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Effect of cadmium and zinc on biomolecular profiles of the freshwater cyclopoid copepod *Thermocyclops decipiens*

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Abstract
The acute toxicity of cadmium and zinc to the freshwater cyclopoid copepod *Thermocyclops decipiens* is studied in relation to biochemical parameters such as protein, RNA, DNA, and free amino acids. Results indicate that cadmium is more toxic than zinc; mortality was at 100% at higher concentrations within 48 hours, whereas the effects of zinc were much milder. Cd exposure resulted in large decreases in the levels of proteins and RNA with a lower RNA:DNA ratio, indicating slowed protein synthesis and cellular activity. The concentration of total free amino acids was reduced by both metals, which can indicate metabolic perturbations. These findings focus attention on the use of biochemical endpoints as indicative measures of the toxicity of heavy metals and place an emphasis on the need to carry out long-term studies regarding the effects of heavy metal exposure on aquatic organisms. This paper contributes to an understanding of how pollution affects the aquatic ecosystem, thereby informing the management strategies against heavy metal contamination.

Keywords: Acute toxicity, heavy metals, cyclopoid, biochemical profile

1. Introduction
Consumption of fish and fishery products has been increasing as they are considered the safest source of proteins of animal origin. Hence, to meet the huge gap between production and demand, the rearing of fishes and prawns of both marine and freshwater species is developed in the branch of aquaculture. Live-food organisms serve as the best source of food for the success of hatchery production [1] as they provide the necessary nutrients for the sustainment, growth, and maintenance of the farmed fish that feed on it. Zooplankton, being the primary consumers in the aquatic food chain, constitute the major portion of feed for the commercially important prawns and fishes. The freshwater zooplankton mostly includes the species of rotifers, copepods, cladocerans, and ostracods. Factors determining the distribution of zooplankton include the depth of the water body, water transparency and pH [2], and predation potential [3]. Zooplankton are rich sources of amino acids, fatty acids, minerals, and enzymes [4]. The enzymes (amylase, protease, exonuclease, and esterase) are inevitable for larval digestion [5], enhancement of metamorphosis of larvae, and for easy digestibility of protein [6]. Both DHA and EPA, essential for the growth and development of fish, are present in significant levels in zooplankton [7].
Aquatic ecosystems are vulnerable to various forms of pollution, including the contamination of heavy metals such as cadmium and zinc. Cadmium is a highly toxic element that can adversely affect the biota, even at low concentrations [8]. Similarly, zinc, although an essential micronutrient, can also be toxic to aquatic organisms in high concentrations.
Cadmium, a non-essential heavy metal, is a ubiquitous environmental pollutant due to industrial and agricultural activities [9]. It has no known biological function and is highly toxic even at low concentrations [8]. Cadmium's toxicity stems from its ability to disrupt enzyme function and metabolic processes [9]. This disruption can lead to decreased amino acid content and total protein levels, indicating impaired protein metabolism. Additionally, cadmium exposure can reduce the RNA:DNA ratio, a key indicator of growth and nutritional status,

suggesting inhibited protein synthesis and cell division.

Zinc, unlike cadmium, is an essential micronutrient for aquatic organisms (Burch *et al*., 1975). However, at elevated concentrations, zinc can become toxic, inducing oxidative stress and negatively impacting biochemical and physiological processes. For instance, research on *Centropages ponticus* revealed that cadmium exposure significantly altered biochemical markers, indicating cellular stress and damage [10]. Another study on *Acartia tonsa* and *A. hudsonica* demonstrated that both cadmium and mercury negatively impacted egg production and hatching success [11-16]. These findings highlight the vulnerability of copepods to heavy metal contamination and the potential consequences for aquatic food webs.

The present study aims to investigate the acute toxicity of cadmium and zinc on the biochemical parameters of the freshwater planktonic cyclopoid copepod *Thermocyclops decipiens*, a key component of aquatic food webs. This research is particularly important as aquatic environments face increasing pressure from industrial activities, agricultural runoff, and other anthropogenic sources of pollution, which can lead to the accumulation of heavy metals and disrupt the delicate balance of aquatic ecosystems [8]. The findings of this study will contribute to a better understanding of the impacts of heavy metal pollution on aquatic ecosystems and inform the development of effective management strategies to protect these vulnerable environments.

2. Materials and methods

2.1 Collection of zooplankton

Zooplankton samples were collected from the Madhuranthakam temple tank, Chennai. The samples were collected during the early hours of the day using a plankton net (75 µm) made up of bolten silk. Samples were collected by towing the plankton net horizontally at a depth of 40 cm for about 10 minutes. Live zooplankton samples were transported to the laboratory within an hour with an insulated polyethylene container.

2.2 Sampling strategies

Thermocyclops decipiens were separated under a dissection binocular stereo microscope using a fine pipette and identified based on key characters [17]. *T. decipiens* were enumerated under the binocular zoom microscope using a fine brush (or) glass dropper.

2.3 Preparation of inoculum

The *T. decipiens* were isolated and maintained in a 1-liter beaker for pure culture, and they were fed with yeast (150 ppm) [18].

2.4 Preparation of culture medium

Fertilized culture medium was prepared by thoroughly dissolving the baker's yeast in filtered water. The medium was stirred thoroughly and used as a stock solution. From the stock solution, the culture medium of 150 ppm concentration was prepared, and *T. decipiens* were inoculated at the rate of 40-50 animals. Fertilized medium was added at an interval of seven days.

2.5 Toxicity of *T. decipiens*

The experiments were carried out to assess the LC_{50} value of cadmium and zinc on *T. decipiens.* In this regard, 5 different concentrations (0.4 mM, 0.2 mM, 0.2 nM, 0.2 µM, and 0.2 pM) of cadmium and zinc were taken, each in triplicate, and introduced with 25 animals. The species were incubated for 24 and 48 hrs in each test medium. The mortality of the species was counted after the incubation period in each concentration. The percentage mortality was calculated and determined the LC_{50} value. The LC_{50} values were calculated using Probit analysis. Based on the LC_{50} value, sublethal concentrations of cadmium and zinc were calculated. The animals were exposed to the sublethal concentration to study the free amino acid [19], protein [20], and nucleic acid analysis [21]**.** The animals were incubated for 24 and 48 hrs. After the time intervals, the animals were filtered, and they were lyophilized for further biochemical studies. The data of mortality mean, standard deviation, and significant difference between the concentration in different durations were calculated using SPSS 21.0 ver.

3. Results

Freshwater *T. decipiens* were treated in different concentrations of (0.4 mM, 0.2 mM, 0.2 nM, 0.2 µM, and 0.2 pM) of cadmium and zinc, and their percentage of mortality is depicted in Table 1.

3.1 Toxicity

The table represents the mortality percentage of the cyclopoid copepod *Thermocyclops decipiens* after exposure to varying concentrations of cadmium and zinc over 24 hrs and 48 hrs. At 24 hrs incubation, the mortality percentage of cadmium-treated *T. decipiens* was minimal (1.33%) at 0.2 pM, while at 0.4 mM, it reaches 100%. Intermediate concentrations show a gradual increase in mortality (14.67% to 92.00%). However, in zinc-treated *T. decipiens, it* was 38.67% (0.2 pM) and 89.33% (0.4 mM). While in the case of 48 hrs treated animals, a similar trend was observed. The mortality at the lowest concentration (0.2 pM) increases significantly to 34.67%, while at 0.4 mM, it remains at 100% in the cadmium-treated animals. The mortality rates were notably higher and reached 100% at zinc concentrations of 0.2 mM and 0.4 mM. At lower concentrations (0.2 pM, 0.2 nM, 0.2 µM), mortality still increases but remains below 100% (Table 1). The mortality at the intermediate concentrations also shows a rise, indicating heightened sensitivity over a longer exposure time. Cadmium exhibits higher toxicity than zinc, with marked reductions in viability at lower concentrations and shorter exposure times. The continuous decrease in viability with increased cadmium concentration and exposure time suggests a direct correlation between cadmium concentration and toxicity. In contrast, zinc may act as a micronutrient at lower concentrations, supporting cell viability. Mortality percentages are consistently higher at 48 hours compared to 24 hours across all cadmium and zinc concentrations, demonstrating that prolonged exposure exacerbates cadmium and zinc's toxic effects on *T. decipiens*.

The LC50 values were calculated from the data indicating the toxicity of cadmium at 24 hrs and 48 hrs. The significant increase in LC50 from 0.244 µM to 0.131 nM suggests that cadmium becomes more toxic with prolonged exposure, which reflects the reduced tolerance of *T. decipiens* over time. The LC50 value of zinc shows 0.976 µm for 24 hours and 0.302 nM for 48 hours (Table 1); it reveals critical toxicological insight into *T. decipiens.* Cadmium exhibits higher toxicity than zinc, with marked reductions in viability at lower concentrations and shorter exposure times. The continuous decrease in viability with increased cadmium concentration and exposure time suggests a direct correlation

between cadmium concentration and toxicity. In contrast, zinc may act as a micronutrient at lower concentrations, supporting cell viability.

Table 1: Percentage of mortality of *Thermocyclops decipiens* treated in different concentrations of cadmium and zinc during 24 hrs. and 48 hrs.

	Duration	0.2 pM	0.2 nM	0.2 µM	0.2 mM	0.4 mM	LC_{50} value
Cadmium	24 Hours	1.33 ± 2.31[a]	14.67 ± 2.31[b]	36.00 ± 4.00[c]	92.00 ± 4.00[d]	100.00 ± 0.00[e]	0.244 µM
	48 Hours	34.67 ± 6.11[a]	58.67 ± 6.11[v]	86.67 ± 2.31[c]	97.33 ± 2.31[d]	100.00 ± 0.00[d]	0.131nM
Zinc	24 Hours	38.67 ± 6.11[a]	46.67 ± 4.62[a]	45.33 ± 12.22[a]	82.67 ± 10.07[b]	89.33 ± 6.11[b]	0.976 µm
	48 Hours	45.67 ± 2.08[a]	48.00 ± 19.29[a]	58.67 ± 2.31[a]	100.00 ± 0.00[b]	100.00 ± 0.00[b]	0.302 nM

Mean ± SD represents a triplicate value; ANOVA followed by DMRT's test was performed; different superscripts in the same row are significantly different at $P < 0.05$ value.

3.2 Biomolecules

The graph represented the concentrations of protein, DNA, and RNA (in mg/ml) under different conditions and time periods, alongside the RNA:DNA ratio of sublethal concentrations of cadmium- and zinc-treated *T. decipiens* (Fig. 1). The sublethal concentrations of cadmium (0.018 µM and 0.044 nM for 24 hrs. and 48 hrs., respectively) and zinc (0.018 µM and 0.044 nM for 24 hrs. and 48 hrs., respectively). The control group shows the highest protein concentration (235.67±6.03 mg/ml). Protein levels remain lower (215.33±2.52 mg/ml) in the cadmium 24 hrs. group but are slightly higher (227.00±6.08 mg/ml) for zinc treatment at 24 hrs. Both cadmium and zinc showed a decrease in protein levels at 48 hrs. compared to their earlier measurements (Fig. 1).

DNA levels appear consistent across the control group (292.67±7.02 mg/ml) and treatment groups, with a slight decrease observed in the 24 hrs. treatment groups. At 48 hrs., DNA levels showed slight decreases in both cadmium and zinc treatments. RNA levels were generally lower than protein and DNA across most groups but exhibited a notable decrease in the cadmium (99.67±1.53 mg/ml) and zinc (107.33±1.53 mg/ml) treatment groups at 48 hrs. relative to 24 hrs. (Fig. 1). Cadmium treatment at 24 hours shows a significant reduction in RNA concentration compared to both the control and zinc. The RNA:DNA ratio starts at about 0.91 in the control and shows minor fluctuations across the treatments. The ratio tends to remain stable at around 0.81 to 0.85 (Fig. 1) through the treatments, indicating relatively consistent cellular processes even when exposed to pollutants. The overall trends suggest that exposure to cadmium and zinc affects protein, DNA, and RNA concentrations, with varying impacts over time. The consistency of the RNA:DNA ratio may indicate a stable balance of nucleic acids despite these external influences. Further investigation could provide insights into the physiological implications of these changes in cellular components.

3.3 Free Amino Acids

The control group exhibited the highest total FAA concentration (2663.96 µg/g). The cadmium exposure *T. decipiens* shows resulted in a significant reduction to 2268.90 µg/g. However, the zinc treatment also reduced total FAA levels, though less severely at 2369.37 µg/g. Aspartic acid showed a marked decrease in both treatments with cadmium (1339.26) and zinc (1289.44) when compared to the control (1476.61). The same trend in the arginine and tyrosine. Serine and histidine showed considerable declines when exposed to cadmium (38.96 and 28.68, respectively), while zinc had a moderate effect on serine (49.32) and better preservation of histidine (38.42) (Table 2). The glycine and threonine showed notably reduced in cadmium but better sustained under zinc treatment. The exposure of *T. decipiens* to cadmium at sublethal concentrations markedly lowers FAA levels, with significant variability among individual amino acids. Zinc treatment, while still resulting in a decrease compared to control, demonstrated a softer impact on FAA concentrations, suggesting potential for better resilience against toxic metal exposure. The varied responses of individual amino acids indicate potential pathways for metabolic adaptation or stress responses in the presence of heavy metals. Further research could examine the underlying mechanisms of these adaptations.

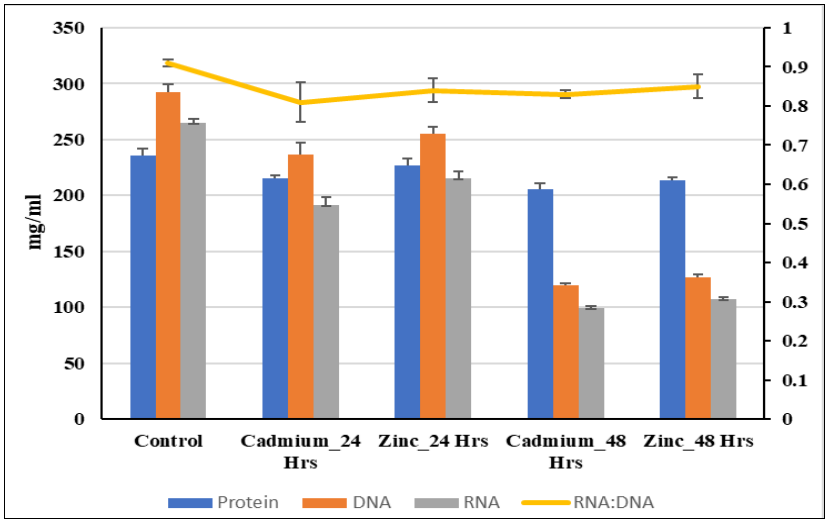

Fig 1: Protein, RNA, DNA, and RNA:DNA ratios of *T. decipiens* treated in sublethal concentrations of cadmium and zinc during 24 hrs. and 48 hrs. of exposure.

Table 2: Free Amino Acid Profile of *T. decipiens* Treated in Sublethal Concentration of Cadmium and Zinc During 24 hrs. and 48 hrs.

S. No.	FAA	Control	Cadmium 48	Zinc 48
1.	Aspartic acid	1476.61	1339.26	1289.44
2.	Glutamic acid	171.65	137.11	136.93
3.	Serine	62.18	38.96	49.32
4.	Histidine	43.28	28.68	38.42
5.	Glycine	81.21	48.18	68.34
6.	Theronine	69.08	49.67	58.34
7.	Alanine	161.55	131.23	148.17
8.	Arginine	125.39	74.13	98.13
9.	Tyrosine	42.94	29.61	41.88
10.	Valine	50.81	55.39	77.62
11.	Methionine	6.54	7.61	12.78
12.	Phenylalanine	46.7	38.56	41.26
13.	Isoleucine	64.33	47.38	54.32
14.	Leucine	119.74	89.81	110.09
15.	Lysine	141.95	153.32	144.33
	Total	2663.96	2268.90	2369.37

4. Discussion

The results of this study provide valuable insights into the acute toxicity of cadmium and zinc on the freshwater planktonic cyclopoid copepod *T. decipiens*. Cadmium, a highly toxic heavy metal, was found to have a more severe impact on the biochemical parameters of *T. decipiens* compared to zinc, an essential micronutrient that can become toxic at elevated concentrations.

The observed decreases in amino acid content, total protein, and RNA:DNA ratio in the exposed copepods suggest that both cadmium and zinc can disrupt protein metabolism, impair cellular growth and division, and ultimately compromise the overall fitness and survival of this important component of the aquatic food web. These findings are consistent with previous studies on other copepod species, such as *Centropages ponticus* and *Acartia tonsa*, which have also demonstrated the detrimental effects of heavy metal exposure on biochemical and physiological processes [10].

The mechanisms underlying these effects are likely related to the ability of heavy metals to interfere with enzyme function, disrupt cellular homeostasis, and induce oxidative stress, ultimately leading to cellular damage and impaired metabolic processes [22]. These results underscore the need for continued research and monitoring of heavy metal pollution in freshwater ecosystems, as the adverse effects on key species like *T. decipiens* can have cascading impacts on the entire aquatic community. Effective management strategies, such as strict regulations on industrial effluents and agricultural runoff, are crucial to mitigate the long-term consequences of heavy metal contamination and protect the delicate balance of aquatic ecosystems.

The observed dose-dependent increase in mortality of *T. decipiens* upon exposure to cadmium aligns with previous studies demonstrating cadmium's high toxicity to aquatic organisms [9, 23]. Cadmium's detrimental effects are attributed to its interference with enzyme function and disruption of metabolic processes [9], ultimately leading to reduced viability. The complete mortality (100%) observed at 0.4 mM cadmium within 24 hours underscores the acute toxicity of this heavy metal. The increased mortality at lower cadmium concentrations over a longer exposure duration (48 hours) further emphasizes the time-dependent nature of cadmium toxicity.

While zinc is an essential micronutrient [24], the observed increase in *T. decipiens* mortality with increasing zinc concentrations and longer exposure times confirms that zinc can also exert toxic effects at elevated levels. This toxicity may arise from oxidative stress induced by high zinc concentrations, negatively impacting various physiological processes. The lower mortality rates observed in zinc-exposed *T. decipiens* compared to cadmium-exposed organisms suggest that cadmium exhibits higher toxicity. This difference in toxicity could be attributed to the fact that cadmium has no known biological function and disrupts essential metabolic pathways, whereas zinc plays a vital role in various cellular processes at lower concentrations.

The higher mortality percentages observed at 48 hours compared to 24 hours across all cadmium and zinc concentrations highlight the importance of exposure duration in heavy metal toxicity. Prolonged exposure allows for greater accumulation of heavy metals within the organism, exacerbating their toxic effects. This time-dependent toxicity has been reported in other studies on heavy metal exposure in aquatic organisms [25, 26].

The findings of this study contribute to the growing body of literature demonstrating the detrimental effects of heavy metal pollution on aquatic organisms. Further research is needed to investigate the specific mechanisms underlying the toxicity of cadmium and zinc in *T. decipiens* and to assess the long-term consequences of heavy metal exposure on population dynamics and ecosystem health.

The observed decrease in protein concentration in *T. decipiens* after exposure to both cadmium and zinc aligns with the general understanding of heavy metal toxicity in aquatic organisms. Heavy metals can disrupt protein metabolism, leading to reduced protein synthesis and increased protein degradation. The more pronounced decrease in protein levels with cadmium exposure compared to zinc exposure further supports the higher toxicity of cadmium observed in the mortality data. This difference in toxicity may be attributed to cadmium's ability to directly bind to critical cellular components and disrupt protein structure and function [27].

The relatively stable DNA levels across the control and treatment groups suggest that cadmium and zinc exposure at sublethal concentrations may not significantly impact DNA integrity within the observed time frame. However, the slight decrease in DNA levels at 48 hours in both cadmium and zinc treatments warrants further investigation to determine if prolonged exposure could lead to more substantial DNA damage. The notable decrease in RNA levels, particularly at 48 hours in both cadmium and zinc treatments, indicates a

potential disruption of RNA synthesis and processing. This finding was consistent with the observed decrease in the RNA:DNA ratio, which was a sensitive indicator of growth and nutritional status in aquatic organisms [28]. A decrease in the RNA:DNA ratio suggests impaired protein synthesis and reduced cellular activity, which could have long-term consequences for the growth and reproduction of *T. decipiens*. The significant reduction in RNA concentration after 24 hours of cadmium exposure compared to both the control and zinc treatment further highlights the higher toxicity of cadmium.

The relatively stable RNA:DNA ratio across the treatments, despite the observed changes in individual RNA and DNA concentrations, suggests a complex interplay between these two nucleic acids in response to heavy metal exposure. While the overall ratio remains relatively constant, the decrease in RNA levels suggests that the cells may be downregulating RNA synthesis to conserve resources and mitigate the toxic effects of cadmium and zinc. This observation warrants further investigation to understand the specific mechanisms regulating RNA and DNA synthesis and turnover in *T. decipiens* under heavy metal stress. Studies on other copepod species have also reported alterations in RNA and DNA levels upon exposure to heavy metals, highlighting the importance of these parameters as indicators of heavy metal toxicity in aquatic organisms [9-11, 22, 29-31].

The decrease in total free amino acid concentration in *T. decipiens* upon exposure to both cadmium and zinc suggests a disruption in amino acid metabolism. This disruption could be attributed to several factors, including impaired protein synthesis, increased protein degradation, or altered amino acid transport [27]. The more pronounced reduction in FAA levels with cadmium exposure compared to zinc exposure is consistent with the higher toxicity of cadmium observed in the mortality and biochemical analyses.

The marked decrease in aspartic acid, arginine, and tyrosine levels in both cadmium and zinc treatments may indicate their importance in heavy metal detoxification or stress response mechanisms. These amino acids could be preferentially utilized for energy production or incorporated into stress-related proteins under heavy metal stress.

The differential effects of cadmium and zinc on serine, histidine, glycine, and threonine levels suggest distinct mechanisms of action for these two heavy metals. The significant decline in serine and histidine levels with cadmium exposure, coupled with the moderate effect of zinc on serine and better preservation of histidine, may indicate that cadmium disrupts specific metabolic pathways involved in the synthesis or utilization of these amino acids. Similarly, the notable reduction in glycine and threonine levels in cadmium-treated organisms but better sustenance under zinc treatment further supports the idea of distinct mechanisms of toxicity.

The overall decrease in FAA levels, coupled with the varied responses of individual amino acids, suggests that *T. decipiens* may employ metabolic adaptations or stress responses to cope with heavy metal exposure. These adaptations could involve altering amino acid metabolism to prioritize essential functions or activating detoxification pathways. Further research is needed to elucidate the specific mechanisms underlying these adaptations and their long-term consequences for the survival and reproduction of *T. decipiens*. Studies on other copepod species exposed to heavy metals have also reported alterations in amino acid profiles [10], highlighting the importance of FAA analysis as a tool for assessing heavy metal toxicity in aquatic organisms.

5. Conclusions

The present study investigated the acute toxicity of cadmium and zinc on the freshwater planktonic cyclopoid copepod *Thermocyclops decipiens*, with a focus on biochemical endpoints, including protein, amino acids, RNA, DNA, and the RNA:DNA ratio. The results demonstrate that cadmium is more toxic than zinc to *T. decipiens*, as evidenced by the higher mortality rates and more pronounced disturbances in biochemical parameters. Cadmium exposure led to a significant decrease in protein and RNA levels, as well as a reduction in the RNA:DNA ratio, indicating impaired protein synthesis and cellular activity. In contrast, zinc exposure had a less severe impact on these parameters. The observed decrease in total free amino acid concentration, along with the differential response of individual amino acids, suggests that *T. decipiens* employs metabolic adaptations to cope with heavy metal stress. The findings of this study highlight the usefulness of biochemical endpoints, such as RNA, DNA, protein, and amino acid analysis, as sensitive indicators of heavy metal toxicity in aquatic invertebrates. Further research is needed to elucidate the specific mechanisms underlying the observed responses and to determine the long-term consequences of cadmium and zinc exposure on the growth, reproduction, and overall fitness of *T. decipiens.*

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Data availability

The data that support the findings of this study are available with corresponding author.

Conflict of interest

The authors declare that they have no conflict of interest and relevant to the content of this article.

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