to U.S. EPA methods. All test water was aerated and prepared for at least 24 hours before initiating a test. Toxicity tests were conducted under the same temperature (26 °C ± 1 °C), dissolved organic carbon (DOC), hardness and alkalinity. These parameters as DOC, hardness, and alkalinity, nickel was analyzed before the exposure test. In terms of temperature, it was controlled by air-conditioner and checking the temperature every day in the domestication tank and exposure chambers by a multi-indicator measuring device (Model HandyLab 680).

The use of ordinary tap water as dilution water, was guaranteed as normal for zebrafish development, according to OECD [17, 19] (pH 6.5-8.5, chlorine < 0.5 mg/L, NH_3 < 5 mg/L, NO_2^- < 0.1 mg/L, NO_3^- < 140 mg/L, hardness 25-250 mg/L, alkalinity 50-150 mg/L) and it was dechlorinated and completely treated. Following dechlorination, total residual chlorine was 0.001 mg/L, and it guaranteed the development of zebrafish. Some parameters in water that affect nickel toxicity, as well as normal growth of zebrafish, were inspected before conducting exposure. As the matter of fact, dissolved oxygen and temperature were carried out at daily (DO 6 ± 1 mg/L; temperature (26 °C ± 1 °C)); NH_4^+ 0.14 mg/L; nitrite (NO_2^-) 0.003 mg/L; nitrate (NO_3^-) 16.04 mg/L; SO_4^{2-} (2.26 mg/L); Ni 0.003 mg/L; Cd 0.001 mg/L; hardness 45 mg/L; alkalinity 60 mg/L, chemical oxygen demand (COD) 9.12 mg/L and DOC 3.5 mg/L and then stored it for about two days before initiating a test.

Tests were carried out in 500-mL graduated polypropylene beakers, each containing 250 mL of test medium. The beakers were randomly positioned at a temperature of 26 °C ± 1 °C, with a white light LED and operated by trigger timers on a 12/12 light/dark. Ten zebrafishes were placed to each beaker to receive water. A small volume of the holding water (approximately 5%) was removed by siphoning, and then replaced slowly over a 10 to 15 min period with dilution water. Dead fish were removed daily. Mortality was also recorded until test termination [17, 19].

Thirteen-day-old zebrafish were used in acute toxicity test and Ni sulfate hexahydrate ($NiSO_4 \cdot 6H_2O$) was used to prepare diverse Ni concentration for exposure [12].

2.3. Water quality

Water hardness, pH, alkalinity, and dissolved oxygen were measured at the beginning and end of every test and each treatment. Water samples were analyzed for total Ni, major anions, major cations, and DOC at the beginning and end of each experiment.

The concentration of Ni was determined by using atomic absorption spectrometer (AAS) and physico-chemical parameters, such as ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), pH, dissolved oxygen (DO), COD, total hardness, alkalinity, chlorine, TSS, temperature, SO_4^{2-} were analyzed using standard techniques (APHA, 1999) [20].

2.4. Experimental design

13-day-old zebrafish were collected randomly (they were the most sensitive stage (ISO 12890; 1999)) and were put into six study groups (a control group and five treatment groups) in the experimental tanks with volume 250 mL (10 zebrafishes/beaker). Three replicates were used for each treatment level by Complete Randomized Design (CRD).

The effect of pH on the toxicity of Ni to 13-day-old zebrafish was investigated by conducting 48-h static-renewal toxicity tests at different pH values of 6.2; 6.8; 7.3;7.7 and under the same DOC 3.5 mg/L, and hardness 45 mg/L, and at constant alkalinity of 60 mg/L, as $CaCO_3$. Because DOC, hardness, and alkalinity affect strongly the toxicity of nickel in an aquatic environment, therefore controlling these parameters is necessary to achieve reliable results [14].

Based on national technical regulation on surface water quality (QCVN 08: 2008/BTNMT), permissible limit of nickel is 100 µg/L and according to OECD guidelines for the testing of chemicals [12], the concentration range by allowing a spacing factor of 1.8, the research proposed the concentration range of Ni (µg/L) used to investigate acute toxicity 0; 200; 360; 648; 1166; 2099 with symbols for control and treatments N_0, N_1, N2, N3, N4, N_5, respectively. Normally, only 5 levels of test concentration with the range that does not exceed the coefficient of 2.2 is sufficient to meet statistical requirements [21].

The stock solution of nickel sulfate ($NiSO_4 \cdot 6H_2O$) was prepared by dissolving the appropriate amount of $NiSO_4 \cdot 6H_2O$ as Ni. Zebrafishes were investigated for various nickel levels with exposed within 48 hours.

Zebrafishes were fed before the experiment taken about 2 hours and they were not fed during the experiment. At the end of this study, survival rates of zebrafish were recorded and statistically analyzed to determine LC_{50}.

2.5. Data analysis

LC_{50} value was calculated by using the Probit method from the mortality data. SPSS 22.0 software (SPSS Inc., USA) and Microsoft Excel 2010 were applied to data analysis with 95% confidence intervals. In addition, the extrapolation method from the graph was applied to calculate LC_{50} and was compared to the results from the Probit calculations. Regression analysis was also done to identify correlation coefficients. Generated data was analyzed with a one-way analysis of variance (ANOVA) to determine whether significant differences existed among experimental groups with different pH (6,2 to 7,7) in the same nickel concentration using the SPSS 22.0 tool. The results were presented as average ± SE.

As described in the experimental design (treatments with Ni and pH at the same time), this study should use Two-way ANOVA in steads of one-way ANOVA for data analysis.

The LD or LC values are calculated using 'Probit Analysis', which was initially developed by Finney (1971) and later discussed in detail elsewhere [22].

The following steps are used in the calculation of LD or LC:

The proportions are corrected for control mortality if it is more than 10% using Schneider-Orelli's (1947)

Formula:

$$\text{Corrected mortality (p)} = \frac{\%\ \text{Responded} - \%\ \text{Responded in Control}}{100 - \text{Responded \% in Control}} \times 100$$

Converting corrected proportions (p) to empirical probits (y).

A dose-response curve is drawn using the log10 doses (x) and empirical probits (y) and the regression equation is derived. Empirical probits less than 1 and more than 7 are ignored as they have little and no significance in the estimation of LD or LC.

$$y = 5 + (x - \mu)/\sigma$$

From the equation of the curve and log10 doses, the expected probits (Yi) are derived

From the expected probits (Yi), the expected mortality proportion followed by the expected number of fishes is derived.

3. RESULTS AND DISCUSSION

Thirteen-day old zebrafishes were dosed to a lethal concentration of nickel for the short term of exposure. The average and corrected mortality rate in control and exposed for 24 and 48 hours had been determined and results presented in Table 2. Undoubtedly, what stands out from Table 2 is that pH value affects considerably the mortality rate of zebrafishes. That means that nickel's acute toxicity was impacted significantly by pH value. Notably within the range of pH 7.3 to pH 7.7 for 48 hours, the results indicate that the influence of pH in this area to nickel toxicity was higher than others during of the surveyed period. As a matter of the fact that when pH increases from 7.3 to 7.7, average mortality proportion of zebrafishes in exposed samples witnessed a plunge from 46.67 ± 14.34; 76.67 ± 14.34; 90.00 ± 0.00; to 33.33 ± 14.34; 53.33 ± 14.34; 80.00 ± 0.00 respectively with nickel concentration (µg/L) at 360; 648, and 1166 (Figure 1). Also, Figure 2 indicates a high correlation between Ni concentration and average mortality (R = 0.96).

Table 2. Average and corrected mortality rate (%) in control and exposed groups of Ni for the period 48 hours to zebrafish

pH	C - Ni (µg/L)	The mortality rate in control (%)	The average mortality rate in exposed (%)	The corrected mortality rate in exposed (%)	pH	The mortality rate in control (%)	The average mortality rate in exposed (%)	The corrected mortality rate in exposed (%)
6.2	0	0.00			7.3	3.33 ± 14.34		
6.2	200	0.00	13.33 ± 14.34	13.33 ± 14.34	7.3	3.33 ± 14.34	23.33 ± 14.34	20.01 ± 14.34
6.2	360	0.00	30.00 ± 0.00	30.00 ± 0.00	7.3	3.33 ± 14.34	46.67 ± 14.34	43.35 ± 14.34
6.2	648	0.00	50.00 ± 0.00	50.00 ± 0.00	7.3	3.33 ± 14.34	76.67 ± 14.34	73.36 ± 14.34
6.2	1166	0.00	73.33 ± 14.34	73.33 ± 14.34	7.3	3.33 ± 14.34	90.00 ± 0.00	86.70 ± 0.00
6.2	2099	0.00	93.33 ± 14.33	93.33 ± 14.33	7.3	3.33 ± 14.34	100.00 ± 0.00	96.70 ± 0.00
6.8	0	3.33 ±14.34			7.7	6.66 ± 14.34		
6.8	200	3.33 ±14.34	20.00 ± 0.00	16.67 ± 0.00	7.7	6.66 ± 14.34	16.67 ± 14.34	10.02 ± 14.34
6.8	360	3.33 ±14.34	36.67 ± 14.34	33.34 ± 14.34	7.7	6.66 ± 14.34	33.33 ± 14.34	26.69 ± 14.34
6.8	648	3.33 ±14.34	46.67 ± 14.34	43,35 ± 14.34	7.7	6.66 ± 14.34	53.33 ± 14.34	46.70 ± 14.34
6.8	1166	3.33 ±14.34	63.33 ± 14.34	60.02 ± 14.34	7.7	6.66 ± 14.34	80.00 ± 0.00	73.39 ± 0.00
6.8	2099	3.33 ±14.34	70.00 ± 24.84	66.69 ± 24.84	7.7	6.66 ± 14.34	100 ± 0.00	93.40 ± 0.00

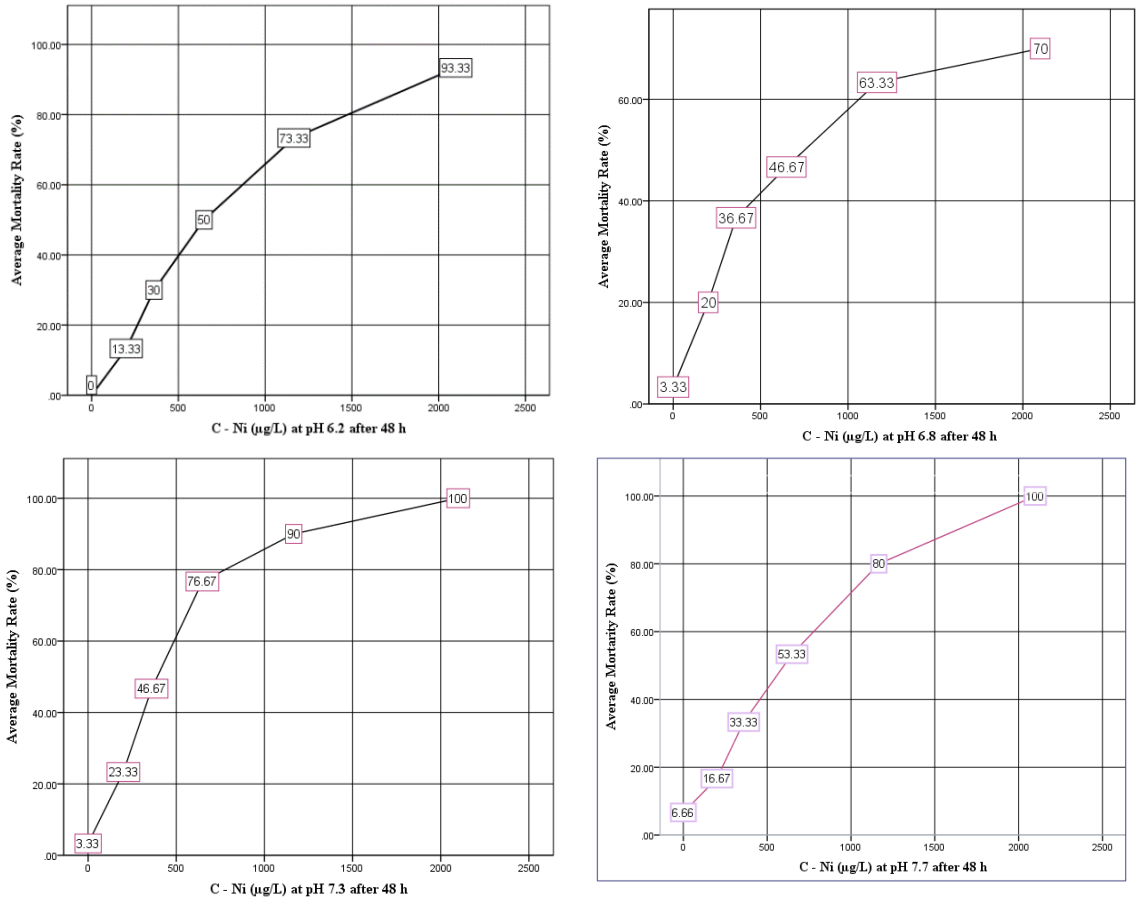

Figure 1. Average mortality rate (%) in control and exposed groups with different Ni values at diverse pH levels for the period 48 hours to zebrafish.

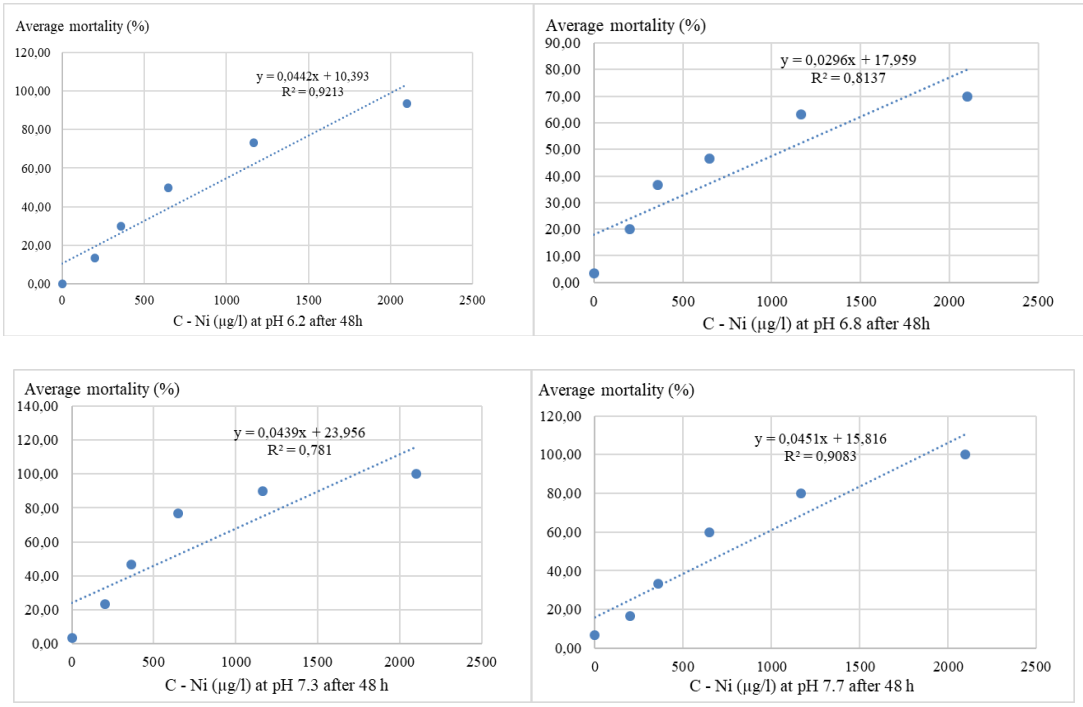

Figure 2. Correlation between concentration and average mortality rate

Figures 3 and 4 shows that there is significant disparity the mortality of zebrafish at pH 7.3 and pH 7.7 when compared to the normal distribution, therefore applying the logit model is more appropriate to calculate LC_{50} value.

The results from Table 3 and Figure 5 show that the LC_{50} values for 48 hours of nickel under normal laboratory conditions were found at 384.63 and 607.57 (µg/L) with pH values 7.3 and 7.7, respectively by logit method. This result was different compared to the studies of Shabnam *et al*. (2015) and Kienle *et al*. (2008), with the 96-h LC50 for larvae of 31.13 mg/L and 15 mg Ni/L in 2h exposure [13, 23]. However, their studies did not report these parameters used in their experiments as well as pH value, hardness, and even though this parameter significantly impacted on nickel toxicity [14]. Exposure time is also not similar among the researches.

Results show that nickel toxicity was higher at pH 7.3 than at pH 7.7, and that there is high correlation between Ni concentration according to log10 and logit value ($R^2 = 0.995$ and $R^2 = 0.999$, respectively pH 7.3 and pH 7.7).

Table 3. LC_{50} values of heavy metal nickel (Ni) as $NiSO_4.6H_2O$ in the period of 48 hours for zebrafish

pH	Method	LC_{50} values (µg/L)	Regression equation
6.2		That is not inadequate to identify LC_{50}	
6.8			
7.3	Logit	384.63	y = 4.44x - 11.48 R^2 = 0.995
	Probit	396.70	y = 1.99x - 0.17 R^2 = 0.98
7.7	Logit	607.57	y = 0.36x + 0.028 R^2 = 0.999
	Probit	694.51	y = 2.14x - 1.07 R^2 = 0.98

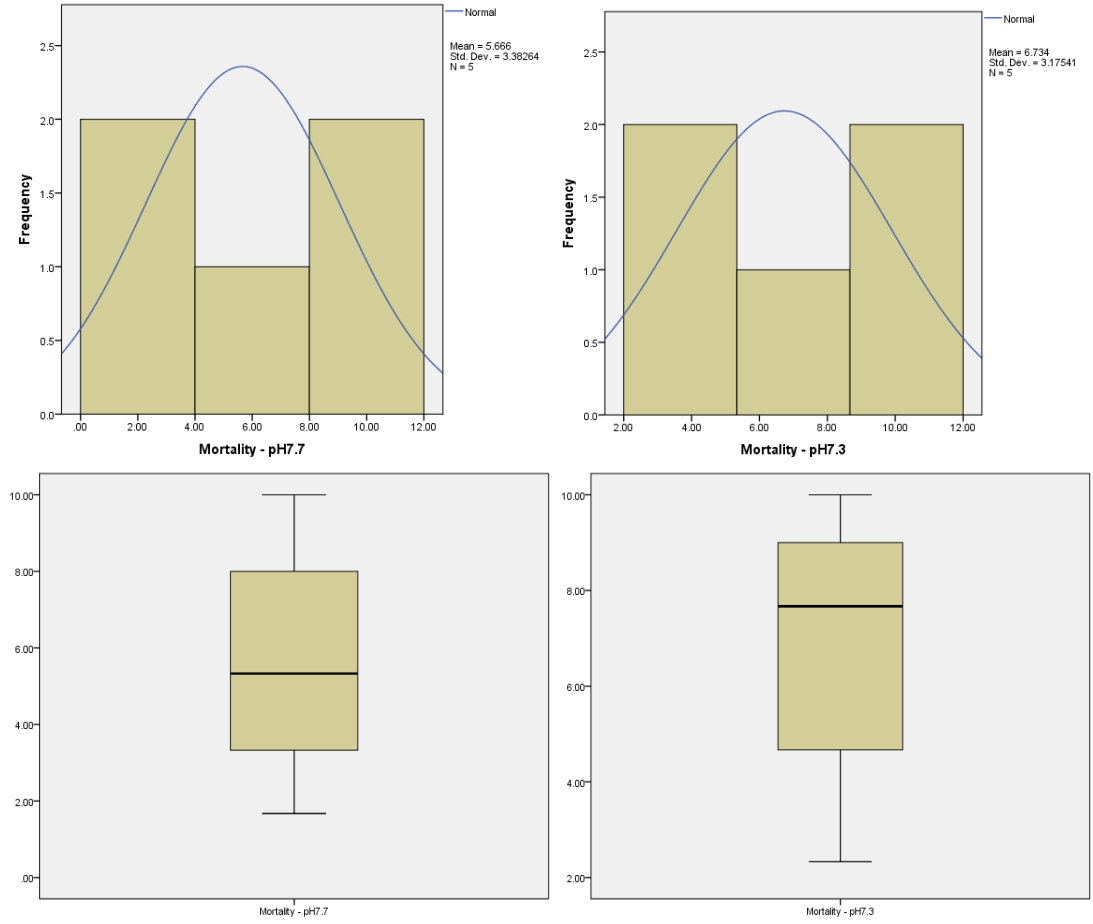

Figure 3. Comparison of the mortality rate of zebrafish with the normal distribution

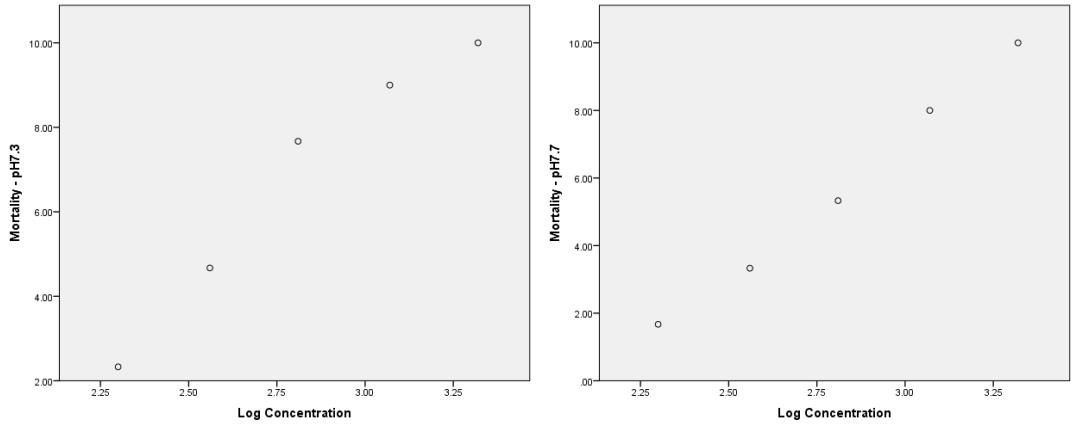

Figure 4. Relationship between the response number and concentration

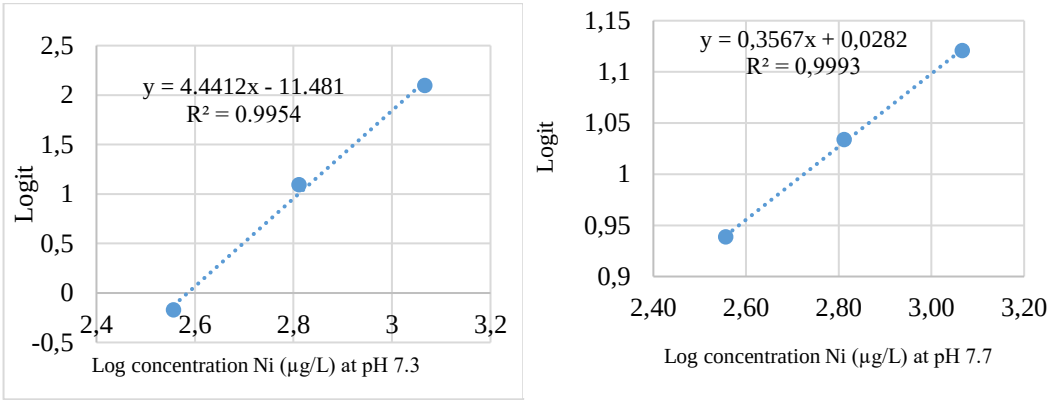

Figure 5. Relationship between concentration nickel and Logit at pH 7.3 and pH 7.7.

The results of one-way ANOVA (Table 4) show that in 48 hours of the surveyed duration, the influence of different pH levels (6.2; 6.8; 7.3 and 7.7) to Ni toxicity was not significant. However, the T-test (Table 5) illustrates that the mortality rate of zebrafish at pH 7.3 and pH 7.7 underwent a considerate disparity (Sig. = .031). That means the morality figures of zebrafishes at pH 7.3 (90.00% at Ni 1166 µg/L) was higher than that at pH 7.7 (80.00% at Ni 1166 µg/L). There is also some similarity between pH 7.3 and pH 6.8 (Mortality rate of zebrafish at pH 7.3 was 20 percent bigger than that at pH 6.8). The significant differences also were recorded comparing pH 7.3 and pH 6.2 (Mortality rate of zebrafish at pH 6.2 was 15% smaller than that at pH 7.3). When the pH level increased from 6.2 to 7.7, the mean dead ratio of zebrafish grew by about 6 percent. By contrast, comparing pH 6.2 with pH 6.8, and between pH 6.8 and pH 7.7, there was same volume mortality of zebrafish at the same Ni concentration. Thus, the general pH level affects substantially on Ni acute toxicity to zebrafish and the mortality proportion was the highest at pH 7.3 under the same experimental conditions.

Table 4. The difference of corrected mortality ratio of zebrafish among different pH values over the observed period for 48 hours

pH	6.2	6.8	7.3	7.7
	Sig. (95% confidence interval of the difference)			
6.8	.809			
7.3	.430			
7.7	.756			
6.2		.809		
7.3		.307		
7.7		.582		
6.2			.430	
6.8			.307	
7.7			.629	
6.2				.756
6.8				.582
7.3				.629

Table 5. The difference of corrected mortality ratio of zebrafish between different values over the observed period 48 hours

Cases	Sig. (2-tailed)
Mortality rate at pH 7.3 - Mortality rate at pH 7.7	.031
Mortality rate at pH 7.3 - Mortality rate at pH 6.8	.023
Mortality rate at pH 7.3 - Mortality rate at pH 6.2	.011
Mortality rate at pH 7.7 - Mortality rate at pH 6.8	.169
Mortality rate at pH 7.7 - Mortality rate at pH 6.2	.009
Mortality rate at pH 6.8 - Mortality rate at pH 6.2	.455

(95% confidence interval of the difference)

4. CONCLUSIONS

With the exposure time of 24 hours, the influence of pH on Ni acute toxicity was indistinctive and not sufficient condition to determine LC_{50}. In terms of 48 hours exposure time coupled with increasing pH from 7.3 to 7.7, the number of dead zebrafishes was recorded with the plunge from 76.67 ± 14.34 to 53.33 ± 14.34% at Ni level as 648 µg/L and LC_{50} increased by 222.94 µg/L, which means that Ni acute toxicity declined by 222.94 µg/L (from 384.63 to 607.57 µg/L). Meanwhile, pH increases from 6.2 to 6.8, had no significant impact on the morality levels of zebrafish.

To conclude, pH level affects substantially on Ni acute toxicity to zebrafishes and the mortality proportion made up the highest at pH 7.3 and 7.7 with Ni 2099 µg/L (100%) under the same experimental conditions in surveyed period.

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TÓM TẮT

XÁC ĐỊNH ĐỘ ĐỘC CẤP TÍNH CỦA NIKEN (Ni) TRONG MÔI TRƯỜNG NƯỚC TỚI CÁ SỌC NGỰA Ở CÁC MỨC pH KHÁC NHAU

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Môi trường nước đang phải đối mặt với nhiều thách thức từ chất thải nguy hại, trong đó thành phần kim loại đang đe dọa đến đời sống thủy sinh. Đặc biệt, các chất thải độc hại như các kim loại nặng (Cd, Ni, Hg, Pb, As) đã gây những ảnh hưởng xấu đến hệ sinh thái dưới nước thông qua chuỗi thức ăn. Đây là nguồn gốc của các bệnh tiềm ẩn và ảnh hưởng đến sức khỏe con người. Hơn nữa, nhiều loài cá đã được áp dụng rộng rãi để đánh giá ảnh hưởng của độc chất đối với hệ sinh thái dưới nước. Vì vậy, trong nghiên cứu này, cá sọc ngựa đã được sử dụng làm sinh vật chỉ thị sinh học. Với mục đích xác định độc tính cấp tính của Niken (Ni) ở các nồng độ khác nhau (0,2, 0,36, 0,648, 1,166, 2,099 mg/L) đối với cá sọc ngựa trong môi trường nước ở các mức pH khác nhau (6,2; 6,8; 7,3; 7,7). $NiSO_4.6H_2O$ đã được sử dụng làm nguồn Ni để thực hiện các xét nghiệm độc tính cấp tính (sau phơi nhiễm 48 giờ [48 giờ LC_{50}]) với cá sọc ngựa 13 ngày tuổi ở cùng một điều kiện độ cứng, carbon hữu cơ hòa tan (DOC) và độ kiềm. Các kết quả nghiên cứu chỉ ra rằng độc tính cấp tính của niken đối với cá sọc ngựa giảm khi pH tăng từ 7,3 lên 7,7 và giá trị LC_{50} tăng tương ứng 384,63 và 607,57 59 µg/L. Bên cạnh đó, kết quả thu được trong nghiên cứu này có thể cung cấp cơ sở hữu ích để tìm ra nguyên nhân chính xác gây tử vong cho cá.

Từ khóa: Độc tính cấp tính, LC_{50}, độc học sinh thái, niken, cá sọc ngựa (*Danio rerio*).