The Effect of pH on Sodium Dodecyl Sulphate (SDS) Toxicity Towards Zebrafish (Danio Rerio) Larvae Development

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Abstract

SDS is a chemical widely used in cleaning and personal care products like soap. Its use in detergents may harm aquatic ecosystems. This research investigates the effects of pH on SDS

toxicity in zebrafish larvae development and observes any phenotypic deformities under different pH conditions. Zebrafish eggs (30 per treatment and control) were exposed to SDS (0.000004%) at pH 5.86 and pH 6.95. The hatching rate ($p<0.218$), free swim rate ($p<0.56$), and survival rate ($p<0.168$) were observed. Results show no significant effects of pH on these rates. Additionally, no significant differences were found in phenotypic deformities (pericardial edema, yolk sac edema, and vertebral bend) under varying pH conditions. However, pericardial and yolk sac edema appeared only in the presence of SDS which resulting in statistically insignificant differences. This study concludes that pH alteration may mask SDS effects on zebrafish larvae development, highlighting its role in controlling pollution.

Keywords: Sodium dodecyl sulphate (SDS); Nonbiodegradable surfactants; Toxicity; Phenotypic deformities; Temporal development

Introduction

Sodium dodecyl sulphate (SDS) is an aniotic surfactant, and it is domestically used as detergents. It is also used for food processing, technology applications, and significantly used for personal care. Its effectiveness in these areas has driven widespread use (Yeltekin & Oğuz, 2022). However, the increasing use of SDS has increased the concerns in regards to the effects of it towards the environment. This can be seen when the waste of non-biodegradable surfactants, contained in SDS from the domestic industries, is still flowing in the freshwater or natural water. Studies indicate that SDS does not readily degrade, meaning it can accumulate in water bodies and lead to harmful effects on aquatic life (Antonatos et al., 2023). Therefore, actions and preventions need to be taken to resolve the problems of chemical waste and its effects towards the environment. The aim of this study is to investigate the effects of pH on SDS toxicity on zebrafish larvae temporal development, and to observe any phenotypic deformities that are caused by SDS towards zebrafish larvae development. This observation is made under different pH condition.

Methodology

Preparation of Sodium Dodecyl sulphate (SDS) stock solution

About 40g of Instant Ocean Sea Salts was added to 1000 mL of distilled water and 60 µg/mL of final concentration, and the solution was shaken vigorously. 99% SDS stock solution that was given was obtained from Sigma Aldrich (St. Louis, Mo, USA). Therefore, to prepare SDS in this experiment, about 10% of SDS solution was prepared as a stock solution. After that, a serial dilution, ranging from 10% to 0.1% dilution was prepared. Next, the mixture of the solution was kept at room temperature.

Maintenance of fish during breeding and feeding

The zebrafish were placed in a small tank for maintenance. Then, they were separated according to gender and identified through their physically distinct body shapes. The differences could be determined by how female zebrafish have large bellies where the eggs are stored. Besides, they are also not active and slower than male zebrafish. The spawning season of zebrafish was according to the photoperiod cycle which was fixed with 10 hours of light

conditions, and 14 hours of dark conditions. Next, lights were switched on at 7.00 a.m. and switched off at 5.00 p.m. The zebrafish were fed twice per day with TETRAMIN pellets.

Treatments

The pH up and pH down solution (Neon) were used in this experiment. These two pH solutions were used to increase and decrease the egg water's pH by ±0.5 concentration. The eggs were collected and placed in a 50 mL petri dish right after the spawning session. The concentration percentage of SDS used for the treatments was 0.000004%. As a start, about 30 eggs were distributed into five different petri dishes. Control and treatments were also included, and all of them had three replicates each. Each treatment had its respective controls. Treatment 1's pH was added by +0.5 while in treatment 2 was reduced by -0.5. In addition, a control was performed with pH 7. This can be shown on the first day after the embryo was exposed to the SDS and pH. After that, any changes in temporal and phenotypic deformities on zebrafish larvae were observed and captured every day until day 7. This observation was performed using a compound microscope (Motic® BA210 Basic Biological Light Microscope), with 4x10 magnification. The capture of images was done with a digital camera.

Statistical Analysis

Survival rate (SR), hatching rate (HR), and free swim rate (FS) were calculated and recorded as follows:

Survival rate % (SR)

$$\frac{\text{Final number of larvae survived}}{\text{Initial number of larvae incubated}} x\ 100$$

Hatching rate % (HR)

$$\frac{\text{Total number of larvae hatching}}{\text{Total number of larvae survived}} x\ 100$$

Free swim rate % (FS)

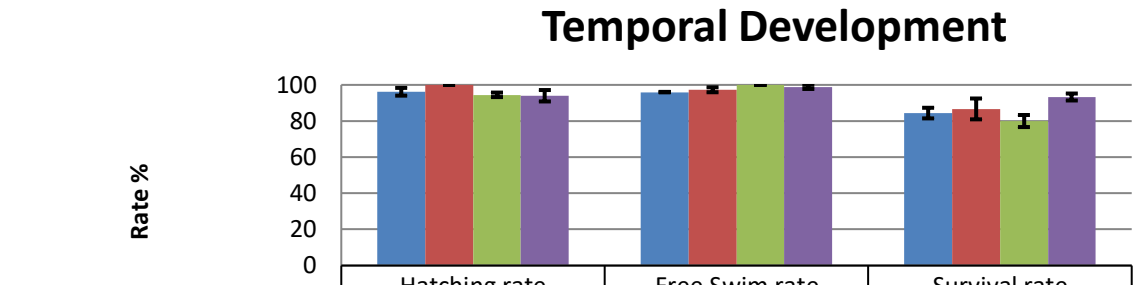
$$\frac{\text{Total number of swim larvae}}{\text{Total number of larvae survived}} x\ 100$$

In this experiment, the deformities of larvae were also observed and recorded using a light microscope. The deformities which were observed were pericardial edema, yolk sac edema, and tail bent. All of these deformities were seen after the larvae hatched. Next, the percentages of temporal development and phenotypic deformities were recorded from three replicates for each calculation. After that, the mean and standard error of these percentages were calculated. Lastly, the significant differences between groups of means were determined through a one-way analysis of variance (ANOVA), followed by Turkey's HSD post hoc test.

Results and Discussion

Temporal development

As shown in Figure 1, throughout this experiment, although the graph displays the differences between the rates of temporal development, statistically, there was no significant difference ($p>0.05$). This shows that the rate of larvae development through hatching, survival, and free swimming was the same between controls and treatments.



	Hatching rate	Free Swim rate	Survival rate
Control A (pH-0.5)	96.2	96	84.4
Treatment A (pH-0.5 + SDS)	100	97.3	86.7
Control B (pH+0.5)	94.5	100	80
Treatment B (pH+0.5 + SDS)	94	98.9	93.3

Figure 1: The temporal development of zebrafish larvae (hatching rate, free swim rate, and survival rate) in controls and treatments. Mean value (± SE) alphabetical indicates a significance difference (One-way ANOVA, $p<0.05$).

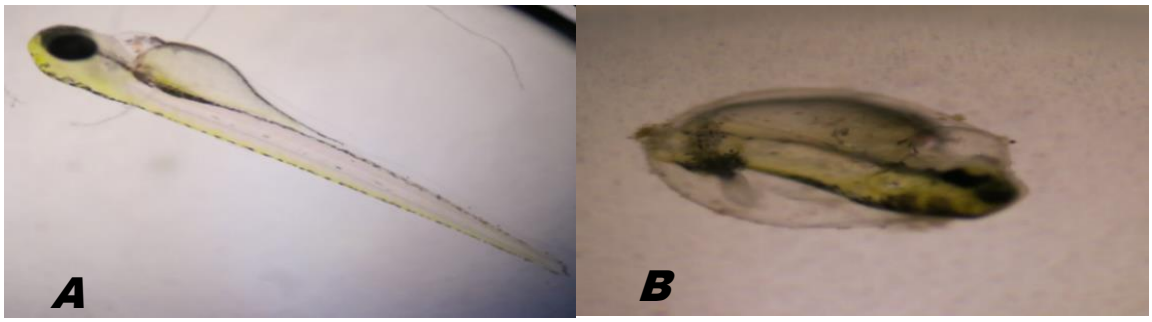


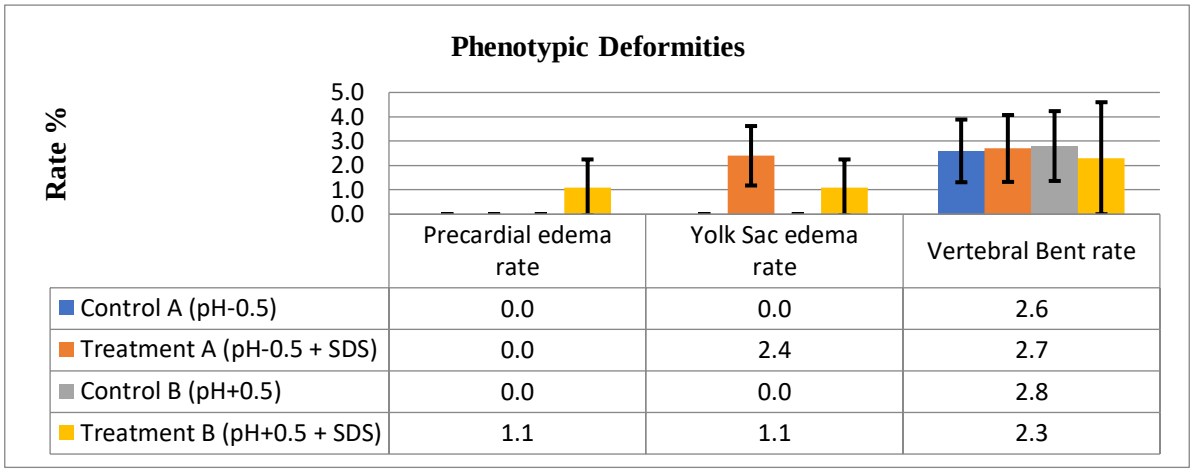
Figure 2: Microscopic image between total hatching of zebrafish larvae (A) and not hatching of zebrafish larvae (B).

The growth and developmental patterns of zebrafish larvae such as hatching (Figure 2), swimming ability, and survival rates showed no statistically significant differences across the various conditions tested, with each rate consistently above 80%. Although both SDS and pH variations are known to affect zebrafish, it's expected that combining these factors could lead to more pronounced developmental impacts (Falfushynska et al., 2021). Research has shown that extreme pH levels can significantly impact fish health, increasing mortality rates and disruptions in growth and reproduction (Shull et al., 2020). SDS exposure alone has also been linked to adverse effects on fish physiology, including damage to gill structures, reduced fertilization rates, and altered metabolic processes (Torres-Ruiz et al., 2023).

However, in this experiment, the combined effect of SDS and pH variations did not result in notable differences between control and experimental groups. However, in this experiment, it was shown that even with the addition of pH with SDS, the result showed no difference between the controls and treatments. Therefore, it is believed that the effects of SDS itself have been expected to be masked by the pH. This is due to the profound effects caused by the pH itself on zebrafish larvae.

Phenotypic deformities

As shown in Figure 3.0, pericardial edema was recorded only in treatment B, and the rate of pericardial edema showed no significant difference in statistical analysis ($p > 0.05$) and the microscopic image of pericardial edema was shown in Figure 4. Furthermore, yolk sac edema was recorded in treatment A and treatment B, and it was recorded neither in control A nor B. On the contrary, there was no significant difference in statistics ($p > 0.05$) and the microscopic image of yolk sac edema was shown in Figure 5. Moreover, Figure 3.0 also shows that vertebral bend was recorded in terms of its controls and treatments. However, there was no significant difference ($p<0.05$) shown by the groups statistically and the microscopic image of vertebral bent was shown in Figure 6. This was due to the smaller number of zebrafish larvae with vertebral bend in their controls and treatments. Figure 3.0 above also displays the phenotypic deformities rate of zebrafish larvae, as well as the result shown by control A and B. Treatment A had vertebral bend while treatment B had pericardial edema, albeit in a smaller amount.



	Precardial edema rate	Yolk Sac edema rate	Vertebral Bent rate
Control A (pH-0.5)	0.0	0.0	2.6
Treatment A (pH-0.5 + SDS)	0.0	2.4	2.7
Control B (pH+0.5)	0.0	0.0	2.8
Treatment B (pH+0.5 + SDS)	1.1	1.1	2.3

Figure 3: The phenotypic deformities rate of zebrafish larvae development (pericardial edema, yolk sac edema and vertebral bent) within 7 dpf. Mean value (± SE) alphabetical indicates a significance difference (One-way ANOVA, $p<0.05$).

Based on this result, even a smaller alteration of pH (ranging from 5.86 to 6.95) could give profound effects on zebrafish larvae. Studies support that pH levels near 5.9 can lead to physical changes in fish, including edema and spinal deformities (Yi et al., 2022). Furthermore, SDS exposure has been linked to organ-level structural changes, such as in the gills, kidneys, and spleen of aquatic organisms, indicating that SDS exposure in this study likely contributed to the observed deformities (Sundarraj et al., 2021).

However, statistically, there was no significant difference ($p<0.05$) between the controls and the treatments. This is on the contrary to the fact that the differences could obviously be identified through the rate, which is recorded in Figure 3.0. The reason for this was probably due to a very low percentage of larvae that survived compared to the number that hatched. Furthermore, in this experiment, it was shown that all the controls and treatments had vertebral bend. Thus, it is believed that vertebral bend may occur to zebrafishes during the larva stage, under both pH and SDS conditions. This finding is consistent with previous research, which associates vertebral curvature in larvae with muscle and cardiovascular developmental factors (Stachurski et al., 2023).

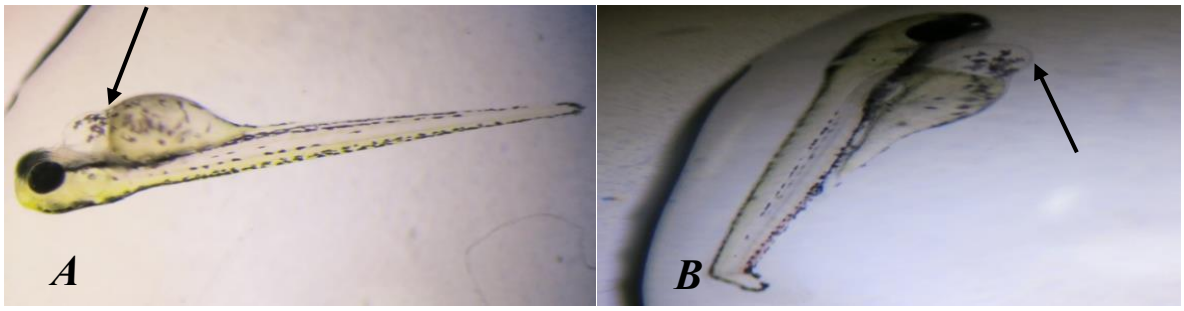


Figure 4: Microscopic image of zebrafish larvae x10 magnification. The arrow in picture A and B showed

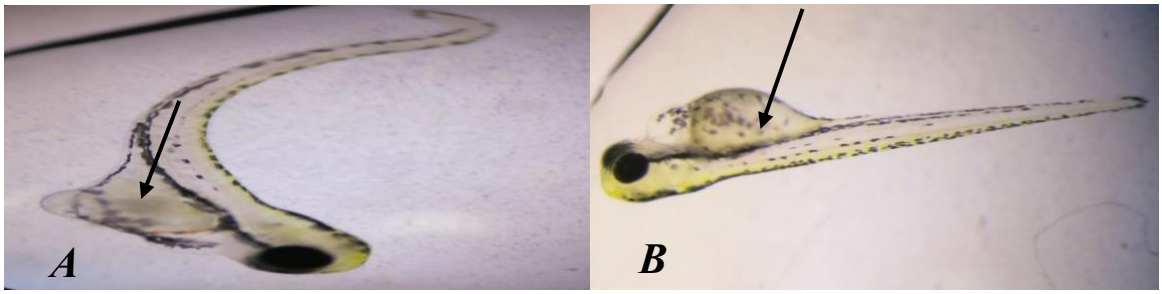


Figure 5: Microscopic image of zebrafish larvae x10 magnification. The arrow in picture A and B showed phenotypic deformities of zebrafish larvae development (yolk sac edema) within 7 dpf.

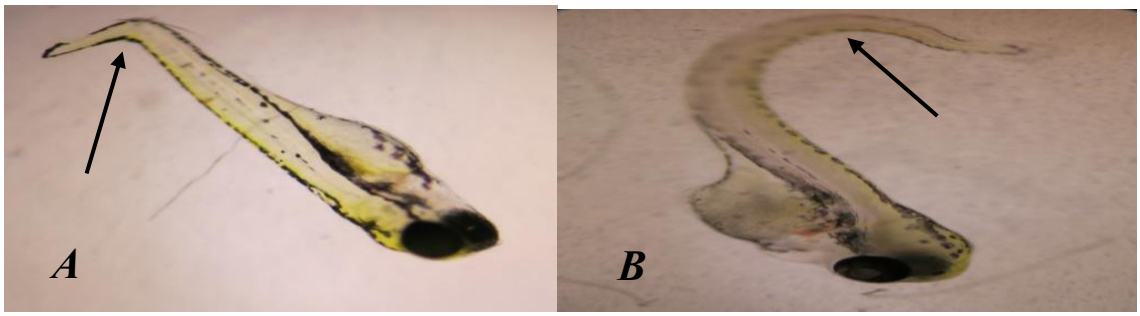


Figure 6: Microscopic image of zebrafish larvae x10 magnification. The arrow in picture A and B showed phenotypic deformities of zebrafish larvae development (vertebral bent) within 7 dpf.

Conclusion

As a conclusion, the pH factor in this experiment was not the effect of Sodium Dodecyl Sulphate (SDS) on zebrafish larvae development. The result which showed the different rates was recorded in terms of hatching, free swim, survival, pericardial edema, yolk sac edema, as well as vertebral bent. However, all of these rates did not show significant differences statistically. This indicates that despite the differences between the rates of development, they are actually the same. The changes in pH itself have officially had extreme effects on zebrafish larvae. Additionally, it is accepted that pH could mask the impact of SDS towards zebrafish larvae development. As a reference for future studies, it is recommended that the pH adjuster be changed, instead of using a commercial pH adjuster on ornamental fish. This is to verify that the zebrafish larvae are only influenced by the pH itself, and not influenced by other chemicals in the pH adjuster.

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