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DAPHNIA MAGNA INDICATOR OF WATER POLLUTION WITH ZINC

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Abstract

Heavy metal contamination represents a persistent threat to aquatic ecosystems due to its toxicity and long-term environmental persistence. Among these metals, zinc plays a dual role as an essential micronutrient at low concentrations but becomes toxic when present in excess. The present study evaluates the acute toxicity of zinc on the freshwater crustacean Daphnia magna, a widely used bioindicator species in ecotoxicological assessments. Toxicity experiments were conducted using standardized Daphtoxkit bioassays in which neonates of D. magna were exposed to zinc concentrations ranging from 1.0 to 6.0 mg/L under controlled laboratory conditions. Mortality rates were recorded at four-hour intervals over an exposure period of 36 hours. The results revealed a strong concentration- and time-dependent increase in mortality. At the lowest concentration tested (1.0 mg/L), mortality reached 20% after 16 hours and increased to 60% after 36 hours. In contrast, the highest concentration (6.0 mg/L) induced rapid toxicity, producing 80% mortality within four hours and complete mortality after twelve hours of exposure. These findings highlight the high sensitivity of D. magna to elevated zinc concentrations and confirm its suitability as an effective biological indicator for monitoring heavy metal contamination in freshwater ecosystems. The results contribute to a better understanding of zinc-induced toxicity mechanisms in aquatic invertebrates and provide valuable information for environmental monitoring and risk assessment of metal pollution.

Key words*: Daphnia magna, mortality rate, toxicity, zinc.*

INTRODUCTION

The contamination of aquatic environments with heavy metals represents one of the most persistent environmental challenges associated with industrialization and modern agricultural activities. Due to their chemical stability and limited biodegradability, heavy metals tend to accumulate in aquatic ecosystems and can exert long-term toxic effects on living organisms (Ali et al., 2019; Pandey & Keshavkant, 2021). Their presence in water bodies may disrupt ecological balance and pose risks to aquatic biodiversity as well as to human health through the contamination of water resources.

Among heavy metals, zinc occupies a particular position because it plays a dual role in biological systems. At low concentrations, zinc is considered an essential micronutrient involved in numerous physiological processes, including enzyme activation, gene expression, and protein synthesis (King, 2011). Several studies have highlighted the importance of zinc in maintaining cellular metabolism and proper organismal functioning (Bellido et al., 2022; Paylar et al., 2022; Oprea et al., 2024). However, when present in elevated concentrations, zinc may become toxic and cause adverse effects on aquatic organisms (DeForest & van Genderen, 2012; Clearwater et al., 2002).

The detection and monitoring of aquatic pollution can be effectively achieved using biological indicators, also known as bioindicators. These are living organisms that respond to environmental changes and can provide valuable information about ecosystem health. Bioindicators may include microorganisms, plants, and animals whose physiological responses can signal the presence of pollutants even at low concentrations (Zaghloul et al., 2020; Simionov et al., 2022). Their use is particularly valuable because they can detect the early stages of environmental contamination and provide insights into the biological effects of pollutants (Wang & Guan, 2010; Parmar et al., 2016; Kadim & Risjani, 2022).

Several aquatic organisms have been widely employed in ecotoxicological testing to assess water pollution. Among these are the freshwater crustacean *Thamnocephalus platyurus*, the

rotifer *Brachionus calyciflorus*, the protozoan *Tetrahymena thermophila*, the cladoceran *Ceriodaphnia dubia*, the aquatic plant *Spirodela polyrhiza*, and the microalga *Pseudokirchneriella subcapitata*. While a variety of aquatic organisms are used as bioindicators, they are mentioned here to provide context, with the present study specifically focusing on *Daphnia magna* due to its high sensitivity and established role in standardized ecotoxicological testing.

One of the most widely used organisms in freshwater ecotoxicology is the cladoceran *Daphnia magna*, commonly known as the water flea. This species plays a crucial ecological role in freshwater ecosystems such as ponds and lakes, where it acts as a primary consumer by filtering suspended particles and microorganisms from the water column. Through this filtering activity, *Daphnia magna* contributes significantly to water clarity and nutrient cycling within aquatic ecosystems

Acute toxicity tests involving freshwater daphnids have become internationally recognized tools for evaluating the toxic effects of chemical substances and for monitoring contaminated water and industrial effluents (Persoone et al., 2009; Fekete-Kertész et al., 2016; Sönmez et al., 2022). The extensive use of *Daphnia magna* in ecotoxicological studies is mainly due to its high sensitivity to pollutants, rapid reproduction, short life cycle, and parthenogenetic reproduction (Koivisto, 1995). In addition, it offers practical advantages such as low experimental costs, standardized protocols, and reproducible results within short testing periods (Kim et al., 2003).

Considering the increasing presence of heavy metals in aquatic ecosystems and the potential ecological consequences of their accumulation, it is essential to evaluate their effects on sensitive aquatic organisms. Therefore, the main objective of the present study was to investigate the toxic effects of zinc at different concentrations on *Daphnia magna* and to assess the relationship between pollutant concentration, exposure time, and mortality rate in this bioindicator species.

MATERIALS AND METHODS

The experimental study was carried out using the *Daphtoxkit F magna toxicity test system,* produced by MicroBioTests Inc. (Belgium, Figure 1). This standardized bioassay kit contains the necessary materials for performing acute toxicity tests on *Daphnia magna*, including concentrated salt solutions used for preparing the test medium, micropipettes, a microsieve, Petri dishes, spirulina owder for feeding the organisms, and dormant ephippial eggs of *Daphnia magna*.

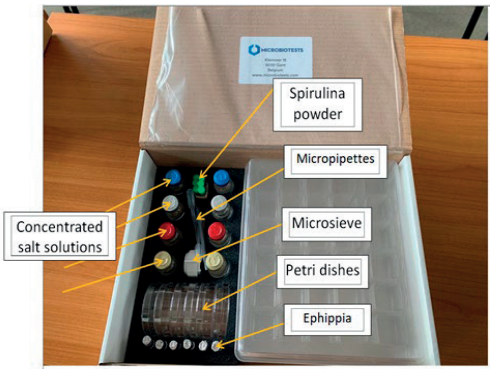


Figure 1. Daphtoxkit *F magna*

Preparation of standard freshwater

A Standard Freshwater solution was prepared according to the protocol described in ISO 6341:2012, which is commonly used in acute toxicity tests involving freshwater crustaceans.

The solution was obtained by adding the following concentrated salt solutions to one liter of distilled water: $NaHCO_3$ with a concentration of 64.75 mg/L (12 mL), $CaCl_2 \cdot 2H_2O$ with a concentration of 294 mg/L, $MgSO_4 \cdot 7H_2O$ with a concentration of 123.25 mg/L, KCl with a concentration of 5.75 mg/L.

After the addition of these components, the solution was diluted with distilled water to obtain a final volume of 2000 mL, and the mixture was thoroughly homogenized to ensure uniform distribution of the salts.

This prepared solution served both as the hatching medium for ephippia and as the dilution medium for preparing pollutant concentrations.

Preparation of zinc pollutant solution

The pollutant solution used in the experiments consisted of zinc sulfate ($ZnSO_4$) dissolved in distilled water. The physicochemical parameters of the test solutions (pH, dissolved oxygen, and conductivity) were maintained within the recommended ranges specified by ISO

6341:2012. A stock solution with a concentration of 104.5184 mg/L $ZnSO_4$ was prepared and subsequently used to obtain the experimental concentrations through serial dilution with Standard Freshwater.

From the stock solution, six working concentrations of zinc sulfate ($ZnSO_4$) were prepared: 1.0 mg/L, 2.0 mg/L, 3.0 mg/L, 4.0 mg/L, 5.0 mg/L, 6.0 mg/L.

Each concentration represented a separate experimental treatment. All experimental variants were performed in four independent replicates in order to ensure reliability and reproducibility of the results.

The prepared solution served both as the hatching medium for ephippia and as the dilution medium for preparing pollutant concentrations.

Hatching of Daphnia magna ephippia

Dormant eggs (*ephippia*) of *Daphnia magna* were first rinsed using tap water in order to remove any residues of the storage medium. The ephippia were then placed in a microsieve and transferred into Petri dishes containing 10 mL of pre-aerated Standard Freshwater.

The Petri dishes were covered with lids and incubated for approximately 80 hours at a controlled temperature of 20–22°C under continuous illumination. These conditions allowed the hatching of the ephippial eggs and the emergence of neonate individuals.

Feeding of Neonates

After the hatching process was completed, the newly emerged *Daphnia magna* neonates were provided with spirulina powder as a food source. Feeding was performed shortly after hatching in order to ensure the physiological stability of the organisms before the toxicity testing began. Two hours after feeding, the neonates were selected for use in the toxicity experiments (Figure 2).

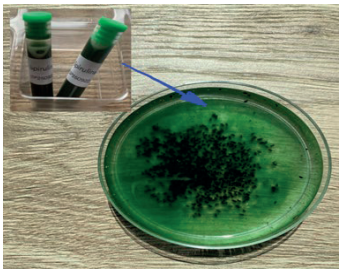


Figure 2. Spirulina feeding

Toxicity testing procedure

For each experimental condition, five neonates of *Daphnia magna* were introduced into test plates containing 10 mL of zinc-contaminated Standard Freshwater at the predetermined concentrations. The experimental procedure followed the standardized protocol described in ISO 6341:2012. The use of five neonates per test plate is consistent with Daphtoxkit guidelines and contributes to the reproducibility and reliability of the results. To prevent evaporation of the test solution during the incubation period, the plates were sealed with parafilm strips and covered with a transparent lid.

The experimental plates were then incubated under controlled conditions in darkness at a constant temperature of approximately 22°C (Figure 3).

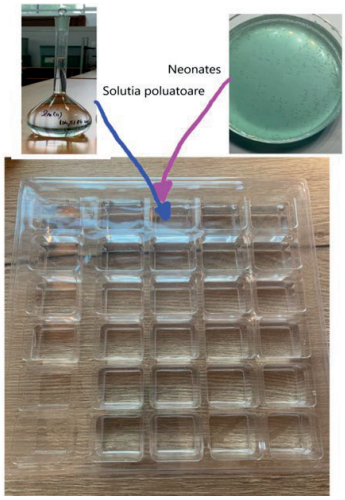


Figure 3. Testing plates

Monitoring of organism response

The response of *Daphnia magna* to zinc exposure was monitored throughout the experimental period. Observations were conducted every four hours, during which the number of surviving and dead organisms was recorded.

Mortality was determined based on the absence of movement of the organisms when observed under standard laboratory conditions.

Calculation of mortality rate

The mortality rate of the organisms was calculated using the following equation:

$$R_m = \frac{N_i - N_f}{N_i} \cdot 100\ (\%) \quad (1)$$

where:
Rm represents the mortality rate expressed as a percentage (%);
Ni represents the initial number of *Daphnia magna* individuals introduced into the test plate;
Nf represents the number of surviving individuals recorded during observation.
In addition to mortality rate, the inhibitory effect of the pollutant was evaluated by comparing the results obtained in the contaminated samples with those observed in the control group, where the organisms were maintained in uncontaminated Standard Freshwater.

RESULTS AND DISCUSSIONS

Effects of zinc exposure on the survival of Daphnia magna

After the hatching period and the initial feeding stage, the newly emerged *Daphnia magna* neonates were transferred to freshwater containing different concentrations of zinc (Figure 4).



Figure 4. *Daphnia magna* (water fleas)

During the experimental exposure period, the organisms were not provided with additional food. The survival of the water fleas was monitored throughout the experiment in order to evaluate the influence of zinc concentration and exposure time on organism mortality.
The observations revealed that *Daphnia magna* individuals were able to survive in zinc-contaminated freshwater for a maximum exposure period of approximately 36 hours. However, their number progressively decreased as both the zinc concentration and exposure duration increased.
The experimental results, summarized in Table 1, indicate a clear concentration-dependent reduction in the number of surviving organisms. The values presented represent mean results obtained from four independent replicates for each concentration and exposure time.

Table 1. Zinc toxicity on *Daphnia magna*

Concentration (mg/L)	Number of *Daphnia magna* in test plates								
	Day 1					Day 2			
	Hour					Hour			
	8 h	12 h	16 h	20 h	24 h	4 h	8 h	12 h	16 h
1.0	5	5	5	4	4	4	4	3	2
2.0	5	5	4	4	3	2	2	1	0
3.0	3	3	2	2	2	1	1	1	0
4.0	2	2	2	1	1	1	0	0	0
5.0	2	1	1	0	0	0	0	0	0
6.0	1	1	0	0	0	0	0	0	0

For example, at the lowest tested concentration (1.0 mg/L Zn), all five individuals survived during the first 12 hours of exposure. After 16 hours, the number of surviving organisms decreased to four individuals, and after 36 hours, only two organisms remained alive.
At a zinc concentration of 2.0 mg/L, the number of surviving individuals began to decrease earlier. While five organisms were initially present, survival declined progressively to zero by the end of the exposure period.
At 3.0 mg/L, the toxic effects became more pronounced. Only three individuals remained alive after the initial exposure period, and the population gradually declined until complete mortality occurred by the end of the experiment.
Higher zinc concentrations caused even faster reductions in survival. At 4.0 mg/L, only two organisms remained alive after the first observation interval, and the entire population was eliminated within the experimental timeframe.
Similarly, at 5.0 mg/L, mortality increased rapidly, with only one organism surviving after the early observation intervals and complete mortality occurring shortly afterward.
The most severe toxic effects were observed at the highest concentration tested (6.0 mg/L). In this case, only one individual survived during the initial observation period, and all organisms died within a few hours of exposure.

These results clearly demonstrate that zinc toxicity in *Daphnia magna* increases significantly with increasing pollutant concentration.

Mortality rate of water fleas

The mortality rate of the water fleas was calculated based on the recorded number of surviving individuals at each observation interval. The results are illustrated in Figures 5 and 6, which show the mortality rates recorded after the first and second days of exposure.

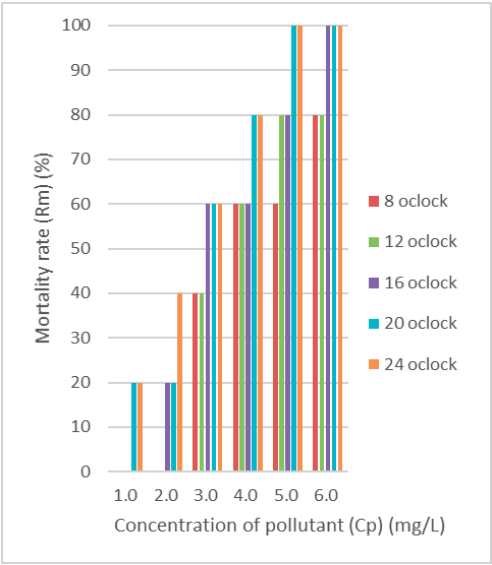


Figure 5. Mortality rate of water fleas after Day 1 of exposure to the pollutant

During the first day of exposure, the mortality rate remained relatively low at the lower zinc concentrations. At concentrations of 1.0 mg/L and 2.0 mg/L, mortality reached approximately 20% only after 16 hours and 12 hours of exposure, respectively.

In contrast, the mortality rate increased substantially at higher zinc concentrations. At the maximum concentration tested (6.0 mg/L), mortality reached approximately 80% after only four hours of exposure and increased to 100% after twelve hours.

The results also indicate that mortality increased significantly during the second day of exposure (Figure 6). After 36 hours, the mortality rate reached 60% at the lowest tested concentration (1.0 mg/L), while at all higher concentrations complete mortality (100%) was observed.

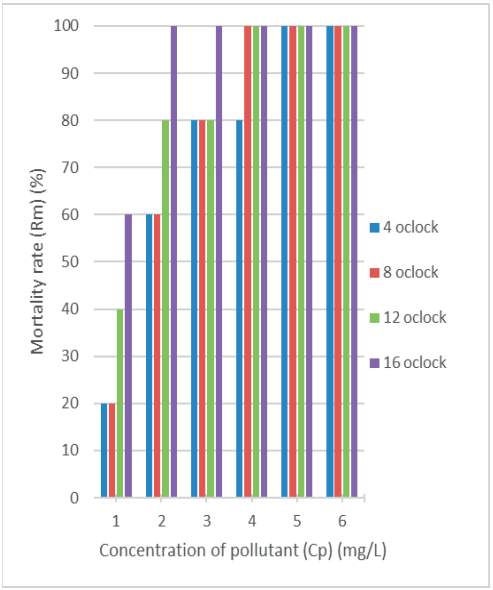


Figure 6. Mortality rate of water fleas after Day 2 of exposure to the pollutant

These findings demonstrate that both exposure time and zinc concentration strongly influence the survival of *Daphnia magna*.

After calculating the mortality rate of water fleas, the LC_{50} (lethal concentration for 50% of organisms) was determined, a standard toxicology indicator used to measure the acute toxicity of a substance. LC_{50} is calculated using statistical methods and is used to compare toxicity and assess ecological and human risks. A lower LC_{50} value indicates higher toxicity.

To calculate the LC_{50} value, the logarithmic method was applied. Using the AAT Bioquest LC_{50} calculator, the concentrations (X values) and the corresponding responses expressed as percentage mortality (Y values) were entered to obtain the equations required for determining the LC_{50} for Zn exposure on Day 1 and Day 2. The resulting equations and LC_{50} values are presented in Table 2.

Table 2. The LC_{50} values for Zn pollution used in the experiment

Type of Pollutant	Equation		LC_{50}	
Zn^{2+}	*Day 1*	*Day 2*	*Day 1*	*Day 2*
	$Y = 0.034 + \frac{1.019 - 0.034}{1 + \left(\frac{X}{2.96}\right)^{4.623}}$	$Y = -0.053 + \frac{4.969 + 0.053}{1 + \left(\frac{X}{0.434}\right)^{1.596}}$	2.969±0.032	0.434±0.063

All values are mean values of repetitions ± S.D.

Relationship between zinc concentration and population inhibition

In addition to mortality rate, the inhibitory effect of zinc exposure on *Daphnia magna* populations was also evaluated by comparing the survival of organisms in contaminated water with that observed in the control sample maintained in uncontaminated freshwater.

Graphical analysis of the relationship between pollutant concentration and inhibition of the organisms revealed a clear correlation between increasing zinc concentration and increasing levels of inhibition. This relationship is illustrated in Figures 7, 8, and 9, which show the correlation between pollutant concentration (Cp) and the inhibition of *Daphnia magna* (I) after 12 hours, 24 hours, and 36 hours of exposure, respectively.

The results indicate that inhibition increased with both zinc concentration and exposure time.

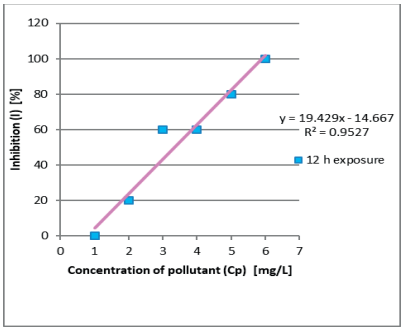


Figure 7. Correlation between the concentration of pollutant (Cp) and inhibition (I) of *Daphnia magna* under the influence of water pollution with Zn after 12 hours of exposure

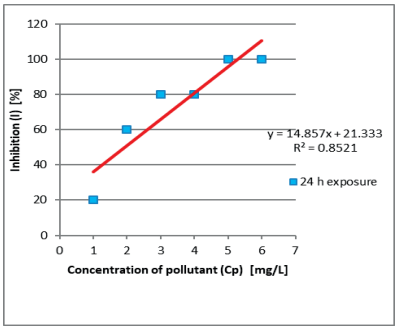


Figure 8. Correlation between the concentration of pollutant (Cp) and inhibition (I) of *Daphnia magna* under the influence of water pollution with Zn after 24 hours of exposure

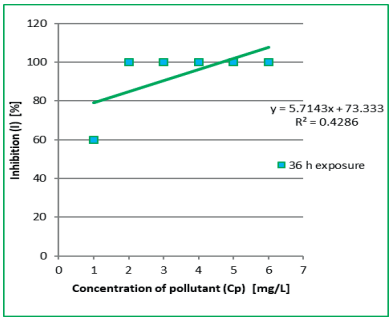


Figure 9. Correlation between the concentration of pollutant (Cp) and inhibition (I) of *Daphnia magna* under the influence of water pollution with Zn after 36 hours of exposure

The relationship between pollutant concentration and inhibition was quantified using the correlation coefficient (R^2), which showed a decreasing trend over time, with values of 0.9527 after 12 hours, 0.8521 after 24 hours, and 0.4286 after 36 hours. This decline suggests that, at longer exposure times, the strength of the correlation weakened due to the high mortality observed, with surviving organisms recorded only at the lowest zinc concentration tested (1.0 mg/L).

Toxicological interpretation

Zinc is widely recognized as a metal that can exert both beneficial and harmful effects on aquatic organisms depending on its concentration in the environment. At low concentrations, zinc functions as an essential

trace element and plays an important role in enzymatic activity and metabolic regulation.
However, when present in elevated concentrations, zinc may become toxic and disrupt important biological processes in aquatic organisms.
From an ecological perspective, increased mortality of *Daphnia magna* may disrupt trophic interactions, as this species represents a key link between primary producers and higher trophic levels. A decline in its population could lead to algal overgrowth and reduced water quality. Prolonged exposure to high zinc concentrations can affect growth, reproduction, and overall physiological functioning. Toxic accumulation of the metal may interfere with essential biological processes such as cellular respiration, ionic balance, and metabolic regulation, ultimately leading to organism mortality.
The results obtained in the present study are consistent with those reported by De Schamphelaere et al. (2004), who demonstrated that excessive zinc exposure can negatively affect the development and reproduction of *Daphnia magna* due to metal accumulation within the organism.
Furthermore, previous research conducted by Simionov et al. (2022) suggests that the toxicity of zinc in aquatic environments can be influenced by environmental parameters such as water alkalinity and salinity, which affect the bioavailability and uptake of metals by aquatic organisms.
The findings of the present study confirm that *Daphnia magna* populations are highly sensitive to zinc exposure. A significant reduction in organism survival was observed as zinc concentrations increased. After 32 hours of exposure, the population decreased by approximately 40% at the lowest concentration (1.0 mg/L), while reductions of approximately 80% were observed at concentrations of 2.0 mg/L and 3.0 mg/L.
At 4.0 mg/L, the population was completely eliminated, resulting in 100% mortality. At the highest concentration tested (6.0 mg/L), toxicity reached its maximum level within only 12 hours of exposure, indicating a rapid and severe toxic effect on the organisms.

CONCLUSIONS

The present study demonstrates that *Daphnia magna* exhibits a pronounced sensitivity to zinc contamination, reinforcing its relevance as a reliable bioindicator for evaluating heavy metal pollution in freshwater environments. Experimental results clearly show that both exposure duration and zinc concentration significantly influence mortality rates in *D. magna*. Even relatively low concentrations of zinc (1.0 mg/L) produced measurable toxic effects within a short exposure period, while higher concentrations caused rapid and complete mortality.
The observed concentration-dependent toxicity confirms that zinc, although biologically essential in trace amounts, can severely disrupt physiological processes when present in elevated concentrations. The observed toxicity could be related to potential disruptions in physiological processes such as ion regulation and metabolic activity; however, these mechanisms were not directly measured and therefore remain speculative. These findings are consistent with previous ecotoxicological studies demonstrating that metal bioavailability and exposure time are critical determinants of toxic responses in aquatic invertebrates.
From an environmental perspective, the results underline the importance of monitoring zinc levels in freshwater systems, particularly in areas influenced by industrial activities, mining operations, or urban wastewater discharges. The rapid toxic response observed in *D. magna* suggests that this organism can serve as an early warning indicator of metal contamination in aquatic ecosystems.
Future research should focus on evaluating the influence of environmental parameters such as water hardness, pH, temperature, and dissolved organic matter on zinc toxicity. These factors may significantly affect metal bioavailability and consequently modify toxic responses in aquatic organisms. Additionally, further studies involving sublethal endpoints such as reproduction, growth, and behavioral changes could provide a more comprehensive understanding of long-term ecological impacts.

Overall, the findings contribute to the growing body of knowledge on metal toxicity in freshwater organisms and support the use of *Daphnia magna* in ecotoxicological monitoring and environmental risk assessment programs.

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