International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 30-07-2022, Accepted: 16-08-2022, Published: 01-09-2022
Volume 7, Issue 9, 2022, Page No. 13-18

Effect of sublethal concentration of zinc on fresh water fish *Channa punctatus*

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Abstract

Channa punctatus, a freshwater fish, was exposed to zinc at three sub-lethal concentrations (10 mg/l, 15 mg/l, and 25 mg/l) over a period of 15 days. At intervals of 8, 10, and 15 days, the effects of this exposure were examined on the bio-accumulation of zinc. In fish from all treated groups, a statistically significant rise in zinc concentration was observed. After switching these fish to regular tap water for an additional two weeks, post-exposure recovery in the fish was seen. Even while the concentration of zinc remained much greater than controls until the end of the experiment, there was a slow removal of zinc. At LC50 for 24, 48, 72, and 96 hours no deaths were noted in the control experiments. When the metals' concentration and duration of exposure rose, the fish mortality went up considerably. Upon exposure of zinc on the fish specimens, the fish displayed behavioral changes during the lethal toxicity trial, including symptoms of fatigue, erratic swimming, jerky movements, restlessness, frequent surfacing, spinning, convulsions, and fin extensions.

Keywords: zinc, *Channa* punctatus, long term exposure, toxicity

Introduction

Untreated municipal trash, the usage of fertilisers and pesticides, as well as the disposal of industrial wastes that are both organic and inorganic, are all significantly increasing environmental contamination. Because of their toxicity, persistence, tendency to accumulate in animals, and food chain amplification, heavy metals have been recognised as potent biological poisons (Kamble and Muley, 2000; Dinodia *et al*., 2002) [1, 2]. They also harm aquatic life, especially fish. Zinc is one of the heavy metals that is used in a variety of ways before ending up in a river or the ocean. Human operations include mining, purifying zinc, lead, and cadmium ores, burning coal, and burning garbage release too much zinc into the environment. Although only modest amounts of zinc are necessary for healthy growth and metabolism (Gupta and Sharma, 1994; Srivastava and Sharma, 1996; Srivastava and Kaushik, 2001; Shukla *et al*., 2002) [3, 4, 5, 6], if the level is higher than what is necessary for physiologic function, it may become hazardous. This causes numerous fish organs to become metabolically and pathologically altered, which results in overall enfeeblement, growth retardation, and other effects (Sharma and Sharma, 1994; Singh and Gaur, 1997) [3, 7, 8]. Any consequence of such pollution would eventually have a negative impact on the nutritional value of fish and on man through their ingestion because fish populations are a crucial part of the food chain.

Heavy metals among other harmful contaminants have a tendency to bio-magnify in the food chain, which makes them particularly harsh in their impact. A significant environmental issue is the global water pollution caused by heavy metals. The majority of water sources are getting poisoned as a result of the agricultural and industrial revolutions (Khare and Singh, 2002). Industrial discharges comprising heavy metals and other harmful and dangerous compounds (Gbem *et al*., 2001; Woodling *et al*., 2001) [9, 10] have a significant negative impact on the health of aquatic ecosystems. Zinc is a necessary element that serves as a structural component and has unique qualities that are necessary for life (Bengari and Patil, 1986) [11]. Because it cannot be physiologically eliminated but only undergoes oxidation in the environment, zinc's toxicity is exacerbated by its almost infinite persistence The risk posed by zinc is increased by the fact that it cannot be biologically eliminated and instead can only be changed from one oxidation state or organic complex to another, making it practically indestructible in the environment. Fish may be toxic to Zn, which disrupts gill tissue, the control of acid-base and ions, and causes hypoxia (Everall *et al*., 1989) [12]. (Hogstrand *et al*., 1994) [13]. Metals' bioaccumulation is a function of how much an organism consumes how they are distributed throughout the various tissues, and how much metal is maintained in each type of tissue. Zinc accumulation has reached a dangerous level and is now responsible for pathologies like Alzheimer's disease. Freshwater species need Zn in particular concentrations to thrive, but too much buildup is dangerous for both exposed organisms and people who ingest them directly or regularly. During the fish mortality test, feeding was stopped 24 hours earlier. The fish were fed earthworm pieces once daily for 30 minutes prior to the replacement of the test water during the accumulation studies, but after that time the food was eliminated.

Material and Method

Channa punctatus a fresh water fish native to Bhopal that is edible was chosen for the study. The choice of the test organism needs to take into account a variety of factors. These include the species' availability, its propensity for survival in a lab setting, its manageable size for handling and keeping in a lab setting, prior knowledge of the species, its biology, which includes its behavior and feeding patterns, ecology, reproduction, development, and life cycle, and its widespread distribution throughout the area of concern, as well as its ecological significance and economic value. One species likely won't meet every one of these requirements. *Channa punctatus* was the test organism chosen for the investigation.

Collection and Laboratory Maintenance of Test Organism

Local residents gathered fingerlings of the freshwater fish. Large aerated polythene bags filled with water were used to capture them and transfer them to the lab with the least amount of disturbance possible. Healthy fish were put in huge aquariums made of glass. The aquariums were first drained and cleansed with potassium permanganate solution ($KMnO_4$ /20ppm). After that, seasoned (chlorine-free) tap water was added to the container. The fish were given a 15-day acclimatization period before the experiment began. Aquarium aerators were used to provide enough aeration. The temperature, pH, and dissolved oxygen levels were held steady at 7.25, 0.08, and 7.88, 0.33 mg/L respectively. The fish are fed commercial fish feed twice daily.

All toxicity trials utilized healthy, alert animals that were roughly the same size that were randomly chosen from the holding tanks. For the experiment, fish with a total length of 4-5 cm and a weight of 10-15 g were chosen. Before the test, the fish were famished for 24 hours. This provided the intestines enough time to empty completely of all food and waste.

Toxicants

Zinc sulphate ($ZnSO_4.7H_2O$), produced by Merk Limited, was chosen as the toxicant for the investigation. White powdered zinc sulphate has a molecular weight of 287.54. In water, both easily dissolve. Prior to the experiment starting, test solutions were made. The test solutions were made with a lower magnitude than the stock solutions, and the stock solutions were prepared in accordance with normal practice (APHA, 2005) [14].

The Experiment

Static renewal bioassay method as mentioned in APHA (2005) [14] was followed in the experiment. This type of bioassay conducted as a short-term toxicity test. The 96-hour test period was used as time interval. The death of the test organism was the criterion used to evaluate the toxicity of the heavy metals. Before the experiment, the previously cleaned aquariums were filled with 40 liters of seasoned (chlorine-free) tap water and scrubbed with potassium permanganate ($KMnO_4$/20). Ten healthy and active Channa Punctatus specimens were moved into various aquaria after the acclimatization period was over. To identify the range of toxicants to be used for the conclusive toxicity test, range finding tests were conducted before to each toxicity experiment. A number of varied concentrations were prepared for the range finding test, and the toxicity of the compounds was assessed using accepted procedures (APHA, 2005) [14]. The concentration for the conclusive toxicity studies was chosen in light of the findings. zinc test concentrations were 1 mg/L, 2 mg/L, 5 mg/L, 10 mg/L, and 15 mg/L. According to the protocol, the exposure water was examined for temperature, pH, and dissolved oxygen (APHA, 2005) [14]. Throughout the exposure, the fish were not fed. Every 24 hours, the water in the aquariums was changed. For the 96-hour test period, the fishes were monitored for signs of death, and the observations were recorded every day. Zinc copy underwent a set of observations. In 96 hours, tests, the measure that is typically employed is death. In the test series, the number of living and dead organisms was counted. The 96-hour LC50 value was determined using the 96-hour mortality data. From this value, it was determined that the sub lethal concentrations were 1/5th, 1/10th, and 1/15th of the LC50 value.

Statistical Analysis

For the mortality study experiment, percentage mortality was calculated for zinc concentration. In the statistical study, the % mortality statistics were employed. Using SPSS software 16.0, the probit mortality was determined. Probit values were plotted on probit paper, and the concentrations of trace metals zinc that kill 50% of test organisms (LC_{50}) during 96 hours of exposure, along with the 95% confidence limit, were calculated in accordance with Finney (1971) [17].

Results

The fish displayed changed behavior during the lethal toxicity trial, including symptoms of fatigue, erratic swimming, jerky movements, restlessness, frequent surfacing, spinning, convulsions, and fin extensions. The fish's schooling behavior was also hampered after exposure to the heavy metals. By using probit analysis, the toxicity of the heavy metal zinc on *Channa punctatu*s, a freshwater fish, was evaluated, and the LC50 for 24, 48, 72, and 96 hours was established. No deaths were noted in the control experiments. When the metals' concentration and duration of exposure rose, the fish mortality went up considerably. This experiment was carried out to gather preliminary data on the toxicity of zinc to freshwater edible fish.

Table I. demonstrates the percentage of death for various exposure times at various zinc concentrations (1 to 20 mg/L). The majority of fish survive the initial onslaught during the early period (i.e., the first 24 h) of the toxicant introduction. Some damages or injuries were evident during the second renewal (48 h exposure), especially in some fish that were exposed to the highest concentrations (10 and 15 mg/L). These wounds certainly make the organisms less resistant to toxins, which cause a considerable amount of death—up to 50%—in the area with the highest concentration. Even at lesser concentrations, deaths become inescapable with progressive exposure after 72 to 96 hours. Stress and the cumulative effects of zinc poisoning may be to blame for this.

For the intervals of 24, 48, 72, and 96 hours, the probit-derived LC50 value for zinc was 19.79, 17.24, 12.36, and 9.47 mg/L 2. The Table II provides the upper and lower bounds. It was discovered that longer exposure times resulted in lower LC_{50} values. Between 24 and 96 hours, the evaluation of the lower and upper confidence limits showed a declining tendency, going from 16.99 to 6.99 and 22.47 to 12.77, respectively.

Table 1: Cumulative percentage mortality of *Channa punctatus* exposed to Zinc

Conc. (mg/L)	24 Hours	48 Hours	72 Hours	96 Hours
1	0	0	0	0
5	0	0	0	20
10	0	0	20	30
15	0	20	40	40
20	40	30	70	100

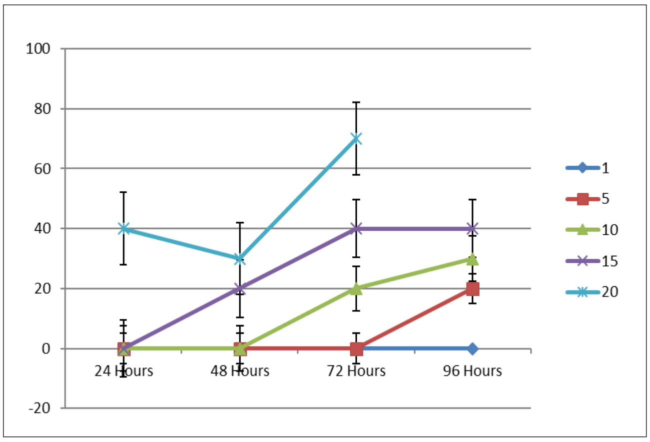

Fig 1

Table 2: Table showing the LC_{50} values, upper and lower confidence limits on exposure to Zinc at different time intervals for the fish *Channa Punctatus*

Parameters	24 Hours	48 Hours	72 Hours	96 Hours
LC_{50} (mg/L)	19.796	17.246	12.369	9.478
Upper confidence limit	22.472	19.997	15.647	12.77
Lower confidence limit	16.990	14.659	9.598	6.99

Table 3: Parameter estimates for the Probit Analysis of 24 hours exposure to Zinc

Parameter		Estimate	St. Error	Z	Sig	95% confidence interval	
						Lower Bound	Upper Bound
Probit	Concentration	9.238	3.012	5.097	.000	5.296	13.180
	Intercept	-11.494	3.615	-5.016	.000	-14.107	-8.880

a. Probit model: PROBIT (P) = intercept + BX (covariates X are transformed using the base 10.000 Logarithm

Table 4: Parameter estimates for the Probit Analysis of 48 hours exposure to Zinc

Parameter		Estimate	St. Error	Z	Sig	95% confidence interval	
						Lower Bound	Upper Bound
Probit	Concentration	7.342	2.801	4.524	.000	3.814	8.867
	Intercept	-7.021	3.258	-4.554	.000	-11.228	-6.764

a. Probit model: PROBIT (P) = intercept + BX (covariates X are transformed using the base 10.000 Logarithm

Table 5: Parameter estimates for the Probit Analysis of 72 hours exposure to Zinc

Parameter		Estimate	Std. Error	Z	Sig	95% confidence interval	
						Lower Bound	Upper Bound
Probit	Concentration	5.736	2.266	4.745	.000	3.257	8.214
	Intercept	-5.997	2.375	-4.637	.000	-7.372	-4.623

a. Probit model: PROBIT (P) = intercept + BX (covariates X are transformed using the base 10.000 Logarithm

Table 6: Parameter estimates for the Probit Analysis of 96- hours exposure to Zinc

Parameter		Estimate	St. Error	Z	Sig	95% confidence interval	
						Lower Bound	Upper Bound
Probit	Concentration	3.924	.790	4.704	.000	2.377	5.471
	Intercept	-2.713	.719	-4.778	.000	-4.431	-2.995

a. Probit model: PROBIT (P) = intercept + BX (covariates X are transformed using the base 10.000 Logarithm

Discussion

Due to the application of trial-and-error approaches using acute and sub lethal exposures of X zinc, the current inquiry has attempted to evaluate the environmental risk of man-made chemicals, primarily focusing on fish. Studies on the median tolerance limits typically give indications of the relative toxicity of various chemicals as well as the sensitivity of different fish species, but they do not represent the highest concentration of harmful compounds that would not influence the metabolism of the fish. Since mortality data would be a practical and practical criterion for evaluating the organism's reaction to fatal concentrations in the current study. The findings showed that zinc is more harmful to Channa punctatus and is more poisonous to the organism. Zinc's LC50 value was 9.238 mg/L. This result is consistent with data on toxicity published by Bryan (1971), Ahsanullah (1976) [15], and Arnott and Ahsanullah (1979) [16]. An organism's tolerance is frequently determined by the amount of a fatal component and how long the animal lives. According to Finney (1971) [17], the connection between the logarithm of the test toxicant (zinc) water concentrations and the logarithm of fish mortality appears to be linear for all treatments (Table 3).

According to published research, normal 96-hour LC50 zinc concentrations for fish range from 1 to 10 mg/liter in soft water (Spear, 1981) [18]. The 96-hour LC50 result for zinc on Channa punctatus in the current investigation, 9.238 mg/L, falls within this range. The acute toxicity of zinc to common freshwater fishes in Lithuania was researched by Gintaras (1999) [19], and the LC50 values for five fish species tested ranged from 3.79 to 11.37 mg /litre. Hardness and water pH are the main physicochemical parameters that alter Zn toxicity (Alabaster and Lloyd, 1980; Spear, 1981; Bradley and Sprague, 1985) [20, 18, 22]. According to Holcombe and Andrew (1978) [21], Spear (1981) [18], and Bradley and Sprague, 1985; Cusimano *et al.*, 1986) [22, 23]. The acute lethality of dissolved Zn increases as water hardness and pH decrease. According to Cusimano *et al*. (1986) [23], the 96-hr LC50 values in soft water (25-44 mg/litre as CaCO3) ranged from 0.066 mg Zn/litre to 0.91 mg Zn /litre (Herbert and Shurben, 1963) [24]. In contrast, they ranged from 3.924 (Holcombe and Andrew, 1978) [21] to 7.21 mg Zn mg/ litre in hard water (179-350 mg/liter as CaCO3), depending on the size of the fish and the test conditions. Rachycentron canadum juvenile and young fish both reported comparable LC50 values of 0.804 mg/L Zn and 0.815 mg/L Zn, respectively (Le *et al*., 2005). Ali *et al*. also established the 96-hour LC50 values of zinc sulphate (ZnSO4.H2O), a significant harmful industrial contaminant, on Poecilia reticulata (2009) [26, 43]. This showed that the LC50 values obtained in the current study were not exactly comparable to those from earlier studies because variations in toxicity could be caused by, among other things: a) different test conditions; b) species specificity of the test chemical, such as, phylogenetic status and immune adaptability; c) the ability of different species to detoxify the compound; d) factors of the medium, such as dissolved oxygen, temperature, hardness, pH, turbidity; and e) age Coins and Scheier's (1964) and Rita and Nair's (1986) findings, as well as the lower 96 hour, 72 hour, and 48 hour LC50 values compared to 24 hour, imply that the fish's resistance decreases as experiment period is extended (1978).

As a result, in stable environmental conditions, different size, weight, and age groups of the organism may have varied toxicity tolerance values. Depending on the period of exposure and the conditions present in the ambient water, metal ions and their complexes have a wide range of toxicity to the organism that can vary from sublethal to lethal (Goel, 1997) [27]. Due to the method of action and responses of the animals, the LC50 values for a given toxicant vary depending on the species (King, 1992) [28]. The size, age (Sanders, 1993) [29], sex (Victoriamma and Radhakrishnan, 1982) [30], nutrient supply (Arunachalam, 1980; Eisler, 1970; Trivedy and Dubey, 1978) [31, 32 34], and genetic characteristics of the individual have also been shown to affect toxicity testing (Newman and Clement, 2008) [33]. The current study shows that zinc, out of the two toxicants under consideration, has significantly harmed fish. This is demonstrated by the fact that a slightly increased zinc concentration in the medium results in the death of a significantly higher proportion of fish.

Typically, predictions on impacts at the ecosystem level cannot be made directly from data on chronic toxicity for a particular aquatic species. However, they typically provide closer approximations of such effects than do the findings of acute toxicity studies. The acute tests should be used for toxicity ranking of chemicals, whereas the chronic tests can give a first approximation of the experimental, laboratory determination of chronic toxicity can be performed using realistic concentrations, i.e., concentrations that can be expected to occur in the environment. This is the difference in usefulness between data from acute and chronic toxicity testing. Additionally, there are many endpoints in chronic tests that can be used to detect deleterious effects in addition to lethality. In order to estimate the mortality of the xenobiotics to the test specimens, short-term toxicity tests had been developed. Lethality is quantified in test protocols for acute (EPA 2002a) [35] and chronic (EPA 2002b) [36] water exposures within these two broad categories of exposure time.

Zinc's lethality has been extensively discussed, and its synergistic interactions with specific metals have also been well-documented. Since these heavy metals are rarely/or never occur in isolation within the environment of influence, further joint action toxicity testing with metals containing zinc should be conducted on various sensitive aquatic creatures to measure departure from its single component toxicity. According to Lloyd (1992) [37], copper and zinc are both poisonous to fish, although copper is more toxic than zinc. The level of toxicity changes depending on whether the metal is in its ionized form, which is more hazardous, or whether it is in contact with an organic component. Additionally, according to a number of authors (Sprague and Ramsay, 1965; Gordon *et al*., 1974; Waiwood and Beamish, 1978; Hansen *et al*., 2002; Karakoç, 1999) [39, 38, 40, 45, 41], the toxic effects of zinc and copper differ depending on the species and age of fish, water temperature, pH, organic matter in the water, and salinity. According to Bat *et al*. (1998), copper is more hazardous than zinc, and this investigation shows that zinc has a far greater lethal dosage limit than copper. The 96-hour LC50 value of ZnCl2 for Chanos chanos was 25 mg/l, according to Herrera *et al*. Lebistes reticulatus, a freshwater teleost, was tested by Khangarot *et al*. (1981) [42] for toxicity to interactions involving zinc, nickel, and copper as well as zinc, nickel, and copper. For each salt, the 48-hour median lethal values (LC50) were 75 mg/l for Zn2+ and 37 mg/l for other salts. Their experiment demonstrated that the toxicity was more than additive in the Zn2+ - Ni2+ mixture when Ni2+ was present in higher proportions. Rasbora sumatrana and Poecilia reticulata fish were shown to be more toxic to zinc, according to a comparison of LC50 values (Gomes *et al*., 2009; Shuhaimi-Othman *et al*., 2010) [46, 44]. Park and Heo (2009) [26, 43] reported that the 24h-LC50 for Zn in P. reticulata was 1.17 mg/L, which was less than in the current study. The findings suggest that strong metallic combinations might endanger fish more toxicologically than the corresponding individual metals. Since water quality, such as water hardness, can affect toxicity (Hodson *et al*., 1982; McCahon and Pascoe, 1988) [47, 48], it is likely that the toxicity reported by other studies differs from that of this study due to the varied species employed, age, size of the organism, test methodologies, and test conditions. There are still significant research that attempt to link LC50 values derived for duration to effects that are significant to ecotoxicologists and risk assessors at other durations (Stark, 2005) [50]. There are methods for converting the several acute lethality measures to chronic fatal effects utilizing a range of estimates (Mayer *et al*., 1995) [49]. Recently, Duboudin *et al*. (2004) [51] performed these extrapolations using species distributions. Despite years of underutilization, these techniques are currently being used more frequently to tackle pertinent issues (Crane *et al*., 2002; Newman, 1995; Newman and Dixon, 1996) [52, 53, 54].

This suggests that the vulnerability of various organisms to heavy metals varies. Metallothionein (MT) production, which is thought to play a protective role against the harmful effects of metals in aquatic species including fish, is one explanation for these variances to trace metals (Roesijadi, 1992; Hollis *et al*., 2001) [55, 57]. In Norway, a thorough investigation of fish and fisheries in a region with numerous copper- and zinc-polluted lakes was conducted (EIFACIFAO, 1977) [59]. The sum of the copper and zinc concentrations in Lake Ringvatnet and Lake Hostovatnet, where there was a healthy fishery, slightly exceeded the water quality standards suggested by EIFACIFAO (Alabaster and Lloyd, 1982) [60]; in Lake Bjorra, where copper and zinc concentrations were significantly higher than EIFAC standards, all fish had disappeared. This shows that the sensitivity of various organisms to heavy metals varies. These discrepancies in trace metals could have a variety of possible causes. Van Loon and Beamish (1977) [56] found no fish in Ross Lake with high concentrations of copper and zinc, but they did observe a slight decline in fish population in Hamell Lake, where, for brief periods of the year, the sum of the metal concentrations exceeded the 95th percentile of the water quality criteria established by EIFAC (1975) [58], while the 50th percentile of the sum of the two metals was 0.5. On the other hand, fish populations were thriving in Lake Cliff, where copper and zinc concentrations were lower. Such findings provide an indirect endorsement of the value of short-term laboratory tests, the results of which were used to establish the relevant water quality criteria.

It is crucial to carry out research with local organisms that may be utilized to gather information on metal toxicity, assess the sensitivity of the organism, and define an acceptable limit for water that can safeguard aquatic communities. The need to conduct toxicity testing on more native fish species under controlled laboratory circumstances is corroborated by the data of numerous other researchers and those of the current study. This would make it possible to use it for hazard assessment, heavy metal or other toxicant testing on wastewater, and real water pollution problems.

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