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Comparative Toxicity Of Lead And Cadimium On Developing Zebrafish Embryos

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ABSTRACT

This study investigated the effects of lead (Pb^{2+}) and cadmium (Cd^{2+}) on zebrafish (Danio rerio) embryos by assessing survival rates and cardiac development. Embryos were exposed to five concentrations of each metal (0.1, 1, 10, 20, and 100 µg/L). Results demonstrated that the toxicity of both metals was strongly time- and dose-dependent. At environmental concentrations (0.1 - 1 µg/L), the impact on survival was negligible within the first 48 hours but became apparent after 72 hours and was particularly severe at the 168-hour larval stage, where survival rates plummeted to below 7% at concentrations ≥10 µg/L. Cadmium showed a slightly higher toxicity trend than lead in this aspect. Regarding cardiac activity, both metals induced dose-dependent tachycardia (increased heart rate), with lead exerting a more potent stimulatory effect than cadmium. Notably, alterations in heart rate were detectable as early as 48 hours post-exposure, establishing this parameter as a sensitive early indicator of toxicity. The study affirms that both lead and cadmium pose significant threats to embryo survival and physiological health, highlighting the critical need for monitoring heavy metal contamination to protect aquatic ecosystems and aquaculture practices.

KEYWORDS: Zebrafish, Embryotoxicity, Heavy Metals, Heart Rate, Lead, Cadmium.

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INTRODUCTION

The pervasive presence of heavy metals in aquatic environments has raised considerable concern due to their detrimental effects on ecological systems and human health (Fatoki et al., 2012). Their persistence and capacity for bioaccumulation in living tissues exacerbate their potential for harm (Swarnalatha et al., 2015). Common metallic contaminants identified in fish populations encompass cadmium, lead, mercury, zinc, and copper, among others (Sfakianakis et al., 2015). It is noteworthy that certain metals, including zinc and copper, are vital micronutrients but become toxic when their concentrations exceed threshold levels (Kennedy, 2011).

Embryonic stages of fish are particularly vulnerable to environmental pollutants, making them a critical model for ecotoxicological risk assessment (Burton, 1991). The relevance of such models is further underscored by regulatory frameworks designed to ensure the ethical use of animals in research (Karatzia, 2017). As sediments often act as spawning sites, assessing contamination using early life stages (ELS) of fish yields crucial data on developmental endpoints like hatching success and growth (Burton, 1991).

The anticipated outcomes of this study are important as they will provide valuable insights into how heavy metals affect fish in their natural environments and their implications for aquaculture. This information is especially relevant for aquaculture because the environments in which fish are raised are subject to continuous changes, with heavy metal concentrations in enclosed sea bays and lakes being a key factor influencing these changes (Liu, Lao et al., 2019).

MATERIAL AND METHODS

Fish breeding and Embryos collection

Zebrafish were maintained under a controlled photoperiod of 14 hours light and 10 hours darkness. Spawning was initiated in tanks with removable dividers, using a sex ratio of one male to two females. After removing the divider and allowing 30 minutes for spawning, the newly fertilized embryos were collected. These embryos were subsequently transferred to 250 mL flasks containing the test solutions at the specified metal concentrations.

Chemicals

The heavy metals examined in this study included $CdNO_3 \cdot 2H_2O$, phosphate-buffered saline (PBS), 65% nitric acid, normal melting point agarose (NMP), and a Pb standard solution with a concentration of 1000 mg/L ($PbNO_3$ in H_2O), which was sourced from the RANGNGEN Company. The research involved both duplicate control and experimental groups.

Evaluation of live embryos

Embryos were considered viable if they displayed a translucent appearance, spherical form, intact chorion, and an evenly distributed yolk sac. Non-viable embryos were discarded. The percentage of surviving embryos was quantified at 48, 72, and 168 hours post-fertilization (hpf). Simultaneously, embryonic heart rates were visualized and documented via microscopy.

Heartbeat rate

For each experimental group, three larvae were randomly chosen for heart rate measurements, which were repeated. Heart rate assessments were conducted 6 hours after hatching, following the methodology outlined by Gouva et al. (2020). Cardiac activity was evaluated on day 6. For this measurement, ten embryos per replicate were randomly selected. Heartbeats were counted in three separate 20-second intervals for each embryo using a stereomicroscope and a cold light source. The room temperature was maintained at 23±1°C. The cumulative beats per minute (bpm) were calculated for each individual, and the mean cardiac activity per replicate was derived from the average of these ten measurements, adhering to established protocols (Barjhoux et al., 2012; Gouva et al., 2020).

RESULTS

3.1. Embryo Survival Rate

Zebrafish embryos were exposed to lead and cadmium at five different concentrations. At the 22-hour time point (Table 1), the difference in survival rate between the control group and the treatment groups was negligible at all concentrations, indicating minimal initial impact.

Table 1: Survival rate of embryos across developmental stages (%, Mean ± SD)

Metal	Group	22 hours	48 hours	72 hours	168 hours
Pb^{2+}	**Control**	97.33 ± 0.58	95.67 ± 0.58	95.67 ± 0.58	93.33 ± 1.53
	0.1 µg/L	96.33 ± 1.53	93.17 ± 1.53	90.67 ± 2.52	77.50 ± 3.61
	1 µg/L	94.83 ± 1.26	91.50 ± 2.00	90.67 ± 2.08	70.00 ± 5.00
	10 µg/L	94.00 ± 2.00	90.67 ± 2.52	81.50 ± 4.04	6.67 ± 1.53
	20 µg/L	93.17 ± 2.52	89.83 ± 3.06	73.17 ± 5.03	5.83 ± 1.26
	100 µg/L	92.33 ± 2.08	87.33 ± 3.06	51.50 ± 5.13	4.17 ± 0.76
Cd^{2+}	**Control**	97.33 ± 0.58	95.67 ± 0.58	94.83 ± 1.26	92.50 ± 2.00
	0.1 µg/L	94.67 ± 1.53	92.33 ± 2.08	89.00 ± 2.00	76.67 ± 3.06
	1 µg/L	94.00 ± 1.00	90.67 ± 1.53	89.00 ± 2.65	69.17 ± 4.04
	10 µg/L	93.17 ± 1.53	89.00 ± 2.65	79.83 ± 3.21	5.83 ± 1.26
	20 µg/L	91.50 ± 2.00	87.33 ± 2.52	72.33 ± 4.73	5.00 ± 1.00
	100 µg/L	90.00 ± 2.65	85.67 ± 3.06	50.67 ± 4.93	4.17 ± 0.29

Analysis from Table 1 shows that the toxic effects of lead (Pb^{2+}) and cadmium (Cd^{2+}) on the survival rate of zebrafish embryos were significant, evolving over time and depending on dosage, while also reflecting differences in the mechanisms of action of the two metals.

At the 22-hour mark, the survival rate between the control and treatment groups showed little disparity (highest 97.33% vs. lowest 90.00%). This indicates that the initial acute impact of the metals was negligible, possibly because the embryos were still protected by the chorion and had not absorbed sufficient metal. However, toxicity began to accumulate over time. By 48 and 72 hours, survival rates gradually decreased, and a catastrophic drop occurred at the 168-hour (7-day) time point. At this stage, embryos exposed to high concentrations (10, 20, 100 µg/L) were almost completely wiped out, with survival rates plummeting to only 4.17% to 6.67%. This demonstrates that the larval stage, when the fish have hatched and begun more complex life activities, is extremely sensitive to the accumulation of toxicity from heavy metals.

The results indicate a clear "toxicity threshold." At low concentrations (0.1 and 1 µg/L), although survival rates decreased over time compared to the control, the extent of this decrease was relatively modest. In contrast, when the concentration reached 10 µg/L, toxicity became very pronounced, especially in the later stages (72h and 168h). This abrupt decline suggests that the self-protection and detoxification mechanisms of the embryos/larvae were completely overwhelmed at this concentration threshold.
Across all concentrations and time points, cadmium (Cd^{2+}) consistently exhibited a trend of being slightly more toxic than lead (Pb^{2+}), although the difference was not substantial. In most groups, the average survival rate of the Cd^{2+} group was lower than that of the corresponding Pb^{2+} group. For example, at 1 µg/L and 168 hours, the survival rate of the Cd^{2+} group was 69.17%, lower than the 70.00% of the Pb^{2+} group. This difference may stem from distinct mechanisms of toxicity: Cadmium is renowned for its ability to cause strong oxidative stress and replace essential metal ions (such as zinc) in enzymes, thereby disrupting crucial biochemical processes more rapidly and profoundly.

The standard deviation (SD) in the table provides important information about data variability. In the control group, the SD was very small (< 2.0), reflecting stable experimental conditions and high reliability of the baseline data. Notably, the SD tended to increase in groups with medium concentrations (1-20 µg/L), particularly at the 72h and 168h time points. This indicates that the response of individual embryos to heavy metal stress was heterogeneous. Some individuals may have had better tolerance, while others were more sensitive, leading to significant variation between replicates. Conversely, at the highest lethal concentration (100 µg/L), the SD at 168h was very low, which is logical because most individuals had died, resulting in very low and consistent survival rates across replicates.

3.2. Effects on Heart Rate

Table 2: Average heart rate of embryos and larvae (beats per minute)

This table presents the mean ± standard deviation (Mean ± SD), calculated from 3 replicates in the original Table 5.

Measurement Stage	Metal	Control	0.1 µg/L	1 µg/L	10 µg/L	20 µg/L	100 µg/L
Live embryos (48h)	**Pb^{2+}**	113.3 ± 1.2	121.3 ± 2.3	127.3 ± 1.2	128.0 ± 2.0	130.7 ± 1.2	134.0 ± 2.0
	Cd^{2+}	112.7 ± 1.5	120.7 ± 1.2	126.7 ± 1.2	127.3 ± 3.1	130.0 ± 2.0	133.3 ± 3.1
Oropharyngeal (72h)	**Pb^{2+}**	122.0 ± 2.0	126.7 ± 2.3	133.3 ± 2.3	139.3 ± 2.3	143.3 ± 1.2	148.0 ± 2.0
	Cd^{2+}	122.3 ± 1.5	125.7 ± 0.6	132.3 ± 2.5	139.0 ± 1.0	140.7 ± 0.6	147.0 ± 1.0

Analysis from Table 2 reveals a prominent and sensitive physiological effect of heavy metals: disruption of cardiac function. Unlike the trend of causing mass mortality, both lead (Pb^{2+}) and cadmium (Cd^{2+}) caused a clear and systematic phenomenon of increased heart rate (tachycardia), providing an important biomarker of sublethal toxicity.

One of the most significant findings was that heart rate increased proportionally with metal concentration. At both monitoring stages (48h and 72h), the average heart rate of the treatment groups was consistently higher than that of the control group. This increase was particularly impressive at the highest concentration (100 µg/L). Specifically, compared to the control group, the heart rate at the 72h larval stage increased from 122.0 to 148.0 bpm (~21% increase) for Pb^{2+} and from 122.3 to 147.0 bpm (~20% increase) for Cd^{2+}. This "tachycardia" is a typical physiological stress response. When embryos are poisoned, their bodies may attempt to compensate by enhancing cardiac activity to maintain blood circulation and oxygen supply to tissues, while also accelerating the detoxification process. This can be seen as a final effort to sustain life under stress.

Although both metals increased heart rate, there was a subtle difference in intensity. At high concentrations (10, 20, 100 µg/L), lead (Pb^{2+}) consistently produced a slightly higher average heart rate than cadmium (Cd^{2+}) at the same concentration. This difference was most evident at 20 µg/L during the 72h stage (143.3 bpm vs. 140.7 bpm). This suggests that lead has a stronger stimulatory effect directly on the sympathetic nervous system controlling the heart or on ion channels in cardiac muscle cells. In contrast, although cadmium also increased heart rate, its mechanism might be more related to causing oxidative stress and cellular damage, leading to a less intense tachycardia response. The increased standard deviation in the Cd^{2+} groups at 10 µg/L and 100 µg/L also indicates that the heart's response to cadmium may be less stable and more variable among individuals.

The phenomenon of increased heart rate appeared markedly as early as the 48-hour stage, before the sharp decline in survival rates in later stages. This makes the heart rate index a sensitive and valuable early-warning biomarker for the toxic effects of heavy metals. Monitoring heart rate can detect embryo stress even when they are still morphologically alive, thereby predicting the risk of subsequent mass mortality.

CONCLUSION

The study provided compelling evidence of the acute and subacute toxicity of lead (Pb^{2+}) and cadmium (Cd^{2+}) on the development of zebrafish (Danio rerio) embryos and larvae, as demonstrated by two main indicators: survival rate and cardiac activity.

Regarding survival rate, the results showed that the toxicity of both metals evolved over time and was strictly dose-dependent. In the early stages (22-48 hours), the impact was negligible, but it became increasingly pronounced in later stages, particularly at the 168-hour (7-day) time point. The catastrophic drop in survival rate to below 7% in groups with high concentrations (≥10 µg/L) confirms that the larval stage is a critical period, extremely sensitive to the accumulation of toxicity from heavy metals. Comparing the two metals, cadmium (Cd^{2+}) exhibited a slightly higher toxicity trend than lead (Pb^{2+}) across most indicators, likely due to its mechanism of causing strong oxidative stress and disrupting essential biochemical processes.

Regarding effects on heart rate, the study observed a prominent physiological impact: the phenomenon of dose-dependent tachycardia (increased heart rate). This is a typical stress response of the body attempting to compensate for the state of intoxication. Among them, lead (Pb^{2+}) had a stronger stimulatory effect, causing a significantly higher heart rate than cadmium (Cd^{2+}) at the same concentration, hinting at differences in their mechanisms of action on the nervous and cardiovascular systems. More importantly, the change in heart rate appeared markedly very early (48 hours), making it a sensitive and valuable early-warning biomarker for toxic effects, preceding the occurrence of mass mortality signs.

The study confirms that both lead and cadmium are dangerous pollutants, directly threatening the survival of aquatic organisms in their early life stages. While cadmium appears more toxic in terms of mortality rate, lead has a more potent impact on physiological function. These findings not only highlight the dangers of heavy metal pollution for aquatic ecosystems and the aquaculture industry but also propose heart rate monitoring as an effective tool in environmental risk assessment and toxicological testing.

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