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Assessing the Lethal Concentration (LC_{50}) of Cadmium Chloride on Mediterranean Mussel (*Mytilus galloprovincialis*)

Levent Bat[1]*, Zeynep Hasançavuşoğlu[1]

[1]*Sinop University, Faculty of Fisheries, Department of Hydrobiology, 57500, Sinop, Türkiye.*

Abstract

(1) Background: Heavy metal pollution of industrial and domestic origin in aquatic ecosystems is a critical environmental problem that threatens human health through the food chain. This study aimed to determine the acute toxicity levels of cadmium chloride and early-stage ecotoxicological responses in the Mediterranean mussel (*Mytilus galloprovincialis*). (2) Methods: Mussels were obtained from the Sinop University Mussel and Oyster Cultivation Facility, with an average length of 40.48±0.82 mm and width of 20.58±0.42 mm. Triplicate 96-hour static bioassays were conducted in filtered seawater maintained at 17.78±0.03°C and pH 7.93±0.05. Organisms were exposed to cadmium concentrations of 0.5, 1, 5, 10, and 15 ppm, alongside a control group. (3) Results: During the first 24 hours of exposure, distinct physiological stress and avoidance behaviours-such as valve closure, immobility, and increased faecal production-were observed, particularly at high doses of 10 ppm and above. Based on mortality data at the end of the 96-hour observation period, a Probit analysis determined the median lethal concentration (LC_{50}) value for *M. galloprovincialis* to be 3.03326 ppm. (4) Conclusions: The findings indicate that *M. galloprovincialis* possesses a relatively high tolerance threshold to acute cadmium exposure. The numerical data obtained serve as an early warning criterion for food safety risk analysis and monitoring programs in regional bivalve aquaculture. Furthermore, this study provides a foundational baseline dataset for future sublethal chronic evaluations at lower concentrations and for elucidating the cellular mechanisms of cadmium toxicity in bioindicator species.

Keywords: *Mytilus galloprovincialis, Cadmium Chloride, Acute Toxicity, Median Lethal Concentration (LC_{50}), Bioindicator*

Introduction

Along with technological advancements, rapid transformations in economic, social, and cultural fields continuously shape human life. The continuity of these changes, combined with a growing global population, drives a quantitative and qualitative increase in the use of chemicals for industrial, agricultural, and domestic purposes. This widespread chemical utilization escalates air, soil, and water pollution through anthropogenic activities, leading to a rapid elevation of metal concentrations in aquatic ecosystems (Vutukuru, 2005; Karthikeyan, 2014). Agricultural runoff, sewage discharges, and the indiscriminate disposal of industrial effluents into water bodies constitute the primary causes of aquatic pollution (Cook et al., 1995). Sedentary aquatic organisms, such as the Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819), are directly affected by this negative trajectory, facing severe exposure to potentially toxic metal pollution resulting from both anthropogenic and geogenic (natural/crustal) activities in marine ecosystems (Bat and Arıcı, 2018).

The introduction of chemical substances into aquatic ecosystems renders toxic exposure inevitable across a broad biological spectrum, ranging from unicellular microorganisms like algae and bacteria to complex vertebrates such as fish and marine mammals. Toxicology, which investigates the damaging and destructive effects of physical or chemical factors on living organisms, plays a critical role at this juncture for environmental and public health. Accordingly, aquatic toxicology tests aim to determine the specific concentrations at which pollutants induce toxicity in the aquatic biota and the extent to which they disrupt the vital functions of these organisms (Bouldin et al., 2008).

As the leading species in bivalve aquaculture in Türkiye, *M. galloprovincialis* stands out as a robust model organism characterized by its lack of need for external feed supplementation, suitability for low-cost production systems, and eco-friendly cultivation features. Its protein-rich content and high nutritional quality render the aquaculture of these organisms highly advantageous compared to both other seafood and terrestrial protein sources. Offering a strategic alternative to meeting the increasing global demand for animal protein since the 1990s, this species is heavily preferred in coastal regions as a commercial and gastronomic seafood asset due to its high protein ratio and healthy unsaturated fatty acid profile (Akdemir, 2025).

In semi-closed basins like the Black Sea, where coastal inputs from industrial and agricultural activities are intense, metal accumulation levels in mussel tissues are recognized as sensitive biological indicators (bioindicators) for assessing environmental pollution and ecosystem health. As filter-feeding organisms, these bivalves accumulate potentially toxic metals and other persistent pollutants from the surrounding environment, serving as a biological bridge that transfers these contaminants directly to humans through the food chain.

Therefore, analysing the pollution loads of the stations where mussels are gathered and regularly monitoring bioaccumulation processes prior to the harvesting and consumption stages are essential to determining the current state of the aquatic ecosystem (Bat et al., 1999; Yarsan et al., 2007; Küçüksezgin et al., 2010). Such monitoring studies constitute a legal and practical imperative for protecting public health, particularly by preventing products harvested from high-risk contaminated areas from entering the market (Jović et al., 2011).

Considering all these ecological, economic, and epidemiological factors, alongside its strategic importance for the region, *M. galloprovincialis* was selected as the live material for this study. Within the scope of this preliminary research, acute toxicology tests using cadmium chloride ($CdCl_2$) were performed on specimens obtained from the Mussel and Oyster Cultivation Facility. Through this preliminary investigation, it is aimed to contribute to the infrastructure for regular health monitoring of local production areas and to reveal potential metal pollution levels using updated, scientific data.

Materials and Methods

Biological Material

The Mediterranean mussels (*Mytilus galloprovincialis*), which constitute the live material of this study, were obtained from the Mussel and Oyster Cultivation Research Facility operating under the Faculty of Fisheries at Sinop University. The specimens were collected manually from the main longlines located within the production area of the facility.

Transportation and Acclimatization

To minimize transport-induced stress factors, the collected live specimens were immediately placed into plastic containers filled with seawater from the sampling site. During transportation to the faculty laboratory, the samples were protected from direct sunlight and maintained under appropriate temperature and dark conditions.

Upon arrival at the laboratory, the mussels were transferred to aquariums filled with natural seawater supported by a continuous aeration and filtration system. Prior to placement in the stock tanks, the outer shell surfaces of each individual were mechanically cleaned to eliminate potential microorganisms, epibionts, and foreign debris. Following the cleaning process, the organisms were transferred to a pre-prepared and conditioned seawater system, where they underwent a 2-day depuration (starvation) period to clear metabolic wastes and evacuate their digestive tract contents.

Acute Toxicity Assays (LC_{50})

Acute lethal concentration (LC_{50}) tests were performed to determine the heavy metal resistance threshold of the laboratory-acclimatized *M. galloprovincialis* individuals. Prior to the definitive experiment, range-finding preliminary tests were conducted to establish the sensitivity limits of the species. Accordingly, a definitive experimental design was established in triplicate, comprising a control group and five cadmium chloride ($CdCl_2$) exposure concentrations: 0.5, 1.0, 5.0, 10.0, and 15.0 ppm (Table 1).

Table 1. Experimental design and exposure concentrations established for cadmium (ppm).

Replicates	*Control*	Cd_1	Cd_2	Cd_3	Cd_4	Cd_5
I	0.0	0.5	1.0	5.0	10.0	15.0
II	0.0	0.5	1.0	5.0	10.0	15.0
III	0.0	0.5	1.0	5.0	10.0	15.0

Before commencing the exposure phase, biometric parameters, including shell length (mm) and width (mm), were precisely measured for each individual. Following these measurements, the mussels were randomly and gradually distributed into the experimental chambers across the respective concentration groups. Throughout the experiment, continuous low-flow aeration was applied to each setup to maintain dissolved oxygen levels within ideal physiological limits.

To ensure that toxicity results were not compromised by degrading water quality, water parameters (pH, temperature, and dissolved oxygen) were monitored daily. Dissolved oxygen levels were maintained above 60% saturation throughout the 96-hour period via continuous gentle aeration. The 2-day depuration period also ensured that no pseudo-faces or excessive metabolic ammonia accumulated in the test chambers prior to chemical dosing.

Organism mortality and behavioural responses were monitored and recorded at regular intervals during the observation period. The acute toxicity tests were conducted via a 96-hour static bioassay method. The exposure concentration ranges, spanning the spectrum between the "no observed mortality rate" and "complete mortality rate," were determined in strict compliance with the standardized guidelines of the USEPA (1998, 2016). Dead individuals were counted and recorded every 24 hours and promptly removed from the experimental chambers to prevent any deterioration of water quality. In accordance with the nature of acute static bioassays, no feeding was provided to the organisms during the 96-hour test period.

Results

Throughout the 96-hour static bioassays conducted in filtered seawater, the average water temperature was maintained at 17.78±0.03°C, and the pH remained stable at 7.93±0.05. The biometric measurements of the experimental mussel specimens revealed an average shell length of 40.48±0.82 mm and an average shell width of 20.58±0.42 mm.

Behavioural and Physiological Alterations (Valve Activity and Stress Responses)

During the acute toxicity tests, prominent dose- and time-dependent behavioural avoidance mechanisms and physiological stress responses were recorded in the mussels due to cadmium exposure.

During the first hour of the adaptation period, individuals in the control group exhibited complete adaptation to the environment. In contrast, mussels exposed to the 10-ppm concentration remained immobile, exhibiting a distinct avoidance behaviour where the foot became trapped between the two valves. By the 6th hour of exposure, valve closure behaviour became dominant in the high-dosage groups (≥10 ppm) as a protective mechanism against toxic stress, accompanied by a noticeable increase in faecal production.

In the early phase of toxicity exposure (first 24 hours), acute avoidance behaviours and physiological stress indices-such as prolonged valve closure, complete immobility, and excessive faecal production-were explicitly recorded, particularly at high concentrations of 10 ppm and above.

Lethal Effects and Alterations in Environmental Parameters

The physiological differentiation between the control and experimental groups became more pronounced from the 24th hour of exposure onward. The control group individuals exhibited a 100% survival rate and maintained normal filtration activities until the end of the experiment.

Conversely, in the cadmium-exposed groups, a complete loss of mobility, an increase in mortality cases, and intensive foaming in the experimental water were observed starting from the 24th hour.

Estimation of the Cadmium Chloride LC_{50} Value

Based on the data obtained from the exposure groups, which included the control and five concentration levels (0, 0.5, 1.0, 5.0, 10.0, and 15.0 ppm), the median lethal concentration (LC_{50}) value of cadmium chloride for *M. galloprovincialis* was determined to be 3.03326 ppm (Table 2). The Probit Analysis method developed by Finney (1952) was utilized to determine the lethal concentration (LC_{50}) values, and calculations were executed via a specialized algorithm software tailored for this methodology (Mekapogu, 2021).

Table 2. Probit analysis results and the 96-hour median lethal concentration (LC_{50}) of cadmium (Cd) for *Mytilus galloprovincialis*.

Lethal Concentration	*Value (ppm)*	*95% Confidence Interval (Lower Limit - Upper Limit) (ppm)*
LC_{50}	3.03326	1.97708 - 4.65367

The relationship between cadmium exposure concentrations and the mortality percentage of *M. galloprovincialis* was further evaluated using a polynomial regression model (Figure 1). The generated regression equation was determined as $y = -0.7002x^2 + 17.157x$, exhibiting an exceptionally strong coefficient of determination ($R^2 = 0.9918$), which demonstrates a highly predictable and dose-dependent lethal effect.

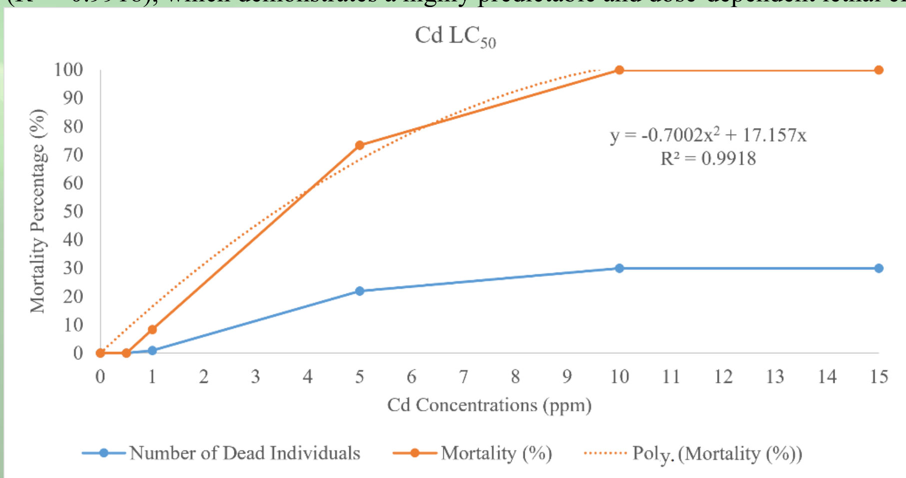

Figure 1. Dose-response curve and polynomial regression model showing the mortality percentage of *Mytilus galloprovincialis* exposed to various cadmium (Cd) concentrations over 96 hours.

Discussion

The immediate behavioural and physiological responses-specifically valve closure (isolation) and foot restriction-observed within the first 1 to 6 hours in mussels exposed to cadmium in this preliminary study confirm the acute physiological disruption induced by this heavy metal. This behavioural transition from protective valve closure to neuromuscular paralysis is strongly supported by Yasmeen and Pathan (2021), who reported that while freshwater bivalves (*Lamellidens marginalis*) tightly close their shells during the initial hours of cadmium exposure as an avoidance mechanism, prolonged stress leads to an irreversible failure of adductor muscles, forcing the valves to remain widely open until death. Furthermore, their observations regarding heavy mucus extrusion and the exacerbating role of elevated temperatures on cadmium toxicity align perfectly with the intense foaming and physiological stress recorded in our experimental groups, confirming that exogenous environmental factors and time-dependent adductor muscle dysfunction collectively dictate bivalve survival thresholds under acute metal stress.

In toxicity assays conducted with aquatic organisms, the parameters LC_{50} (Median Lethal Concentration), representing the toxicant concentration that kills 50% of the test population, and EC_{50} (Median Effect Concentration), representing the concentration that induces a specific target response in half of the population, are widely utilized as standard benchmarks for toxicity assessment.

The relatively high acute tolerance threshold (LC_{50} = 3.03326 ppm) exhibited by *M. galloprovincialis* in this study aligns with the well-known physiological resilience of bivalves. This resistance is often attributed to internal detoxification mechanisms, such as the synthesis of metallothioneins—low-molecular-weight, cysteine-rich proteins that safely bind and immobilize free cadmium ions within intracellular compartments (e.g., lysosomes), thereby preventing systemic enzyme inhibition. Additionally, the low-salinity nature of the Black Sea may influence ionoregulation and the bioavailability of free metal ions (Cd^{2+}), which warrants further physicochemical investigation.

The incorporation of a post-exposure monitoring phase in toxicological bioassays is crucial for capturing delayed lethality and long-term recovery dynamics, which are often masked during standard observation windows (Alonso, 2023). Evaluating post-exposure outcomes reveals a heightened sensitivity in aquatic organisms, as physiological injuries sustained during initial contact can progress over time, even after the toxicant is removed. Conversely, the behavioural and metabolic recovery observed in surviving individuals highlights the activation of efficient internal detoxification pathways among highly tolerant phenotypes. This latent toxicity framework suggests that the 96-h LC_{50} threshold determined for *M. galloprovincialis* in the current study could trigger progressive, non-immediate stress, emphasizing that environmental risk assessments must account for both post-exposure latency and individual detoxification capacities to accurately predict bivalve population survival.

Estimating ecotoxicological thresholds such as the LC_{50} value for *M. galloprovincialis*, a species with a high potential for transferring metals to humans via the food chain, plays a fundamental role in evaluating both the pollution pressure exerted on marine ecosystems and the indirect risks posed to public health. The adverse physiological reactions and toxicity rates detected in these bivalves, even under short-term exposure, function effectively as an ecological early warning system capable of identifying potential environmental hazards prior to severe ecological degradation.

In a similar toxicological context, Bat et al. (2013) calculated LC_{50} values during acute (96-hour) and chronic (28-day) bioassays investigating the toxicity of copper, lead, and zinc in Mediterranean mussels. They emphasized that such ecotoxicological monitoring parameters are crucial for shedding light on legal regulations, ensuring the safe consumption of seafood, and managing environmental pollution. The high tolerance threshold (LC_{50}= 3.03326 ppm) determined for cadmium in the present study further contributes to understanding the bioindicator capacity of *M. galloprovincialis* harvested from regional aquaculture structures under acute chemical stress.

The observed variations in acute tolerance and the subsequent failure of neuromuscular control in larger bivalves can be closely linked to the complex toxicokinetic feedback between body size, age, and weight-specific filtration rates. Although smaller or younger individuals possess a higher surface-area-to-volume ratio, they process a structurally lower absolute volume of contaminated water during acute exposure. Conversely, larger individuals subject their expansive gill surfaces to continuous, high-volume water currents, rapidly accelerating the influx of free Cd^{2+} ions. Consistent with these size-dependent dynamics, younger bivalves often exhibit higher baseline metallothionein levels than their older counterparts, providing a greater physiological buffer against rapid heavy metal influx (Wallace et al., 2003). This enhanced cellular buffering capacity in smaller individuals is frequently accompanied by an accelerated growth dilution effect, wherein the rapid increase in soft tissue mass prevents internal concentrations of contaminants from reaching lethal thresholds (Otchere, 2022).

On the other hand, larger bivalves face increased physiological stress due to their reduced metabolic activity, which leads to longer equilibration times and slower depuration of accumulated metals (Bates et al., 2021). In these larger organisms, the massive volumetric water exposure leads to early tissue saturation, triggering a 'spillover' effect where unbound cadmium overwhelms the calcium-regulated pathways essential for adductor muscle contraction (Wallace et al., 2003; Geffard et al., 2007).

Interestingly, while Wallace and Luoma (2003) also hypothesized that size-dependent uptake rates could concurrently increase the sensitivity of smaller bivalves under specific scenarios, our findings with adult *M. galloprovincialis* clearly demonstrate that the physical advantage of a lower absolute filtration volume combined with enhanced cellular buffering decisively overcomes such risks during acute cadmium pulses. This outcome underscores that variations in efflux rates and age-dependent physiological constraints are not strictly uniform across different life stages and species (Cadmus et al., 2019; Alonso, 2023). This allometric complexity is further illuminated by Cao et al. (2025), who revealed that age exerts a more pronounced influence on trace metal accumulation dynamics than organismal size alone. Their findings indicate that older bivalves exhibit a significantly diminished bioaccumulation capacity, fundamentally driving negative correlations between body size and metal concentration. In the context of our study, this age-dependent decline in metabolic efficiency explains why larger, older *M. galloprovincialis* present a lower threshold of tolerance during acute shocks; their compromised internal machinery is less capable of dynamically partitioning and binding influxing contaminants compared to younger cohorts. Consequently, incorporating age-driven physiological shifts alongside pure physical dimensions is paramount for refining the interpretation of marine biomonitoring data and accurately evaluating environmental risk factors.

Conclusion

Unregistered activities within the bivalve aquaculture sector in Türkiye complicate the precise determination of consumption rates, while potential toxic metal pollution in mussels harvested from unmonitored areas poses a severe gap in public health protection (Aytekin et al., 2019; Bat et al., 2023). The bioaccumulation and biomagnification characteristics of *M. galloprovincialis* present a chronic threat to aquatic ecosystems, and the consumption of contaminated organisms serves as a direct source of heavy metal exposure for humans (Aytekin et al., 2019; Bat et al., 2023). Because *M. galloprovincialis* possesses a high potential to transfer metals to humans via the food chain, an in-depth investigation of this risk is a critical imperative for food safety (Bat et al., 2023). Accordingly, static acute and chronic toxicity tests play a fundamental role in evaluating the pollution pressure exerted on mussels and their potential for food chain transfer by establishing baseline toxicity benchmarks such as the LC_{50} value (Bat et al., 2023).

In comparative risk assessments, the wet weight-based limits established by FAO/WHO (1986) standards (upper limits of 0.1–0.5 mg kg^{-1} for Cd, 20 mg kg^{-1} for Cu, and 1 mg kg^{-1} for other trace elements) and current national/international regulations (EC, 2023; TGK, 2023; setting a maximum limit of 1.0 mg kg^{-1} for Cd) serve as foundational guidelines for determining acceptable risk thresholds for human health (Bat et al., 2021). To ensure the sustainability of public health and food safety, enforcing legal prohibitions on the human consumption of mussels harvested from high-risk, contaminated regions that exceed these established limits is mandatory.

This preliminary study calculating the median lethal concentration provides a concrete ecotoxicological foundation for the effective enforcement of legal thresholds and the preservation of the Black Sea marine ecosystem. The generated data establish a fundamental and referential baseline dataset for future sublethal chronic bioassays designed at lower concentrations, which are required to elucidate the cellular-level toxic mechanisms of cadmium in bioindicator species.

In conclusion, the numerical benchmarks generated in this preliminary study do not only provide a reference baseline for future sublethal and cellular-level chronic bioassays, but they also serve as vital mathematical inputs for localized human health risk models, such as Target Hazard Quotient (THQ) and Estimated Daily Intake (EDI) assessments, ensuring the long-term economic and biological safety of regional bivalve aquaculture.

Declarations

Ethical Approval

The authors declare that the experimental procedures conducted in this study do not require Ethical Committee approval.

Author Contribution Statement

- Zeynep HASANÇAVUŞOĞLU: Data curation, Investigation, Writing – original draft.
- Levent BAT: Formal analysis, Supervision, Conceptualization, Methodology, Writing – review & editing.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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