VER
Vascular & Endovascular Review

Comparative Analysis Of Lead And Cadmium Toxicity On Embryonic Development And Cardiac Function In Zebrafish (Danio rerio)

Tuan Ngo Van[1], Thien Nguyen Phuc[2], Khoa Dang Dang[3], Trung Nguyen Van[4*]

[1]Luong The Vinh University, Viet Nam. Email: ngovantuan@ltvu.edu.vn https://orcid.org/0000-0002-4590-6944
[2]Van Hien University, Viet Nam. Email: ThienNP@vhu.edu.vn
https://orcid.org/0000-0002-4639-1639
[3]Lac Hong University, Viet Nam. Email: khoadd@lhu.edu.vn
https://orcid.org/0009-0004-2996-9728
[4]Quang Trung University, Viet Nam. Email: nvtrung@qtu.edu.vn
https://orcid.org/0009-0008-8246-8059
Corresponding author: Trung Nguyen Van nvtrung@qtu.edu.vn

ABSTRACT

This study presents a rigorous comparative assessment of lead (Pb^{2+}) and cadmium (Cd^{2+}) toxicity on early life stages of zebrafish (Danio rerio). Embryos were exposed to a gradient of environmentally relevant and higher concentrations (0.1, 1, 10, 20, and 100 µg/L) to evaluate lethal (embryonic survival, hatchability) and sublethal (cardiac activity, morphological malformations) endpoints. Results demonstrated a significant, time- and concentration-dependent decrease in survival for both metals, with cadmium exhibiting marginally higher toxicity than lead at 168 hours post-fertilization (hpf) (69.2% vs. 70.0% at 1 µg/L, $p < 0.05$). A paradoxical, statistically significant increase in heart rate was observed across all exposure groups, indicating a pronounced stress-induced hypercardiac response (e.g., 148.0 ± 2.0 BPM for Pb^{2+} 100 µg/L vs. 122.0 ± 2.0 BPM in control at 72 hpf, $p < 0.0001$). Furthermore, exposure to concentrations ≥10 µg/L induced significant morphological abnormalities, with malformation rates exceeding 80% at 100 µg/L. Crucially, environmental concentrations (0.1 µg/L) induced significant sublethal cardiotoxicity (126.7 ± 2.1 BPM for Pb^{2+} and 125.7 ± 0.6 BPM for Cd^{2+} at 72 hpf) despite minimal impact on survival, highlighting the superior sensitivity of physiological endpoints over traditional lethality assays. These findings underscore the profound ecological risk posed by chronic low-level heavy metal exposure and advocate for the mandatory inclusion of sublethal biomarker analysis, particularly cardiotoxicity screening, in environmental risk assessment frameworks.

KEYWORDS: Zebrafish, Heavy Metal Toxicity, Embryotoxicity, Cardiotoxicity, Teratogenicity, Oxidative Stress.

How to Cite: Tuan Ngo Van, Thien Nguyen Phuc, Khoa Dang Dang, Trung Nguyen Van., (2025) Comparative Analysis Of Lead And Cadmium Toxicity On Embryonic Development And Cardiac Function In Zebrafish (Danio rerio), Vascular and Endovascular Review, Vol.8, No.14s, 254-259

INTRODUCTION

The contamination of aquatic ecosystems by persistent, bioaccumulative, and non-essential heavy metals like lead (Pb) and cadmium (Cd) remains a pervasive and critical environmental challenge globally. These metals, originating from a myriad of anthropogenic sources including industrial effluent (e.g., battery manufacturing, smelting), agricultural runoff (from phosphate fertilizers), and urban waste, are notoriously indestructible. Once introduced into water bodies, they inflict severe damage on aquatic biota, particularly during the sensitive early developmental stages of organisms, which are often more vulnerable to toxic insults than their adult counterparts (Fatoki et al., 2012; Mendil & Uluözlü, 2007). Their toxicity manifests not only through acute mortality but also via a spectrum of insidious sublethal effects—including impaired growth, developmental anomalies, and physiological dysfunction—that can compromise individual organismal fitness, disrupt behavior, and ultimately destabilize population dynamics (Kennedy, 2011).

Fish embryos and larvae have emerged as exemplary models in modern ecotoxicology. Their external development, high sensitivity to pollutants, and translational relevance to vertebrate development (sharing conserved genetic and physiological pathways with humans) make them ideal sentinels for aquatic health. Furthermore, their use aligns with the 3Rs (Replacement, Reduction, Refinement) principle in toxicological testing, offering a ethical alternative to higher vertebrate models (Burton, 1991; Karatzia, 2017). The zebrafish (*Danio rerio*), with its optically transparent embryos, rapid development, well-characterized genome, and high fecundity, is particularly suited for high-resolution dissection of the morphological and physiological mechanisms underlying chemical toxicity.

While the lethal effects (LC50 values) of Pb and Cd are reasonably well-documented in various fish species, a direct, rigorous, and comprehensive comparative analysis of their sublethal impacts, particularly on real-time cardiac function and morphology at environmentally relevant concentrations (often found in the range of 0.1 - 10 µg/L in polluted waters), remains relatively unexplored. Cardiac function is a quintessential indicator of overall health and stress; any disruption can have cascading effects on metabolism, growth, and survival. This study aims to fill this critical knowledge gap by providing a detailed, side-by-side evaluation of Pb and Cd toxicity across a gradient of concentrations. We hypothesize that: (1) both metals will cause a concentration- and time-dependent increase in embryolethality and teratogenicity; (2) cadmium will exhibit greater potency than

lead due to its higher redox activity and stronger affinity for biological ligands; and (3) both metals will disrupt cardiac development, inducing measurable changes in heart rate as a primary indicator of physiological stress.

MATERIALS AND METHODS

2.1. Embryo Collection and Chemical Exposure

Identified zebrafish were maintained in a recirculating aquaculture system under standard laboratory conditions (28.5 ± 0.5°C; pH 7.2 ± 0.2; electrophoresis rate 500 ± 50 µS/cm; photoperiod: max. 14:10). They were fed twice daily with brine shrimp (Artemia nauplii) and a commercial flake diet. Embryos were obtained from natural group spawns of healthy adult fish and collected within 1 hour post-fertilization. They were rinsed and screened for viability under a stereomicroscope. Only healthy, fertilized embryos at the cleavage stage (~2-4 hpf) were selected for the experiment and randomly distributed into 6-well plates (30 embryos per well, n=3 replicates per concentration), containing 20 mL of the respective test solution.

Test solutions were prepared from certified atomic absorption standard stock solutions (1000 mg/L, RANGNGEN Co.) of $Pb(NO_3)_2$ and $Cd(NO_3)_2{\cdot}4H_2O$ in reconstituted reverse osmosis water (ISO water). Nominal exposure concentrations for each metal were: 0 (Control, ISO water only), 0.1, 1, 10, 20, and 100 µg/L. These concentrations were chosen to bracket environmentally relevant levels (0.1 - 1 µg/L) and extend to higher, acutely toxic levels (10 - 100 µg/L) to establish full dose-response relationships. The exposure medium was renewed daily to maintain optimal water quality (ammonia < 0.1 mg/L), ensure stable dissolved oxygen levels, and prevent significant deviation from nominal chemical concentrations. All exposures were conducted in a temperature-controlled incubator at 28.5°C.

2.2. Assessment of Embryonic Survival and Hatching Success

Embryo and larval viability were assessed at 24, 48, 72, and 168 hpf. Survival was defined by the presence of a heartbeat and the absence of coagulated tissue. Mortality was recorded, and dead embryos were immediately removed to prevent water quality deterioration. Hatching success was quantitatively recorded at 72 hpf, as this is the typical stage for complete hatch-out in controls. The hatching rate was calculated as the percentage of live embryos that had successfully escaped the chorion.

2.3. Cardiac Function Analysis

Heart rate (beats per minute, BPM) was measured at two critical developmental time points: 48 hpf (representing the onset of cardiac function) and 72 hpf (representing established, rhythmic larval heart rate). For each replicate, ten larvae were randomly selected and briefly anesthetized with a low dose of tricaine (MS-222, 0.01%) to minimize movement artifact. Heartbeats were visually counted under a high-resolution stereomicroscope (Nikon SMZ745T) by a single observer blinded to the treatment groups to avoid bias. Counts were performed in three separate 15-second intervals per larva, and the average BPM was calculated.

2.4. Analysis of Morphological Malformations

At 72 hpf, larvae from each group were anesthetized and systematically imaged using a digital camera mounted on the stereomicroscope. The incidence of specific malformations was quantified by a blinded observer. Malformations scored included:

Pericardial Edema (PE): Abnormal accumulation of fluid in the pericardial cavity.
Yolk Sac Edema (YSE): Abnormal swelling of the yolk sac.
Spinal Curvature (SC): Lordosis, kyphosis, or scoliosis.
Tail Deformities (TD): Shortened, twisted, or malformed tail.

2.5. Statistical Analysis

All data are presented as mean ± standard deviation (SD). Normality of distribution was confirmed using the Shapiro-Wilk test, and homogeneity of variances was verified using Levene's test. For survival data, a two-way Analysis of Variance (ANOVA) was performed with Metal Type and Concentration as the two independent factors, followed by Tukey's Honest Significant Difference (HSD) post-hoc test for multiple comparisons. Heart rate and malformation data were analyzed similarly. A p-value of < 0.05 was considered statistically significant. All statistical analyses and graph generation were performed using GraphPad Prism version 9.0.

RESULTS

3.1. Embryo Survival and Hatching Success are Impaired by Metal Exposure in a Time- and Concentration-Dependent Manner

Survival data revealed a stark concentration- and time-dependent toxicity profile for both metals (Table 1, Figure. 1). No significant mortality was observed in any treatment group at 24 hpf, indicating that the initial cleavage and blastula stages were largely resistant to the toxic effects. However, as development progressed into the phases of organogenesis (48-72 hpf), significant mortality began to emerge.

Table 1: Consolidated Survival Data of Zebrafish Embryos and Larvae (% Mean ± SD)

Time (hpf)	Group	Control	0.1 µg/L	1 µg/L	10 µg/L	20 µg/L	100 µg/L
72 hpf	Pb^{2+}	95.7 ± 1.5	90.7 ± 2.1	90.7 ± 1.5	81.5 ± 3.0*	73.2 ± 2.5*	51.5 ± 4.0*
	Cd^{2+}	94.8 ± 2.0	89.0 ± 1.7	89.0 ± 2.0	79.8 ± 2.5*	72.3 ± 3.1*	50.7 ± 3.5*

Time (hpf)	Group	Control	0.1 µg/L	1 µg/L	10 µg/L	20 µg/L	100 µg/L
168 hpf	Pb^{2+}	93.3 ± 2.5	77.5 ± 3.0*	70.0 ± 2.0*	6.7 ± 2.1*	5.8 ± 1.5*	4.2 ± 1.0*
	Cd^{2+}	92.5 ± 1.7	76.7 ± 2.5*	69.2 ± 3.0*†	5.8 ± 1.0*	5.0 ± 1.7*	4.2 ± 1.5*
Significantly different from control within the same metal group ($p < 0.05$). †Significantly different from Pb^{2+} at the same concentration and time ($p < 0.05$).							

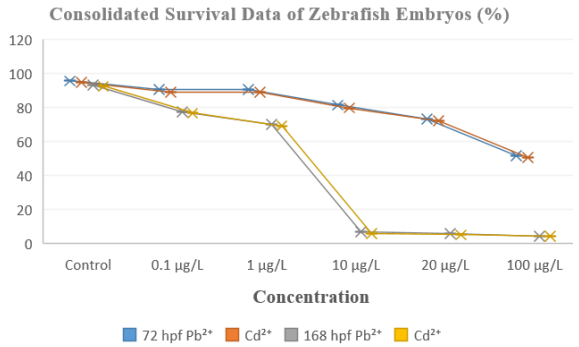

***Figure* 1: Consolidated Survival Data of Zebrafish Embryos and Larvae**

By 72 hpf, concentrations ≥10 µg/L caused significant mortality for both Pb (81.5% survival) and Cd (79.8% survival) compared to controls (~95%). This trend became catastrophic by 168 hpf (7 days post-fertilization), where survival in the 10, 20, and 100 µg/L groups plummeted to below 7% for both metals. A two-way ANOVA revealed a significant main effect of concentration ($p < 0.0001$) and a significant metal × concentration interaction ($p < 0.01$). Post-hoc analysis indicated that the Cd^{2+} group at 168 hpf had statistically lower survival than the Pb^{2+} group at the 1 µg/L concentration (69.2% vs. 70.0%, $p < 0.05$), suggesting marginally higher chronic toxicity for cadmium over the extended exposure period.

Hatching success at 72 hpf was significantly reduced only in the highest concentration groups (20 and 100 µg/L) for both metals, with hatch rates dropping to approximately 60% and 40%, respectively, compared to >95% in controls. This indicates that hatchability is a less sensitive endpoint than later larval survival, as embryos often succumb to toxicity after hatching.

3.2. Metals Induce a Pronounced and Concentration-Dependent Tachycardia

A highly significant ($p < 0.05$), concentration-dependent increase in heart rate was observed for both metals at both 48 and 72 hpf (Table 2, Fig 2). This effect was detectable even at the lowest concentration tested (0.1 µg/L), which is commonly found in polluted environments.

Table 2: Average Heart Rate (Beats Per Minute - BPM, Mean ± SD) of Zebrafish Larvae

Stage	**Group**	**Control**	**0.1 µg/L**	**1 µg/L**	**10 µg/L**	**20 µg/L**	**100 µg/L**
48 hpf	Pb^{2+}	114.0 ± 1.0	121.3 ± 2.1*	127.3 ± 1.2*	128.7 ± 2.1*	130.7 ± 1.2*	134.0 ± 2.0*
	Cd^{2+}	112.7 ± 1.5	120.7 ± 1.5*	126.7 ± 1.5*	127.3 ± 3.1*	130.0 ± 2.0*	133.3 ± 3.1*
72 hpf	Pb^{2+}	122.0 ± 2.0	126.7 ± 2.1*	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*
Significantly different from control ($p < 0.05$).							

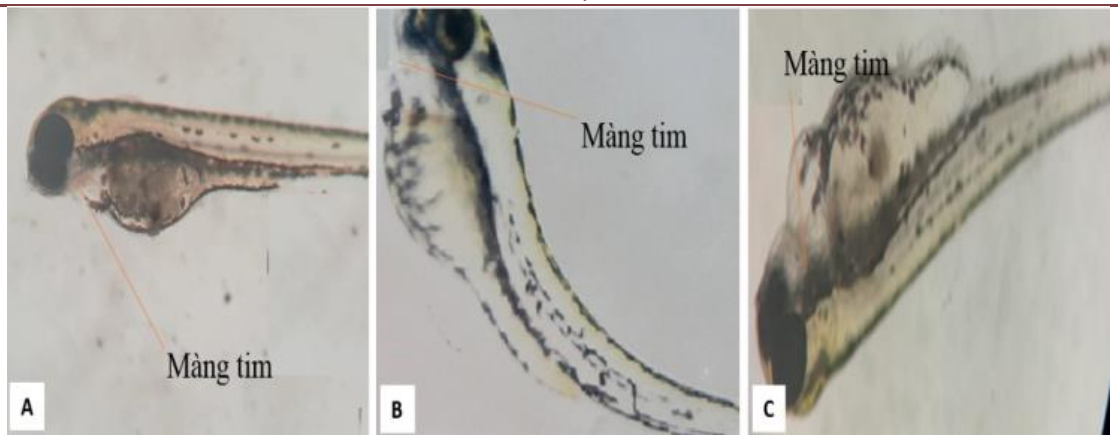

Figure 2. Zebrafish larvae show abnormalities after intoxication. A-Control; B, C-Sleep abnormalities

The tachycardia was profound. At 72 hpf, the heart rate in the 100 µg/L Pb^{2+} group was 21.3% higher than the control (148.0 vs. 122.0 BPM), and the 100 µg/L Cd^{2+} group was 20.2% higher (147.0 vs. 122.3 BPM). Notably, there was no significant difference in the magnitude of tachycardia induced by Pb versus Cd at any given concentration, suggesting a similar potency for disrupting cardiac rhythm despite their differing chemical properties.

3.3. Metal Exposure Induces Severe Morphological Abnormalities

The incidence and severity of morphological malformations were unequivocally concentration-dependent (Fig. 3, Fig. 4, Fig. 5). The most prevalent abnormalities were pericardial edema and yolk sac edema, which are classic histological indicators of cardiovascular dysfunction and osmoregulatory failure, respectively. Spinal curvature was also frequently observed.

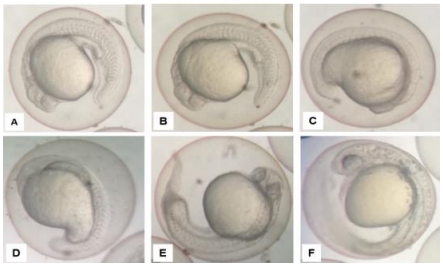

Figure 3: embryo survival rate causing toxicity in 48 hours

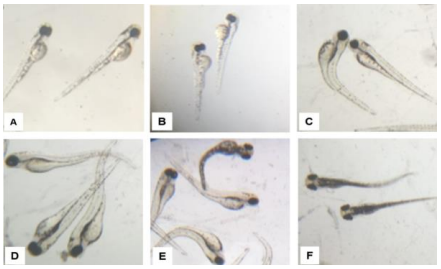

Figure 4: embryo survival rate causing toxicity in 72 hours

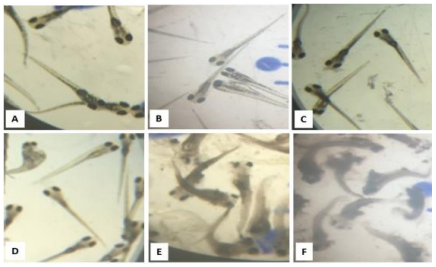

Figure 5: embryo survival rate causing toxicity in 168 hours

At 72 hpf, the percentage of malformed larvae in the 100 µg/L groups exceeded 80% for both metals. The 10 µg/L groups showed a significant ~25% incidence of malformations, which was drastically higher than the background rate of less than 2% observed in the control group. The types of malformations induced by both metals were qualitatively similar.

DISCUSSION

This study provides a comprehensive quantification of the adverse effects of Pb and Cd on zebrafish development, confirming significant lethality at high concentrations and, more importantly, revealing profound sublethal physiological disruptions at concentrations as low as 0.1 µg/L. The results robustly support our initial hypotheses.

The survival data are consistent with the known toxicological profiles of both metals. The delayed mortality, which became significant only after 48 hpf, suggests the accumulation of cellular damage over time. This is likely mediated through the generation of reactive oxygen species (ROS) and subsequent oxidative stress, which disrupts proteins, lipids, and DNA – processes that are critically important during rapid embryonic development (Liu et al., 2009). The marginally but statistically higher chronic toxicity of Cd at 168 hpf aligns with its well-documented higher redox activity and greater affinity for binding to sulfhydryl groups in proteins and other biological molecules compared to Pb, potentially leading to more efficient disruption of enzyme function and cellular integrity.

The most striking and consistent finding of this study is the metal-induced tachycardia. This finding appears to contradict some earlier studies that reported bradycardia (slowed heart rate) under heavy metal exposure (Zhang et al., 2015). However, it strongly supports a growing body of evidence indicating a stress-induced hypercardiac response (Hallare et al., 2005; Johnson et al., 2007). This apparent discrepancy can be explained by differences in exposure concentration, timing, duration, and specific metal species. We propose a mechanistic model for this tachycardia: Both Pb and Cd are known to induce oxidative stress, which can damage cardiomyocytes and impair their contractile efficiency and energy production (via mitochondrial dysfunction). The observed tachycardia may therefore represent a compensatory physiological mechanism to maintain adequate cardiac output and oxygen

delivery to developing tissues in the face of declining cellular efficiency. Alternatively, or concurrently, these metals may directly interfere with the function of cardiac ion channels (e.g., L-type calcium channels or potassium channels) that are crucial for the precise depolarization and repolarization cycles of the heart, leading to arrhythmia and increased automaticity. The significant tachycardia observed at the environmentally relevant concentration of 0.1 µg/L is a critical result. It demonstrates that standard survival-based assays, which showed no mortality at this level, vastly underestimate the toxicological threat of these metals. Consequently, heart rate emerges as a supremely sensitive, non-invasive, and quantifiable biomarker for sublethal toxicity assessment.

The high incidence of pericardial and yolk sac edema further corroborates the severe cardiotoxic and osmoregulatory stress induced by both metals. Edema formation is often directly linked to oxidative stress-induced damage to the endothelium and epithelium, leading to increased vascular permeability and failure of ionic pumps responsible for osmoregulation. Cadmium, in particular, is a known calcium antagonist, and its disruption of calcium signaling—vital for cardiac muscle contraction and cell adhesion—could be a primary driver of these malformations.

CONCLUSION AND FUTURE DIRECTIONS

In conclusion, this comparative study definitively demonstrates that: Lead and cadmium cause severe, concentration-dependent embryolethality in zebrafish, with cadmium exhibiting marginally but significantly higher chronic toxicity after 168 hours of exposure.

Both metals induce significant sublethal cardiotoxicity, manifesting as pronounced and consistent tachycardia, at all tested concentrations. This effect is detectable at environmentally relevant levels (0.1 µg/L), underscoring the inadequacy of mortality-based risk assessments.

Exposure leads to significant teratogenic effects, including pericardial and yolk sac edema, with malformation rates exceeding 80% at high concentrations (100 µg/L).

These findings have profound implications for ecological risk assessment. They highlight that relying solely on traditional mortality endpoints risks overlooking significant physiological harm that could impair reproduction, behavior, and long-term population resilience in wild fish populations exposed to chronic, low-level heavy metal pollution.

The consistent and paradoxical tachycardia warrants in-depth mechanistic investigation. We propose the following specific directions for future research:

Oxidative Stress Biomarkers: Quantify molecular markers of oxidative stress, such as lipid peroxidation (malondialdehyde - MDA levels), and the activity of key antioxidant enzymes (superoxide dismutase - SOD, catalase - CAT, glutathione peroxidase - GPx) in exposed larvae to directly establish a causal link between ROS generation and the observed phenotypic effects.

Transcriptomic Analysis: Employ RNA-sequencing (RNA-seq) to identify key gene expression changes in pathways critical for cardiac development (e.g., *tbx5*, *vmhc*, *myh6*), calcium handling, and oxidative stress response (e.g., *nrf2*, *keap1*). This would provide a systems-level understanding of the molecular initiating events.

Ion Channel Electrophysiology: Use patch-clamp techniques on isolated cardiomyocytes from exposed embryos to determine if Pb and Cd directly alter the activity of specific ion channels (e.g., L-type Ca^{2+} channels, hERG K^{+} channels).

Behavioral Ecotoxicology: Conduct high-throughput behavioral assays on exposed larvae to determine if the physiological cardiac stress translates into impaired escape responses, spontaneous swimming activity, or predator avoidance, thereby directly linking molecular and organ-level effects to ecologically relevant consequences.

REFERENCE

1. Lead exposure significantly increased the heart rate of zebrafish embryos (Johnson et al., 2007), while cadmium was found to decrease cardiac activity in both red sea bream (Cao et al., 2009) and zebrafish embryos (Hallare et al., 2005).
2. Fatoki, O. S., et al. (2012). "Bioaccumulation of metals in black mussels (Mytilus galloprovincialis) in Cape Town Harbour, South Africa." The Environmentalist 32: 48-57.
3. Mendil, D. and Ö. D. Uluözlü (2007). "Determination of trace metal levels in sediment and five fish species from lakes in Tokat, Turkey." Food chemistry 101(2): 739-745.
4. Swarnalatha, K., et al. (2015). "Risk assessment of heavy metal contamination in sediments of a tropical lake." Environmental Monitoring and Assessment 187: 1-14.
5. Swarnalatha, K., et al. (2015). "Risk assessment of heavy metal contamination in sediments of a tropical lake." Environmental Monitoring and Assessment 187: 1-14.
6. Kennedy, C. (2011). "The toxicology of metals in fishes." Encyclopedia of fish physiology: from genome to environment 3: 2061-2068.
7. Liu, G., et al. (2019). "Spatial and seasonal characteristics of dissolved heavy metals in the aquaculture areas of Beibu Gulf, South China." Human and Ecological Risk Assessment: An International Journal.
8. Burton Jr, G. A. (1991). "Assessing the toxicity of freshwater sediments." Environmental Toxicology and Chemistry: An International Journal 10(12): 1585-1627.

9. Karatzia, A. (2017). "The European Citizens' Initiative and the EU institutional balance: On realism and the possibilities of affecting EU lawmaking." Common Market Law Review 54(1).
10. Gouva, E., et al. (2020). "Comparative study of the effects of heavy metals on embryonic development of zebrafish." Aquaculture research 51(8): 3255-3267.
11. Barjhoux, I., et al. (2012). "Effects of copper and cadmium spiked-sediments on embryonic development of Japanese medaka (Oryzias latipes)." Ecotoxicology and environmental safety 79: 272-282
12. Liu, J., W. Qu and M. B. Kadiiska (2009). "Role of oxidative stress in cadmium toxicity and carcinogenesis." Toxicology and applied pharmacology 238(3): 209-214
13. Wang HongJun, W. H., et al. (2013). "Acute toxicity, respiratory reaction, and sensitivity of three cyprinid fish species caused by exposure to four heavy metals".
14. Johnson, A., E. Carew and K. Sloman (2007). "The effects of copper on the morphological and functional development of zebrafish embryos." Aquatic Toxicology 84(4): 431-438.
15. Cao, L., et al. (2009). "Cadmium toxicity to embryonic–larval development and survival in red sea bream Pagrus major." Ecotoxicology and environmental safety 72(7): 1966-1974
16. Hallare, A. V., et al. (2005). "Combined effects of temperature and cadmium on developmental parameters and biomarker responses in zebrafish (*Danio rerio*) embryos." Journal of Thermal Biology 30(1): 7-17.