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Original Article

Impact of pH and Water Hardness on Lead (Pb) Accumulation and Growth of Juvenile Tilapia (*Oreochromis niloticus*) under Laboratory Conditions

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Abstract: Lead (Pb) is a pervasive aquatic contaminant, and its bioavailability and toxicity are strongly influenced by water chemistry. This study investigated the effects of pH (5.0, 5.5, 6.0, and 7.0) and hardness (0–60, 80–100, 200–220, and 300–330 ppm $CaCO_3$) on Pb accumulation and growth in juvenile tilapia (*Oreochromis niloticus*) exposed to 0.05 mg/L Pb for 60 days. The results showed that both pH and water hardness strongly modulate Pb bioaccumulation and growth performance in tilapia, but with distinct temporal and mechanistic patterns. Accumulation was highest at moderately acidic pH (5.5–6.0), while extreme acidity (pH 5.0) reduced Pb uptake and fish growth. Growth performance was inversely related to Pb levels, with near-neutral pH supporting the best outcomes. Hardness had a slower but consistent effect: prolonged exposure to very hard water (300–330 ppm) minimized Pb accumulation, however, soft or very hard water impaired growth. Very hard water caused cyclical growth fluctuations, reflecting ionic imbalance and stress. Together, these results show that pH acts as a fast regulator of Pb solubility and toxicity, while hardness exerts a gradual modulatory influence. Optimal fish growth and minimized Pb accumulation occurred at near-neutral pH and moderate hardness, underscoring the critical role of water chemistry in managing Pb risks in aquatic ecosystems and aquaculture.

Keywords: Tilapia, water hardness, Lead, pH, accumulation.

1. Introduction

Lead (Pb) is a non-essential and potentially toxic metal, even at low concentrations. Its bioavailability to aquatic organisms is largely influenced by environmental factors such as pH, alkalinity, water hardness, and natural organic matter [1]. In freshwater fish, Pb can impair antioxidant enzyme activity, disrupt Deoxyribonucleic Acid (DNA) transcription enzymes involved in vitamin D synthesis, and affect calcium metabolism and cell membrane integrity [2-6]. Among these environmental factors, pH plays a critical role because it regulates both chemical speciation and biological processes in aquatic ecosystems [6].

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It influences membrane permeability, cellular metabolism, enzyme activity, and ATP synthesis, while also determining the solubility and bioavailability of lead (Pb), which are closely linked to Pb accumulation in aquatic organisms [6, 8]. In natural environments, water pH is affected by various factors, including domestic wastewater, industrial discharge, agricultural runoff, medical waste, and chemical transformations [6]. Most aquatic species thrive within a pH range of 6.5 to 8.5 [7], but acidic conditions can increase Pb solubility and uptake, thereby enhancing its bioavailability and toxicity [8].

In addition to pH, water hardness is another key environmental factor that influences fish physiology and metal bioaccumulation. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions are the main components of water hardness and play important roles in aquatic life. Ca^{2+} is essential for freshwater fish, as it regulates ion balance by controlling membrane permeability and minimizing ion loss to the surrounding water [9]. Furthermore, Ca^{2+} is also involved in bone formation, hematopoiesis, blood clotting, and other cellular processes crucial for fish growth and survival [9, 10]. According to the World Health Organization (WHO), freshwater hardness typically ranges from 15 to 375 ppm as calcium carbonate ($CaCO_3$), with water is classified as soft (0–75 ppm), moderately hard (75–150 ppm), hard (150–300 ppm), or very hard (>300 ppm) [11]. Previous studies have shown that both pH and water hardness influence the bioaccumulation and toxicity of metals in fish [12–16].

Oreochromis niloticus (*O. niloticus*), a member of the Cinclidae family native to African freshwater ecosystems, is a globally important aquaculture species due to its high nutritional value, disease resistance, adaptability to diverse environments, and efficient feed conversion [17]. It is widely cultivated and plays a vital role in food security and economic development. In Vietnam, Pb accumulation in *O. niloticus* has been reported from several lakes in Hanoi [18], along with evidence of Pb-induced alterations of enzymatic activities involved in detoxification processes [19, 20]. These findings highlight the potential risks of Pb and its toxic-controlling factors on humans through fish consumption. However, the effects of pH and water hardness on Pb accumulation and fish growth remain poorly understood, particularly under controlled laboratory conditions. Therefore, the present study investigated Pb accumulation in tilapia (*O. niloticus*) and fish growth under laboratory conditions across acidic pH levels (5.0–7.0) and a range of water hardness (50–330 ppm $CaCO_3$). Our goal was to assess the combined effects of Pb with pH or water hardness on juvenile tilapia, thereby providing insights into the physiological responses of this species to ecological stressors.

2. Experimental

2.1. Fish Management

Sixty-day-old tilapia (*O. niloticus*) with an average weight ± SD: 7.81 ± 1.31 g per individual were obtained from the Research Institute for Aquaculture No. 1 (Bac Ninh, Vietnam). Prior to the experiment, fish were acclimated to laboratory conditions for seven days. Each experimental tank, with a capacity of 100 liters each housing 45 fish. Throughout the experiment, the fish were fed a commercial pelleted diet twice daily at a feeding rate of 3–4% of their body weight [2]. To maintain water quality and adequate dissolved oxygen levels, each tank was continuously aerated using an oxygen pump operating 24 hours a day.

2.2. Fish Treatment with Pb, pH and Water Hardness

Lead (II) nitrate [$Pb(NO_3)_2$], a crystalline compound with 99.9% purity, was purchased from Sigma Chemical Company. A stock solution was prepared by dissolving $Pb(NO_3)_2$ in double-distilled water and subsequently diluted with tap water to obtain a final Pb concentration of 0.05 mg/L in accordance with the Vietnamese National Technical Regulation on Surface Water Quality (QCVN

08:2015/BTNMT) [7]. This concentration was selected to evaluate the tolerance of tilapia to lead-contaminated water.

Experimental tanks were divided into two groups:

Group 1: pH Variation

Water pH was adjusted to four different levels: 5.0, 5.5, 6.0, and 7.0, with pH 7.0 serving as the control. The selected pH range was based on Dawson (1986) [21], and previous research indicating that pH values below 6.0 can negatively impact fish growth [22].

Group 2: Water Hardness Variation

Water hardness (expressed as $CaCO_3$) was adjusted to four levels: 50 - 60, 80 - 100, 200 - 220, and 300 -330 ppm, with 80 -100 ppm serving as the control, following the methodology of Adhikari *et al.*, (2007) [16].

Water in each tank was partially renewed (two-thirds of the volume) every two days. After each water change, appropriate amounts of $Pb(NO_3)_2$ and $CaCO_3$ were added to maintain the target experimental concentrations. Dissolved oxygen (DO) and pH were monitored daily using a calibrated DO meter and pH meter, while water hardness was monitored by Sera gH test kit.

2.3. Sample Collection and Analysis

Fish were sampled on days 0, 15, 30, 45, and 60 of Pb exposure under the designated pH or water hardness conditions. At each sampling point, five fish were randomly selected from each tank and body weights were measured using an analytical balance.

The specific growth rate (SGR) was calculated according to Hopkins (1992) [23] using the following formula:

$$SGR\ (\%\ day^{-1}) = \frac{\ln(w_t) - \ln(w_i)}{t} * 100$$

Where: w_t is the fish weight at time t

w_i is the initial weight

t is the time interval (days) between samplings.

After weighing, fish were dissected, and approximately 100–200 mg of wet muscle tissue was collected. Samples were transferred to Teflon digestion tubes and digested in a digestion box (Bio-carrier, Nagel, Bremen, Germany) with 2.5 mL of aqua regia (concentrated HNO_3 and HCl, v/v 1:3) and 200 µL of 30% H_2O_2 at 120 °C for at least 5 hours. The digested samples were then diluted to 20 mL with double-distilled water, filtered, and analyzed for Pb concentration using Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Varian Ultra Mass 700, Victoria, Australia) [24].

2.4. Statistical Analysis

Data were processed by Excel software and presented as mean ± standard deviation (SD). Statistical analyses were performed using two-way ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$ using GraphPad Prism 8.

3. Results and Discussion

3.1. Accumulation of Pb in Tilapia Across Varying pH Conditions

The data indicates that Pb accumulation in tilapia muscle tissue was influenced by both exposure duration and water pH (Figure 1). Concentrations increased significantly over time, peaking at day 30 ($p < 0.0001$), then declined at day 45 ($p < 0.001$), with a slight resurgence at day 60 ($p < 0.01$). This pattern suggests rapid initial uptake followed by detoxification or excretion, but longer exposure may trigger re-accumulation as fish remain in contaminated water. Pb accumulation peaked at day 30, which corresponded with slight reduction in catalase (CAT) activity as reported in our previous report [25], indicating Pb-induced oxidative stress in this species. This initial inhibition may result from direct binding of Pb to the enzyme or indirect induction of oxidative stress, both of which can impair enzymatic activity [26]. By day 45, although Pb levels had declined, CAT activity increased

[25], reflecting a sustained physiological defense response as reported in previous study [27]. Interestingly, enhanced CAT activity likely reduced reactive oxygen species (ROS) levels and lipid peroxidation, thereby protecting cellular membranes, and indirectly limiting Pb accumulation in certain tissues [28]. This observation underscores that CAT functions not as a direct detoxification pathway for Pb, but as a protective antioxidant mechanism that mitigates oxidative stress and helps regulate metal bioaccumulation.

Water pH also significantly influenced Pb accumulation *($p < 0.05$)*, with the highest levels observed at pH 5.5 in day 30 (*$p < 0.01$*). No consistent trend was observed regarding the effect of pH on Pb accumulation across all experimental time points. During the accumulation cycles (day 15, 30 and 60), Pb concentration tended to be higher under moderately acidic conditions (pH 5.5 - 6) compared to neutral condition (pH 7), possibly because acidity enhances the solubility of Pb^{2+} and reduces competition from carbonate and hydroxide complexes, thereby facilitating uptake. This explanation aligns with Slaveykova et al., (2003), who reported that bioavailable Pb speciation accounted for over 90% of total Pb within the pH range of 5-6.5 [29]. However, our data showed that Pb uptake decreased under more acidic condition (pH 5) compared to higher pH levels, suggesting that extreme acidity may cause additional physiological limitations. Similar inhibitory effects of very low pH on metal uptake have been documented, such as high proton concentrations (H^+) can compete with Pb^{2+} for binding sites on the gill surface, decreasing metal uptake efficiency, strong acid stress can damage gill epithelia and induce excessive mucus secretion, forming a barrier that limits metal penetration, and other environmental variables such as temperature and water hardness... may also affect dissolved Pb level [2].

Overall, these results indicate that both exposure duration and water pH strongly influence Pb accumulation. While moderately acidic conditions (pH 5.5 - 6) promote maximal uptake, extremely acidic conditions (pH 5.0) may reduce Pb accumulation due to competitive and physiological inhibitory mechanisms. The slight rebound at day 60 further suggests that prolonged exposure may allow partial re-accumulation despite initial depuration, highlighting the complex dynamics of metal uptake and regulation under long-term exposure.

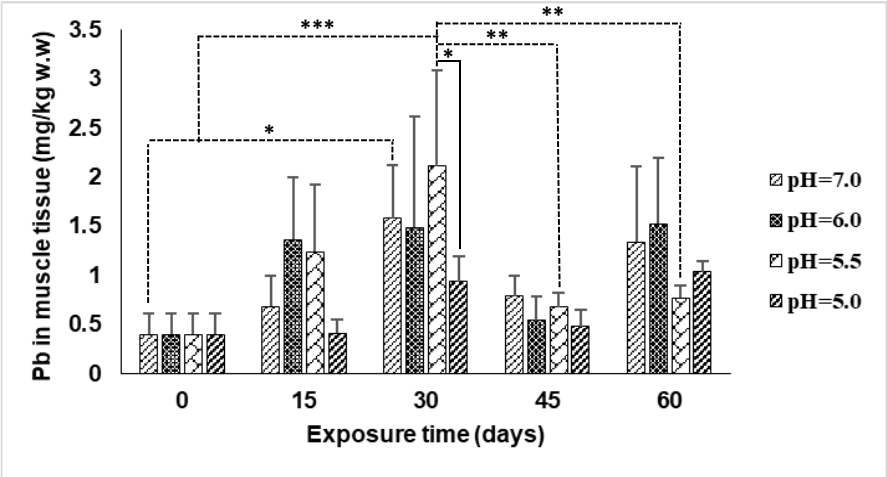

Figure 1. Tilapia's ability to absorb Pb in the environment containing Pb =0.05 mg/l and acidic to neutral pH levels (n=5, mean ±SD); Asterisks indicate statistical significance: (*) for *$p<0.05$*; (**) for *$p<0.01$*; (***) for *$p<0.001$.*

3.2. Growth of Tilapia Under Pb Exposure and Different pH Conditions

Juvenile Tilapia exposed to Pb under different pH conditions showed clear differences in growth performance as demonstrated by body weight (Figure 2A) and growth rates (Figure 2B). As expected, fish weight increased steadily across all treatments, however, the final weight was consistently higher at neutral and near-neutral pH (7.0 and 6.0) compared to acidic conditions (5.5 and 5.0). At pH 5.0, fish exhibited the lowest body weight, indicating that strong acidification suppressed growth. In terms of growth rate, growth rates varied significantly depending on both the duration of exposure ($p < 0.0001$) and the pH levels ($p < 0.05$). Growth rates peaked at day 15, declined markedly by day 30, showed a slight recovery at day 45, and then decreased again by day 60, approaching zero or even becoming negative under acidic conditions (Figure 2B). Thus, while fish continued to gain weight, prolonged acid exposure strongly inhibited growth, with near-neutral pH most favorable for both weight gain and growth rate.

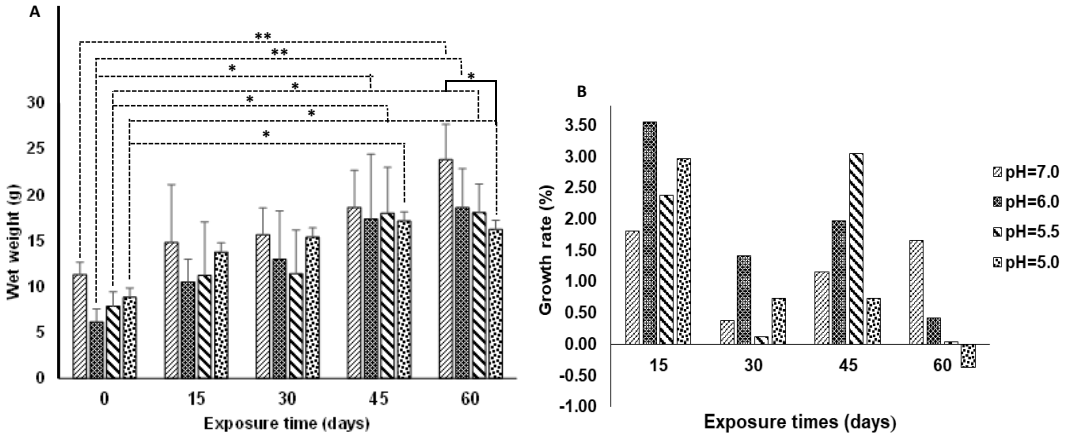

Figure 2. Effect of pH and Pb accumulation on the growth (mean ±SD, n=5) of tilapia. Weight (A) and growth rate (B) of fish on the time exposure with Pb and different pH; Asterisks indicate statistical significance: (*) for $p<0.05$; (**) for $p<0.01$.

When integrated with Pb accumulation patterns, growth rate displayed an overall inverse relationship with Pb bioaccumulation. The sharp decline in growth rate at day 30 coincided with peak Pb accumulation in fish muscle, suggesting Pb-induced oxidative or endocrine disruption as documented in previous studies [25–27]. The partial rebound in growth observed at day 45 paralleled a temporary reduction in Pb burden, likely reflecting activation of antioxidant and detoxification defense systems (e.g., CAT and Glutathione S-Transferase (GST) enzymes), which helped reduce oxidative damage and restore homeostasis [28]. By day 60, however, Pb accumulation increased again, coinciding with a renewed suppression of growth. The only exception was at pH 5.0, where both Pb accumulation and growth remained low, likely due to reduced Pb bioavailability under strongly acidic conditions coupled with physiological stress from acidification. This observation is consistent with established water quality guidelines, which identify pH 5.0 as below the acceptable range for sustaining the viability of aquatic organisms [7, 8].

These findings demonstrate that pH exerts a dual regulatory effect, influencing both Pb bioavailability and growth performance. The highest Pb accumulation occurred at moderately

acidic to near-neutral conditions (pH 5.5–6.0), while growth was optimal at slightly higher pH (6.0–7.0). Overall, growth rates tended to decrease as Pb accumulation increased, indicating a time-dependent and pH-modulated interaction between heavy metal uptake and fish growth. Importantly, these results suggest that growth suppression in tilapia is driven primarily by Pb bioaccumulation and its physiological impacts, rather than by acid stress alone.

3.3. Impact of Water Hardness on Pb Accumulation in Tilapia

Pb accumulation in tilapia muscle tissue exhibited clear temporal and hardness-dependent patterns (Figure 3). At day 0, Pb concentrations were negligible across all treatments. By day 15, Pb levels increased particularly in fish exposed to soft water hardness (50–60 ppm) ($p < 0.01$), while accumulation remained relatively lower at hardness levels (200–220 ppm and 300–330 ppm). A temporary decline was observed at day 30, followed by a sharp increase at day 45 ($p < 0.0001$), when Pb concentrations peaked. The highest values (approximately 10–12 mg/kg w.w.) were recorded in fish maintained at low hardness, whereas significantly lower levels (<7 mg/kg w.w.) were found under high hardness conditions. By day 60, Pb concentrations dropped substantially across all treatments, suggesting either enhanced detoxification and excretion processes or reduced Pb bioavailability over prolonged exposure.

With respect to hardness level, Pb concentrations generally declined as water hardness increased. During the higher accumulation phases (days 15 and 45), the lowest Pb levels were observed at 300–330 ppm $CaCO_3$ (Figure 3). This trend is partly consistent with Adhikari et al., (2007) [30], who reported reduced Pb accumulation in *Labeo rohita* at 350 ppm hardness before rising again at 750 ppm. The decline in Pb uptake under harder water may result from Ca^{2+}/Pb^{2+} competition at uptake sites and the formation of insoluble Pb–carbonate complexes which aligned with previous reports of Karthikeyan *et al.*, (2007) [13] for other metals such as Ni, Cd, and Cu in *Cirrhinus mrigala*, *Carassius auratus*, and *Salmo gairdneri*.

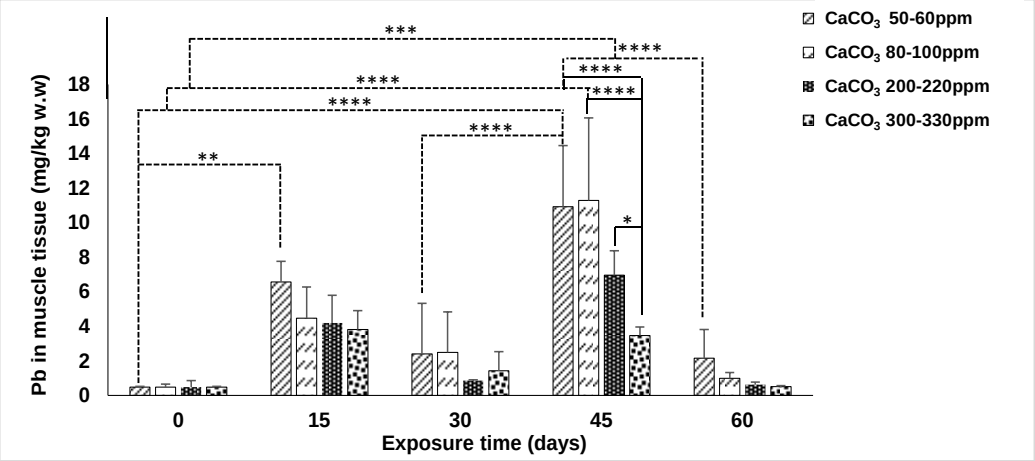

Figure 3. Tilapia's ability to absorb lead in the environment containing lead = 0.05 mg/l and different hardness levels (n=5, mean ±SD); Asterisks indicate statistical significance: (*) for $p<0.05$, (**) for $p<0.01$, (***) for $p<0.001$, (****) for $p<0.0001$).

When compared with the pH treatments, Pb accumulation in tilapia was regulated not only in magnitude but also in temporal dynamics. Under varying pH conditions, responses were immediate, with sharp increases already evident by day 15 and peaks occurring around day 30,

followed by fluctuations at later time points. This rapid response indicates that pH primarily regulates Pb bioavailability through solubility and speciation effects. In contrast, the impact of water hardness developed more gradually: although Pb levels rose by day 15, the clearest differences among hardness treatments were observed at day 45, these declined again at day 60. This delayed effect suggests that hardness modulates Pb accumulation through slower mechanisms, such as Ca^{2+}–Pb^{2+} competition at uptake sites, carbonate precipitation, and the progressive partitioning of Pb into particulate-bound forms [13, 31]. Overall, pH acted as a fast driver of Pb bioaccumulation, while hardness exerted a slower but sustained modulatory effect.

3.4. Growth of Tilapia Under Pb Exposure and Different Water Hardness Conditions

Tilapia growth performance, assessed through body weight and specific growth rate (SGR), is shown in Figure 4. Overall, fish exposed to 0.05 mg/L Pb under different hardness conditions gained weight over time (Figure 4A), but growth rate patterns varied markedly depending on both hardness level and exposure duration (Figure 4B). In soft water (50–60 ppm $CaCO_3$), growth was initially stimulated until day 30, followed by a sharp decline to negative values at day 45. By day 60, growth partially recovered, suggesting compensatory adjustments coinciding with reduced Pb accumulation. This biphasic response may be linked to osmoregulatory and mineral availability constraints: low ionic strength initially reduces ion-transport costs, allowing more energy for growth [32, 33]. However, prolonged exposure leads to Ca^{2+} and Mg^{2+} deficiency, impairing skeletal mineralization and metabolic stability, which in turn suppresses growth [32]. In moderately hard water (80–100 ppm), SGR increased steadily from day 15 to day 45 before declining at day 60, reflecting more stable growth compared to soft water. This suggests that moderate ionic availability better supports mineral demands without imposing excessive ion-regulatory stress. Similar patterns have been observed in carp and tilapia, where intermediate hardness optimized growth [34, 35]. A comparable but less pronounced trend occurred under hard water (200–220 ppm), with overall lower SGR values. In very hard water (300–330 ppm), growth rates fluctuated cyclically, with stimulation at day 15, suppression at day 30, recovery at day 45, and renewed decline at day 60, possibly reflecting cumulative interactions of high Ca^{2+} and Pb exposure.

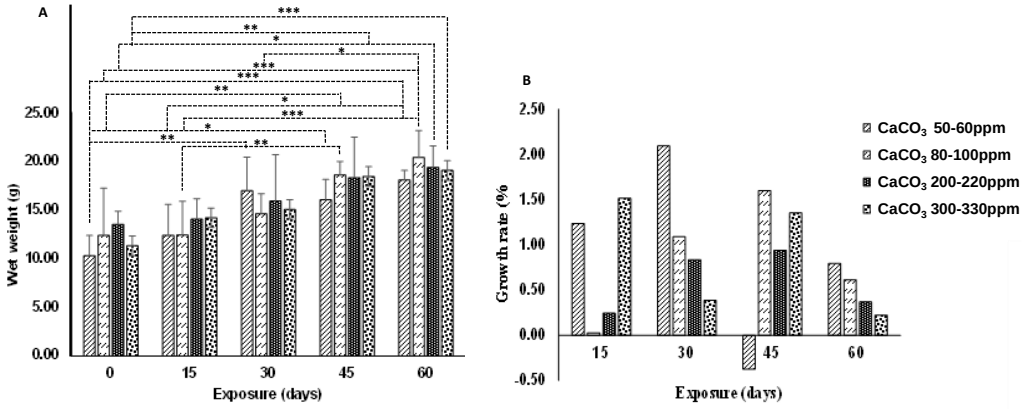

Figure 4. Effect of water hardness and Pb accumulation on the growth (mean ±SD, n=5) of tilapia. Weight (A) and growth rate (B) of fish on the time exposure with Pb and different water hardness; Asterisks indicate statistical significance: (*) for $p<0.05$, (**) for $p<0.01$, (***) for $p<0.001$.

When compared with pH, the effects of hardness on growth were more complex and time-dependent. Neutral and near-neutral pH (6.0–7.0) consistently supported the highest growth, whereas acidic conditions (pH 5.0–5.5) strongly suppressed it, reflecting a rapid and direct impact of pH via Pb solubility and acid stress. In contrast, hardness produced variable outcomes: soft water initially stimulated but later suppressed growth, moderate hardness provided more stable support, and very hard water induced cyclical fluctuations. Thus, pH acted as a fast and consistent regulator of growth, while hardness influenced growth in a slower, more dynamic manner.

Overall, inadequate hardness (<60 ppm) may constrain growth by limiting calcium availability for physiological functions, whereas excessively high hardness (>300 ppm) may induce stress through altered ionic balance and reduced nutrient bioavailability, potentially leading to growth suppression or mortality. These findings are consistent with Rajkumar *et al.*, (2018), who reported enhanced growth of *Labeo rohita* at hardness levels of 125–150 ppm [36]. Recommended hardness ranges for aquaculture are 30–180 ppm by Stone and Thomforde (2004) [37], with optimal conditions between 75–150 ppm by Bhatnagar et al., (2004) [38]. Maintaining appropriate hardness is therefore critical for supporting fish growth and health. Further field-based studies are needed to validate these controlled results and clarify the underlying physiological mechanisms.

4. Conclusion

This study demonstrates that both pH and water hardness strongly modulate Pb bioaccumulation and growth performance in tilapia, but with distinct temporal and mechanistic patterns. pH exerted a rapid and consistent influence, with moderately acidic conditions (pH 5.5–6.0) promoting maximal Pb uptake, while extreme acidity (pH 5.0) suppressed both Pb accumulation and growth. In contrast, water hardness regulated Pb uptake and growth more gradually. Increasing hardness generally reduced Pb bioaccumulation, but very hard water (>300 ppm) induced cyclical growth fluctuations, likely reflecting ionic imbalance and cumulative stress. Optimal growth was observed under moderately hard water (80–100 ppm) and near-neutral pH (6.0–7.0). Overall, these findings highlight the importance of maintaining water quality within ecologically relevant ranges to mitigate Pb toxicity and sustain fish health, with implications for both ecotoxicological risk assessment and aquaculture management.

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