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RESEARCH PAPER

Sperm Quality of Bocachico (*Prochilodus magdalenae*) Subjected to Acute Exposure to Cadmium and Lead

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Abstract

Heavy metal contamination, particularly cadmium (Cd) and lead (Pb), threatens aquatic ecosystems in Colombia. This study evaluated the acute effects of Cd, Pb, and their combinations on sperm quality of the vulnerable endemic species *Prochilodus magdalenae*. Treatments included three Cd doses (0.2, 0.8, 1.4 µg/kg), three Pb doses (2.0, 8.0, 14.0 µg/kg), five Cd+Pb combinations, and a control group (0.9% saline), administered via intramuscular injection. Sperm kinematic parameters, angularity indexes, and head oscillation were assessed 72 hours post-exposure using computer-assisted sperm analysis. Combined treatments significantly reduced motility; notably, the 0.2+8.0 µg Cd+Pb/kg group showed 78.1% motile sperm compared to 99.3% in controls. Reductions in sperm velocities (VCL, VSL) and alterations in lateral head amplitude (ALH) indicated cellular damage compromising sperm kinematics. Even low and moderate mixtures induced significant changes, suggesting additive or synergistic toxicity. Canonical discriminant analysis separated exposed groups from controls, confirming the sensitivity of sperm quality as a biomarker. Pb had a stronger individual impact on motility and movement duration, while Cd primarily affected velocity. The DP3 combination (0.8 µg Cd/kg + 14.0 µg Pb/kg) caused the most severe impairment across all variables. Short-term exposure to environmentally relevant Cd and Pb concentrations may seriously compromise sperm quality and, therefore, fertilizing capacity in *Prochilodus magdalenae* males.

Introduction

Heavy metals pose a serious threat to aquatic ecosystems due to their increasing industrial use. Their accumulation in organic tissues and limited metabolization represent significant risks to both human health and the environment. Effective strategies are needed to address the persistence of these contaminants and their ability to biomagnify in the food chain (Ferreira et al., 2023; Venkateswarlu & Venkatrayulu, 2020). While some metals like zinc, copper, nickel, cobalt, iron, and chromium are essential at low concentrations, excessive levels become toxic. In contrast, metals such as lead, cadmium, tin, and mercury have no known biological function and with toxicity increasing with concentration (Ali et al., 2019; Ferreira et al., 2023; Paschoalini & Bazzoli, 2021).

Water resources in Colombia suffer various environmental impacts, including chemical pollution, sediment accumulation, increasing turbidity, reduced flows, and alterations to natural waterways. Mining discharges contain high concentrations of heavy metals such as arsenic, cadmium, copper, chromium, iron, manganese, mercury, nickel, lead, and zinc, contaminating both exploited areas and interconnected environments through surface and groundwater flow (Mancera-Rodríguez & Álvarez-León, 2006). The Magdalena River, Colombia's main fluvial artery, receives harmful compounds from agricultural, mining, and livestock activities through direct discharges and runoff, threatening human populations and aquatic biota (Noreña Ramírez, 2012).

Among the affected species is the bocachico (*Prochilodus magdalenae*), a rheophilic species endemic to the Magdalena-Cauca basin, whose life cycle depends on migration and is one of the most economically important species in Colombian inland fisheries (Figure 1). Since the 1970s, habitat degradation due to anthropogenic factors—such as organic and inorganic pollution and loss of river connectivity—has caused a progressive decline in catches and reduction in average capture size, classifying the species as vulnerable (Mojica et al., 2012).

Heavy metals from water and their diet are accumulated by fish like *P. magdalenae*, which are prominent in the aquatic food chain (Authman, 2015). These contaminants enter the organism through gills, skin, and digestive tract, bioaccumulation in various tissues. The extent of this accumulation depends on environmental and biological factors, including water quality, temperature, species, and developmental stage (Shahjahan et al., 2022). The presence of heavy metals in aquatic organisms can cause adverse effects such as oxidative stress, cellular damage, and disruption of essential physiological processes, negatively affecting reproduction and population survival (Chakraborty, 2021; Dash et al., 2018; Gautam & Chaube, 2018).

Heavy metals such as lead, cadmium, chromium, and mercury are endocrine disruptors by interfering with hormonal signaling. With no known biological function, these elements alter protein conformation and protein-protein interactions, compromising endocrine homeostasis (Chakraborty, 2021; Schug et al., 2015; Vetillard & Bailhache, 2005). Their widespread presence in the environment and capacity for bioaccumulation in living organisms underscore the urgent need to elucidate the molecular mechanisms of their toxicity and develop effective strategies to mitigate their adverse effects on human and environmental health (Chakraborty, 2021).

Cadmium (Cd) is naturally released through processes like mineral weathering, forest fires, and volcanic activity, but its environmental concentration increases significantly due to human activities such as mining, fertilizer production, fossil fuel combustion, and waste disposal. These anthropogenic sources amplify its toxic effects on aquatic ecosystems and public health (Chakraborty, 2021; Mondal et al., 2018; Roy et al., 2021). Studies show Cd interferes with steroidogenesis, affecting the endocrine system and is classified as a metalloestrogen capable of altering reproductive processes in various organisms (Ankley & Johnson, 2004; Chakraborty, 2021), impairing reproductive function and leading to infertility in fish, birds, and mammals (Luo et al., 2015; Marettová et al., 2015; Vicentini et al., 2022).

Evidence indicates Cd exposure significantly affects fish reproduction, reducing gonadal steroid production

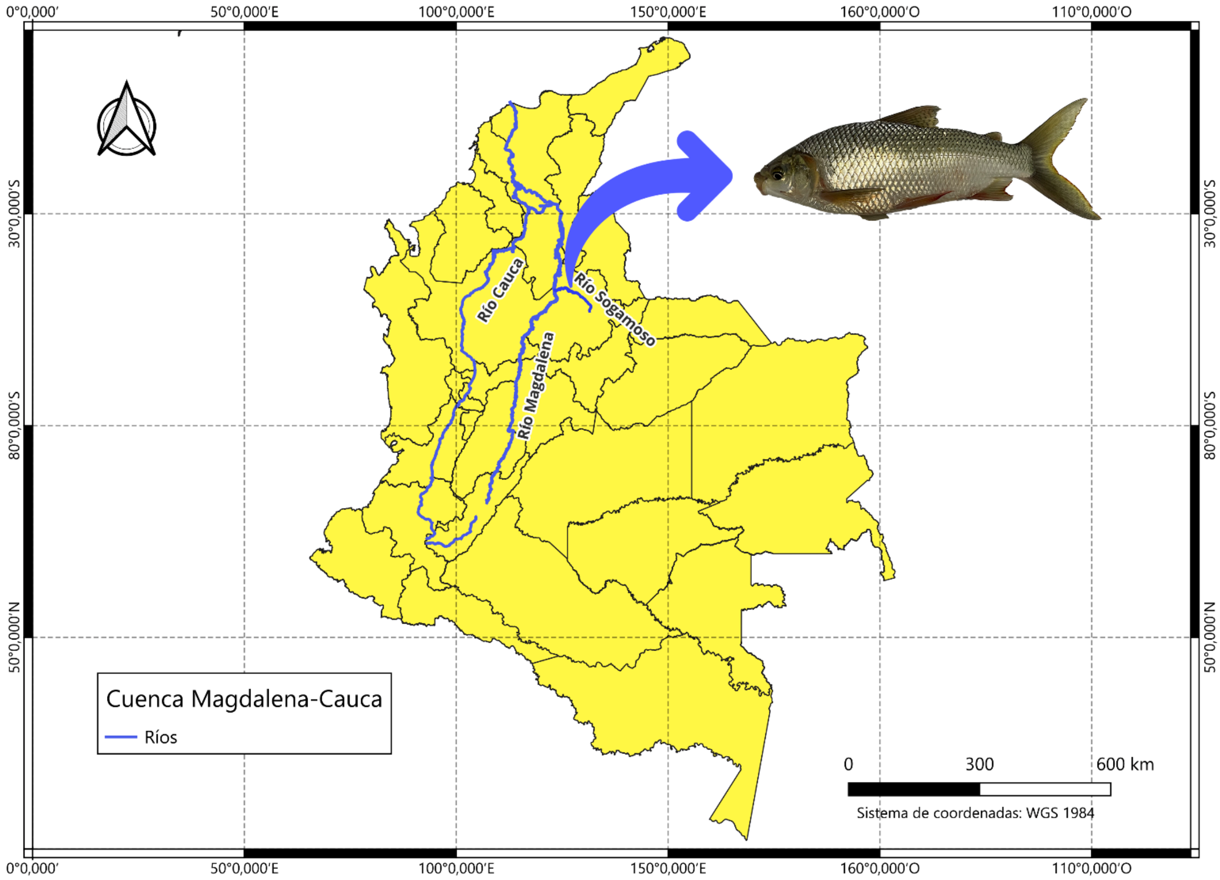

Figure 1. Distribution of Prochilodus magdalenae in the Magdalena-Cauca Basin, Colombia.

in both fish exposed to long-term pollution and those in polluted environments. Research in tilapia suggests even low Cd levels in semen can impair sperm quality and male fertility (Luo et al., 2015; Su et al., 2023). These effects may result from disturbances to the hypothalamic-pituitary-gonadal axis (Chakraborty, 2021). Additionally, Cd exposure induces histological alterations in gonads, including fibrosis and degeneration of spermatogenic elements, reducing sperm count, motility, and sperm viability (Gárriz et al., 2019; Sierra-Marquez et al., 2019).

Lead (Pb) is a prevalent substance in the environment, and it primarily accumulates in aquatic ecosystems due to human activities (Dignam et al., 2019; Ferreira et al., 2023). Pb concentrations in water bodies have greatly increased as a result of PVC-related processes, and the main sources are PVC pipes in sanitation systems and Pb-based paints from PVC recycling. Although PVC itself doesn't contain Pb, its widespread use and improper disposal contribute to environmental lead release, further aggravated by industrial activities like mining and battery manufacturing (Gumpu et al., 2015; Kumar et al., 2024).

Thus, it is necessary to conduct a study on acute exposure, since most existing research has focused on sperm quality under chronic exposure conditions. Notably, in the Magdalena-Cauca basin, where *P. magdalenae* is an endemic species, concentrations of heavy metals such as Cd and Pb have been increasing significantly. This trend is largely attributed to the expansion of hydroelectric, oil, and mining-energy industries in the surrounding areas. These contaminants can enter aquatic ecosystems suddenly, potentially impacting the fish physiology of resident species and, particularly, their reproductive capacity.

Therefore, considering the ecological importance of *P. magdalenae* and the increasing contamination of freshwater ecosystems with Cd and Pb, it was hypothesized that acute exposure to environmentally relevant concentrations of Cd and Pb would impair critical aspects of sperm motility. The primary objective was to determine whether changes in sperm motility could serve as sensitive and early biomarkers of sublethal metal exposure in native fish species.

Materials and Methods

The experiments were conducted on two-year-old mature *P. magdalenae* males, maintained in captivity at a density of 0.3 kg of fish/m³. The selected individuals (n=48) were allocated to twelve 250-L tanks (4 fish per tank), comprising 11 treatment groups and 1 control group (Table 1). Key water parameters, including dissolved oxygen (6.48±0.97 mg/L), oxygen saturation (80.9±11.2%), temperature (27.0±1.3°C), and pH (7.4±0.2), remained within the optimal range for *P. magdalenae* culture. The fish's initial mean weight, standard length (SL), and total length (TL) were 181.1±49.1 g, 22.1±2.1 cm, and 26.9±2.4 cm, respectively.

Broodstock fish from the Fishculture Research Institute of the University of Córdoba – CINPIC (Montería, Colombia) were transferred to a toxicology laboratory to establish baseline levels of Cd and Pb in their tissues. The purpose was to determine whether these metals were present in the fish before experimental exposure. The results indicated that Cd and Pb were not detected in any of the specimens analyzed. Based on the detection limits of the analytical instruments, experimental concentrations were defined as multiples above and below these thresholds. To ensure fish viability throughout the trial, lethal doses were not applied.

Cd was administered at three doses: 0.2 (Cd1), 0.8 (Cd2), and 1.4 µg/kg (Cd3), while Pb was administered at 2.0 (Pb1), 8.0 (Pb2), and 14.0 µg/kg (Pb3). Additionally, five Cd+Pb combinations were tested: 0.2–2.0 (Cd1+Pb1), 0.2–8.0 (Cd1+Pb2), 0.8–2.0 (Cd2+Pb1), 0.8–14.0 (Cd2+Pb3), and 1.4–8.0 µg/kg (Cd3+Pb2) (Table 1). Cd, Pb, Cd+Pb combinations, and a 0.9% saline solution (control) were administered via injection at the base of the pelvic fin. After 72 hours of exposure, sperm was collected by applying gentle abdominal pressure in a cephalocaudal direction. Fish were euthanized by thermal shock through immersion in water at -4°C. All handling and euthanasia procedures followed ethical guidelines for animal research and were approved by the Ethics Committee of the Universidad de Antioquia.

Table 1. Doses of Cd, Pb, and Cd+Pb combinations applied to *P. magdalenae*

Treatments	(µg/Kg)
Cd1	0.2
Cd2	0.8
Cd3	1.4
Pb1	2.0
Pb2	8.0
Pb3	14.0
(DP5) Cd1+Pb1	0.2+2.0
(DP1) Cd1+Pb2	0.2+8.0
(DP2) Cd2+Pb1	0.8+2.0
(DP3) Cd2+Pb3	0.8+14.0
(DP4) Cd3+Pb2	1.4+8.0
C (control)	0.0

At the end of the experiment, sperm samples were collected from each treatment group for sperm quality analysis. A 0.25 µL semen aliquot was placed in a Makler chamber (Sefi Medical Instruments Ltd., Haifa, Israel), activated with 75 µL of Milli-Q water (1:300 dilution), and analyzed using a computer-assisted sperm analysis system (SCA, Microptic SL, Barcelona, Spain) with a phase-contrast microscope (Nikon Eclipse E50i, Tokyo, Japan). The assessed parameters included total motility, motility type (rapid, medium, slow), total progressivity, curvilinear velocity (VCL), linear velocity (VSL), and average path velocity (VAP). Additionally, trajectory indexes (linearity [LIN], straightness [STR], and wobble [WOB]) and head oscillation/angularity parameters (mean angular displacement [MAD], beat-cross frequency [BCF], and lateral head displacement amplitude) were measured.

Sperm motility was classified as rapid for velocities exceeding 100 µm/s, moderate for velocities between 50 and 100 µm/s, and slow for velocities ranging from 10 to 50 µm/s. Spermatozoa with velocities below 10 µm/s were considered immotile. Sperm kinematic parameters were measured between 4 and 8 second post-activation. Motility duration was defined as the time elapsed from semen activation until approximately 90% of the spermatozoa ceased movement. Analyses were performed in triplicate for each male per treatment (n=1–4 fish).

Statistical Analysis

To assess differences among treatments, an analysis of variance (ANOVA) was performed after confirming data normality (Kolmogorov-Smirnov test) and homogeneity of variances (Bartlett's test). Proportional data were transformed using the arcsine square root method before conducting ANOVA. When assumptions were not met, the Kruskal-Wallis test was applied, followed by Dunn's test for multiple comparisons with the control group. The significance level was set at $P<0.05$. Data are presented as the median±standard error (SE).

Results

Table 2 presents the semen quality parameters of *P. magdalenae* following exposure to different doses of Cd and Pb in the Pb3 and Cd1+Pb2 treatments, compared to the negative control group (C). The results indicate that several parameters, including total motility (Mt), rapid motility (Rapid), slow motility (Slow), the proportion of immotile spermatozoa (Im), total progressivity (Pt), curvilinear velocity (VCL), average path velocity (VAP), wobble index (WOB), lateral head displacement amplitude (ALH), and beat cross frequency (BCF), showed significant alterations ($P<0.05$) in the treated groups compared to the control.

For instance, total motility was significantly reduced in the Pb3 (83.9±9.5%) and Cd1+Pb2 (78.1±12.0%) treatments compared to the control (99.3±0.5%). Conversely, rapid motility increased substantially in Cd1+Pb2 (32.0±7.5%) and Pb3 (22.3±9.8%) relative to the control (4.3±1.4%). However, other parameters, such as VSL, STR, and LIN, showed no significant differences between the Pb3 and Cd1+Pb2 treatments ($P>0.05$), suggesting a differential response depending on the combination and concentration of the evaluated metals.

The results indicated that sperm lateral head displacement amplitude (ALH) was significantly greater in the control group (2.01±0.12 µm) than in the DP1 (1.36±0.33 µm), P3 (1.48±0.45 µm), and P1 (1.60±0.26 µm) treatments, which exhibited significantly lower values. A similar trend was observed for head area, with the control group (16.46±0.27 µm²) displaying significantly higher values than P3 (14.29±0.62 µm²).

The beat cross frequency (BCF) was significantly higher in the control group (10.16±0.82 Hz) than in the DP1 (6.53±0.91 Hz) and P3 (7.60±1.53 Hz) treatments. Similarly, the percentage of hyperactivated spermatozoa was markedly greater in the control group (3.09±0.64%) compared to DP2 (0.86±0.74%) and DP1 (0.86±0.95%).

The linearity (LIN), wobble (WOB), and straightness (STR) indexes showed significant alterations in most treatments. For example, LIN was significantly higher in DP2 (48.09±6.41%) and D3 (47.37±3.16%) than in DP1 (35.20±1.53%). Similarly, WOB was also affected, with higher values observed in DP2 (87.53±1.82%) compared to DP1 (69.97±5.46%).

The percentage of immotile spermatozoa (IM) was higher in treatments such as DP1 (21.89±12.04%) compared to the control (0.66±0.47%). This pattern was further supported by motility (M), which was significantly reduced in DP1 (78.11±12.04%) relative to the control (99.34±0.47%). In contrast, DP4 showed a significant increase in motility (99.64±0.15%).

Regarding progressivity (PR), the DP4 (86.68±1.48%) and DP2 (84.30±6.22%) treatments exhibited a significant increase compared to the control (80.05±13.71%), whereas DP1 (36.84±19.19%) and P3 (49.16±21.57%) showed a significant decrease (Table 2).

Curvilinear velocity (VCL), linear velocity (VSL), and average path velocity (VAP) were also affected by exposure to Cd and Pb. VCL was significantly higher in the control group (142.23±7.83 µm/s) than in DP1 (75.05±26.39 µm/s). Similarly, VSL showed a decreasing trend in DP1 (29.53±7.52 µm/s) compared to the control (60.87±5.44 µm/s), while DP2 (65.67±13.33 µm/s) exhibited a significant increase. Regarding VAP, both DP1 (61.32±23.01 µm/s) and P3 (77.70±19.94 µm/s) showed a significant reduction relative to the control (122.38±6.85 µm/s) (Table 2).

Sperm velocities exhibited significant variations. The binary treatment DP1 (30.53±21.12 µm/s) showed a significant reduction in rapid velocity compared to the control group (83.93±0.83 µm/s). However, DP4 presented a similar value (83.93±0.83 µm/s) to the

Table 2. Sperm quality of *Prochilodus magdalenae* following acute exposure to different doses of Cd, Pb, and their combinations. Mt, total motility; Im, immotile spermatozoa; Pt, total progressivity; VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average velocity; LIN, linearity index; STR, straightness index; WOB, wobble index; ALH, lateral head displacement amplitude; BCF, beat cross frequency. Different letters indicate significant differences between columns (P<0.05). Treatments: Cd1 = 0.2 µg/kg, Cd2 = 0.8 µg/kg, Cd3 = 1.4 µg/kg, Pb1 = 2 µg/kg, Pb2 = 8 µg/kg, Pb3 = 14 µg/kg, Cd1+Pb1 = 0.2+2.0 µg/kg, **Cd1+Pb2 = 0.2+8.0 µg/kg**, Cd2+Pb1 = 0.8+2.0 µg/kg, Cd3+Pb2 = 1.4+8.0 µg/kg. Cd2+Pb3 = 0.8+14.0 µg/kg (individuals in this treatment did not survive)

Para-meter	C	Cd1	Cd2	Cd3	Pb1	Pb2	Pb3	Cd1+Pb1	Cd1+Pb2	Cd2+Pb1	Cd3+Pb2
Mt (%)	99.3±0.5^{a}	93.9±4.0^{ab}	93.5±10.2^{ab}	98.0±0.6^{a}	94.7±5.1^{ab}	97.6±1.9^{a}	83.9± 9.5^{b}	95.3±3.7^{ab}	**78.1±12.0^{c}**	96.9±0.4^{ab}	99.6±0.2^{a}
Rapid (%)	4.3±1.4^{a}	19.2± 8.0^{b}	11.1±10.3^{ab}	10.8±2.7^{ab}	20.4±11.6^{b}	12.1±8.0^{ab}	22.3±9.8^{b}	12.5±5.7^{ab}	**32.0±7.5^{c}**	6.4±1.1^{a}	6.7±2.3^{a}
Medium (%)	10.4±5.2^{ab}	14.9±1.6^{ab}	15.2±6.6^{ab}	12.3±0.4^{ab}	11.9± 4.2^{ab}	12.0±6.7^{ab}	12.5±3.3^{ab}	10.3±3.4^{ab}	15.5± 6.1^{ab}	10.8±8.2^{ab}	9.0±1.7^{a}
Slow(%)	85.3±4.4^{a}	66.0±7.2^{b}	73.7±15.9^{ab}	76.9±2.6^{ab}	67.4±14.8^{b}	75.9±9.5^{ab}	65.3±13.2^{b}	77.2±10.5^{ab}	**52.5±14.0^{c}**	82.9±8.5^{a}	84.9±2.2^{a}
Im (%)	0.7±0.5^{a}	6.1±4.0^{b}	6.5±10.2^{b}	2.0±0.6^{ab}	5.3± 5.1^{b}	2.4±1.9^{ab}	**16.1±9.5^{c}**	4.7±3.7^{b}	**21.9±12.0^{c}**	3.1±0.4^{ab}	0.4±0.2^{a}
Pt (%)	80.1±13.7^{a}	65.6±10.6^{b}	73.8±22.2^{ab}	79.8± 2.3^{a}	62.0±17.4^{b}	80.5±11.2^{a}	**49.2±21.6^{c}**	74.1±9.1^{ab}	**36.8±19.2^{c}**	84.3±6.2^{a}	86.7±1.5^{a}
VCL (µm/s)	73.7±9.1^{a}	51.9±7.5^{bc}	58.0±19.3^{ab}	67.5±5.7^{a}	51.7±17.8^{ab}	69.5±6.2^{a}	**41.6±19.5^{c}**	60.6±9.2^{ab}	**36.2±6.6^{c}**	69.1±11.0^{a}	74.9±3.7^{a}
VSL (µm/s)	30.2±7.6^{a}	22.0±1.6^{ab}	26.3±12.6^{ab}	33.0±5.1^{a}	24.2±6.7^{ab}	31.9±7.3^{a}	16.9±6.6^{b}	26.1±8.3^{ab}	12.8±2.9^{b}	28.4±4.3^{a}	33.8±7.1^{a}
VAP (µm/s)	45.1±8.3^{ab}	31.8±9.8^{b}	40.9±6.1^{ab}	44.2±3.6^{ab}	33.8±8.5^{b}	39.7±5.2^{ab}	**29.4±4.8^{c}**	41.6±6.2^{ab}	**28.1±5.7^{c}**	43.5±4.7^{ab}	46.7±3.9^{a}
LIN (%)	43.0 ± 5.1^{ab}	39.5±4.4^{b}	44.1±6,0^{ab}	47.4±3.2^{a}	45.3± 3.8^{ab}	42.9±3.1^{ab}	37.8±2.3^{b}	46.3±6.0^{a}	35.2±1.5^{b}	48.1±6.4^{a}	43.8±4.6^{ab}
STR (%)	50.0 ± 5.3^{ab}	47.9±5.8^{ab}	53.1±6.0^{a}	54.4±2.9^{a}	54.9±4.4^{a}	50.2±3.5^{ab}	47.7±3.0^{b}	54.7± 6.3^{a}	48.5±3.8^{ab}	53.4±5.9^{a}	50.7±5.2^{ab}
WOB (%)	84.4±0.6^{a}	80.9±1.4^{ab}	82.4±4.8^{ab}	84.8±1.4^{a}	80.2± 2.4^{b}	83.6±1.3^{a}	77.4±3.12^{b}	82.2±1.2^{ab}	**70.0±5.5^{c}**	87.5±1.8^{a}	84.6±2.0^{a}
ALH (µm)	2.0±0.1^{a}	1.6±0.3^{ab}	1.8± 0.4^{ab}	1.7±0.2^{ab}	1.6±0.3^{ab}	1.8±0.2^{ab}	1.5±0.5^{b}	1.8±0.2^{ab}	1.4±0.3^{b}	1.7±0.2^{ab}	1.9±0.2^{ab}
BCF (Hz)	10.2±0.8^{a}	8.2± 0.1^{bc}	10.1± 0.9^{a}	9.9± 0.4^{ab}	9.8± 0.7^{ab}	9.5±0.9^{ab}	7.6±1.5^{bc}	10.0±1.6^{a}	**6.5±0.9^{c}**	10.0± 0.8^{a}	10.0±1.0^{a}

control. Additionally, the pronounced decrease in rapid velocity in DP1 led to an increase in slow velocity (32.04±7.46 µm/s). Regarding individual treatments, P3 exhibited a significant reduction in rapid velocity (44.85±22.41 µm/s) alongside an increase in slow velocity (22.28±9.76 µm/s) (Table 2).

Discussion

Heavy metal contamination, particularly from Cd and Pb, significantly impacts fish sperm quality, compromising fertilizing capacity. These metals disrupt the hypothalamic-pituitary-gonadal (HPG) axis, a key regulator of reproductive function, by inducing oxidative stress and altering the hormonal communication between the brain and the gonads (M. Darbandi et al., 2018; Kumar et al., 2024). In addition, the high levels of reactive oxygen species (ROS) not only damage cellular structures directly but can also interfere with the pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, ultimately affecting the secretion of gonadotropins such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (da Costa et al., 2021; M. Darbandi et al., 2018).

In response to the above, sperm motility can be affected by heavy metals, considering that this is a fundamental indicator of male fertilizing capacity. In *P. magdalenae*, it was observed that the control group (C-) exhibited optimal seminal parameters, with 99.34% motility and 80.05% progressivity spermatozoa. However, exposure to Cd and Pb, especially in combined treatments such as DP1 (0.2 µg Cd/kg + 8.0 µg Pb/kg), led to a substantial decline in both motility (78.11%) and progressivity (36.84%). This decline is indicative of compromised sperm functionality, likely impairing fertilization success (Ferreira et al., 2023; Su et al., 2023). In the same way, the observed alterations in sperm kinematics are closely associated with oxidative stress, a mechanism by which both Cd and Pb exert their toxic effects. These metals increase the generation of reactive oxygen species (ROS) or reduce antioxidant defenses, leading to lipid peroxidation, protein damage, and mitochondrial dysfunction (M. Darbandi et al., 2018; S. Darbandi & Darbandi, 2016). Mitochondria are essential for ATP production, which fuels sperm motility; thus, their impairment can significantly reduce sperm velocity and movement efficiency (M. Darbandi et al., 2018).

In the same way, the decrease in progressivity is associated with a reduction in linear velocity (VCL) and average path velocity (VAP) (Acosta et al., 2016). For example, in P3 (1.4 µg Pb/kg), linear velocity dropped to 37.0 µm/s, compared to 60.87 µm/s in the control group. This suggests that Pb-induced toxicity impairs the ability of spermatozoa to maintain a linear trajectory, which is crucial for reaching the oocyte and achieving successful fertilization (Cosson et al., 2008). Furthermore, the lower linear velocity observed in binary treatments such as DP1 (0.2 µg Cd/kg + 8.0 µg Pb/kg) may be linked to an increased proportion of non-progressive spermatozoa (41.04%), indicating motor function deterioration. In addition, ROS not only exert a direct cytotoxic effect on sperm cells but can also disrupt hormonal regulation via the HPG axis. Cd and Pb have been shown to interfere with the pulsatile release of GnRH from the hypothalamus, altering the secretion of key gonadotropins such as FSH and LH (da Costa et al., 2021; Kumar et al., 2024). This disruption can compromise testicular steroidogenesis and impair spermatogenesis. Additionally, ROS generated during normal steroidogenic processes, if produced in excess, can inhibit further steroid production and damage spermatozoa membranes (Hanukoglu, 2006; Luo et al., 2006).

The straightness (STR) and linearity (LIN) indexes are key indicators of a spermatozoon's ability to maintain a direct and efficient trajectory toward the oocyte (Cosson et al., 2008). In the control group, the STR was 50.01%, while the LIN was 42.96%. However, in treatments such as DP1 (0.2 µg Cd/kg + 8.0 µg Pb/kg), both indices decreased (48.47% and 35.19%, respectively), indicating a negative impact of heavy metal exposure. This reduction may be associated with flagellar dysfunction, which affects sperm propulsion and its ability to swim in a straight path. Since a higher beat cross frequency (BCF) is correlated with increased sperm velocity, alterations in this parameter could further compromise motility (Boryshpolets et al., 2018; da Silva Castro et al., 2024). Consequently, morphological changes in sperm head area and flagellar function further support the presence of toxic stress. In DP1, head area and lateral head displacement (ALH) decreased relative to the control. While ALH is less characterized in fish species, studies in mammals suggest its association with fertilization potential (Cejko et al., 2013). Reduced ALH may reflect impaired flagellar motion and lower propulsive force, exacerbating the decline in progressive motility.

Another key parameter, mucus penetration, which reflects the capacity of spermatozoa to traverse the female reproductive environment (Ola et al., 2003), also declined in Cd- and Pb-exposed groups. In DP1, penetration dropped to 5.51%, compared to 11.23% in controls. This decrease aligns with the loss in progressivity and suggests that the ability of sperm to reach the oocyte under natural conditions may be significantly compromised.

Based on these findings, the alterations in the quality observed in spermatozoa exposed to Cd and Pb may result from cellular damage induced by the toxicity of these metals. Such alterations could compromise the ability of male gametes to maintain their integrity and functionality during fertilization. The reduction in movement amplitude suggests a diminished capacity of spermatozoa to generate the propulsive force necessary to reach and penetrate the oocyte, potentially leading to lower fertilization rates. Furthermore, this study demonstrates that acute exposure to Cd and Pb induces significant alterations in the seminal quality of *Prochilodus magdalenae*, affecting key parameters such as motility, velocity, and structural integrity. These findings highlight the importance of considering the sublethal effects of heavy metals on aquatic species reproduction and emphasize the need for environmental management measures to protect biodiversity and safeguard the health of aquatic ecosystems.

These findings highlight the complexity of heavy metal effects on aquatic species reproduction, particularly in combined exposure scenarios where Cd and Pb exhibit synergistic interactions. The registered decline in seminal quality in these treatments underscores the need to investigate pollutant interactions to better understand their effects under real environmental conditions better. Moreover, these results emphasize the importance of considering biological factors such as gonadal status and reproductive phase, which may influence fish responses to metal exposure.

The relevance of these findings lies in their potential application to environmental management and ichthyofauna conservation. By identifying the impact of heavy metals on the sperm quality of *P. magdalenae*, this study highlights the need for monitoring and regulatory strategies to mitigate aquatic pollution, particularly in areas where this species serves as a sentinel of ecosystem health. Moreover, integrating sperm motility assessment into toxicity studies represents a significant advancement in the early detection of contaminant exposure and assessment of aquatic ecosystem health.

Finally, sperm motility assessment has become a fundamental tool in evaluating the toxicity of environmental contaminants, particularly in aquatic species. Alterations in sperm movement induced by stressors such as heavy metals can serve as early indicators of exposure to harmful substances and as biomarkers for assessing the health of aquatic ecosystems.

Conclusions

In conclusion, this study shows that acute exposure to Cd and Pb negatively affects sperm quality in *P. magdalenae,* altering the kinematics of the sperm. The reduction in progressivity, motility, curvilinear velocity (VCL), and beat cross frequency (BCF) suggests impaired motor function, possibly linked to cellular damage and dysfunction of the hypothalamic-pituitary-gonadal axis. Additionally, decreased mucus penetration capacity and morphological alterations indicate that metal toxicity compromises fertilization potential. In fishculture, this may reduce the effectiveness of captive breeding programs, leading to economic and ecological consequences. Future studies should assess both acute and environmentally relevant exposures to understand the underlying mechanisms better and develop effective mitigation strategies.

Ethical Statement

The animal study was reviewed and approved by the National Aquaculture and Fisheries Authority—AUNAP of Colombia (Permit Number: 00071 of 2025).

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Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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