Archives of Ecotoxicology, Vol. 5, No. 1, pp. 13-18, 2023

SCICELL

Archives of Ecotoxicology

Journal homepage: https://office.scicell.org/index.php/AE

OPEN ACCESS

Evaluation of Toxicity of Lead Salts ($PbCl_2$ and $PbNO_3$) Exposed Common Carp (*Cyprinus carpio*) in Two Seasons (Summer and Winter) of Different Temperature Conditions

Gagandeep Kour[a]*, Rekha Shrivastav[b], Vipin Vyas[a]

[a]*Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal, Madhya Pradesh, India*
[b]*Department of Zoology, MVM Bhopal, Madhya Pradesh, India*

Article info
Received 11 February 2023
Revised 23 March 2023
Accepted 30 March 2023
Published online 30 June 2023

Regular article

Keywords:
Counterions, LC 50,
Seasonal temperatures,
Cyprinus carpio,
$PbNO_3$, $PbCl_2$

Abstract

The main objective of the intended research was to ascertain the toxic effects of Lead salts ($PbCl_2$ and $PbNO_3$) in two seasons of varying temperatures in the Indian subtropical region of Bhopal. The toxicity test was carried out at concentrations of 250, 300, 350, 400,450, and 500 mg/L for the determination of LC 50 of $PbCl_2$ and $PbNO_4$ in summer at high temperature (HT) and in winter with low temperature (LT) conditions in common carp (*Cyprinus carpio).* The LC 50 values for $PbCl_2$ for 24 hours in winter (LT) were 487.98 ± 1.17 mg/L and decreased to 466.66 ± 1.31 mg/L, 436.11 ± 1.68 mg/L, 416.49 ± 1.61 mg/L, for 48, 72, and 96 hours respectively whereas in summer (HT) the LC 50 values were calculated as 396.83 ± 1.38 mg/L, 359.42 ± 1.71 mg/L, 345.95 ± 1.80 mg/L and 321.67 ± 1.24 mg/L for 24, 48, 72 and 96 hours respectively. The LC 50 values for $PbNO_3$ in winter (LT) were calculated as 427.76 ± 2.02 mg/L, 400.13 ± 1.01 mg/L, 373.42 ± 1.76 mg/L, and 359.92 ± 1.36 mg/L for 24, 48, 72, and 96 hours and in summer (HT) LC 50 values calculated as 357.93 ± 1.69 mg/L, 329.76 ± 0.83 mg/L, 300.47 ± 1.41 mg/L and 274.29 ± 2.06 mg/L for 24, 48, 72 and 96 hours respectively. This study discovered that the counterions play an important role in toxicity due to which $PbNO_3$ toxicity was high as compared to $PbCl_2$ in both seasons as well effect of temperature in enhancing the toxicity of both Lead salts ($PbCl_2$ and $PbNO_3$) in summer was studied.

1. Introduction

Toxicology is the science that recognizes the repercussions of toxic chemicals on humans, animals, and the environment as well as understands how a toxic substance will affect or doesn't affect an individual, the amount and time of exposure, sensitivity, as well age-dependent effect of a particular chemical substance (**Bruckner, 2000; Rozman and Doull, 2000; Scheuplein *et al.*, 2002**). Toxicology figures out the mechanisms by which substances exert toxic effects as well to ascertain the presence of toxic substances in various samples. As well toxicity of a chemical substance is dependent upon counterions in association with a heavy metal salt (**Bongers *et al.*, 2004; Peredney and Williams, 2000; Schrader *et al.*, 1998**)

To figure out the mechanisms by which substances exert toxic effects as well as to ascertain the presence of toxic substances in various samples, aquatic toxicology is a multidisciplinary science that syndicates the toxicology, the chemistry of water, and the ecology of water. Standardized tests estimate the acute to chronic toxicity with different exposure systems like static, re-circulatory, renewal, and flow through as well the pathway whether water-borne toxicity or administered through feed provide qualitative and quantitative figures on deleterious effects of chemical substances on aquatic organisms with several standardized methods widely accepted by international agencies like American Public Health Association (APHA), U.S Environmental Protection Agency (EPA). The science of aquatic toxicology understands the detrimental impacts of toxic chemicals on aquatic organisms including fish, and shellfish. It is also well understood that the toxicity of a particular chemical or a substance differs among fish species, and their different developmental stages, and there can be a difference in toxicity level with temperature differences, and ionic composition of water (**Lewis and Morris, 1986; Mebane et al., 2012; Patra *et al.*, 2015; Reynolds *et al.*, 2018; Weeks-Santos *et al.*, 2019; Xing *et al.*, 2022**). Dose significantly plays an important role in toxicology as stated by 'Paracelsus', the father of modern toxicology "Only the dose is the poison" meaning dose is the prime factor to assort the toxicity of various chemicals (**Hunter, 2008**). The terms LD 50 and LC 50 are significantly used by toxicologists to determine the amount of a substance that causes the death of 50% of the population of the tested group in a particular duration of time. J.W Trevan in 1927 first created a test to determine the median lethal dose of LD 50 which is usually performed on mice and expressed as a mass of chemical substance administered in milligrams per kg body weight or Nanograms for bacteria.

LD 50 is an ethical procedure for evaluating acute toxicities, whereas LC 50 means the lethal concentration that determines 50 % of mortalities in water-related toxicities when exposed to chemical substances usually expressed as mg per cubic meter or ppm (parts per million). Lead (Pb) toxicity gets induced at high

*Corresponding author: gagandeepkour5@gmail.com
https://doi.org/10.36547/ae.2023.5.1.13-18

temperatures leading to oxidative and metabolic stress in fish (**Kumar *et al.*, 2018**). The objective of the study was to evaluate Lead (Pb) toxicity in different temperatures (seasonal) conditions as well the toxicity of Lead (Pb) and impact of counterions on toxicity at different seasonal temperatures is less studied.

2. Material and methods

2.1 Experimental fish

Common carp (*Cyprinus carpio*) was selected as an experimental animal. Common carp (*Cyprinus carpio*) is a freshwater fish belonging to the family Cyprinidae is a very resistible fish, can tolerate a wide range of temperatures as well as able to survive in low oxygen concentrations, can spawn throughout the year, and is one of the most cultivable species throughout the world (**Froese and Pauly, 2002**), making it a suitable candidate species for toxicological studies.

2.2 Acclimatization

Based on prior studies and laboratory prerequisites, a total of 2000 fish were brought from local aqua farms of Bhopal and were maintained under laboratory conditions for acclimatization for 20 days in de-chlorinated water by aging water for 6 days in circular tanks of capacity 500 liters. Fish were fed thrice 3 % of body weight with Growel GROWIN feed a day during the acclimatization period of 20 days with continuous aeration as each tank was fitted with air compressors. For this 1200 fish were used in two static experiments at different temperature conditions (summers and winters). Six replicates with 20 fish per replica with one control were maintained in 100 liters aquariums of dimensions 1m × 1m × 0.5m.

2.3 Chemicals

For toxicity analysis, $PbCl_2$ was purchased from Fizmerk, India. A stock solution of $PbCl_2$ was prepared in double distilled water. Other chemicals and reagents used were from Himedia, Pvt Ltd, Mumbai, Maharashtra, India.

2.4 Evaluation of LC 50 in winters (LT)

Evaluation of LC 50 at mean room temperature 23.33 ± 1.24 °C during the day and 9.33 ± 1.24°C at night, water temperature of 7.2 ± 0.75 °C fish of size 10.15 ± 0.54 cm and weight 12.63 ± 0.29 g were selected for static test in which no food was given to the fish. LC 50 at different concentrations of 250, 300, 350, 400, 450, and 500 mg/L of toxicant $PbCl_2$ and $PbNO_3$ were set for the evaluation of LC 50 values for 24, 48, 72, and 96 hours respectively using Probit analysis by **Finney, (1952**) and regression analysis in excel.

2.5 Evaluation of LC 50 in summers (HT)

Fish of size 10.2 ± 0.58cm and 12.46 ± 0.44g were selected. LC 50 values at a mean room temperature of 39 ± 0.81 °C during the day and 33.66 ± 0.47°C at night with a mean water temperature of 27.66 ± 0.47°C at the same concentrations of 250, 300, 350, 400, 450, and 500 mg/L of $PbCl_2$ were evaluated.

2.6 Temperature, Ammonia, and Dissolved oxygen (DO) assessment

Ammonia levels and dissolved oxygen content (Winker's method) according to **APHA, (1985**). Water temperature was accessed using a mercury thermometer from LAB WORLD and the room temperature was measured using a LABPRO wall thermometer.

2.7 Analysis of data

Statistical analyses were performed using one-way ANOVA and Tukey post hoc pairwise comparison at P<0.05 significance level. Data are presented as mean with ± Standard deviation (S.D).

3. Results

3.1 Morphological and behavioral changes

The toxicity of both salt of Lead induced behavioral changes like change in swimming movement with irregular moves, hyperactivity and loss of balance. The color of the fish faded with the increase in toxicity period, lots of mucous secretion, loosening of skin whereas in control no behavioral or morphological changes were observed.

3.2 Toxicity in winter (LT)

In winter at low temperature conditions with different concentrations of 250, 300, 350, 400, 450 and 500 mg/L $PbCl_2$, the LC 50 values were 487.98 ± 1.17 mg/L for 24 hours and value decreased to 466.66 ± 1.31 mg/L at 48 hour. The 72 hour LC 50 was observed as 436.11 ± 1.68 mg/L and 416.49 ± 1.61 mg/L for 96 hours respectively. Whereas the LC 50 values of $PbNO_3$ at the same concentrations were 427.76 ± 2.02 mg/L, 400.13 ± 1.01 mg/L, 373.42 ± 1.76 mg/L and 359.92 ± 1.36 mg/L for 24, 48, 72 and 96 hours respectively as depicted in Figure 1.

3.3 Toxicity in summer (HT)

The LC 50 values in summer at high temperature at five different concentrations of 250, 300, 350, 400, 450 and 500 mg/L $PbCl_2$ were 396.83 ± 1.38 mg/L, 359.92 ± 1.71 mg/L, 345.95 ± 1.80 mg/L, 321.67 ± 1.24 mg/L for 24, 48, 72 and 96 hours respectively. The LC 50 values were lower in $PbNO_3$ at the same concentrations as 357.93 ± 1.69 mg/L, 329.76 ± 0.83 mg/L, 300.47 ± 1.41 mg/L and 274.29 ± 2.06 mg/L for 24, 48, 72 and 96 hours respectively as depicted in Figure 1.

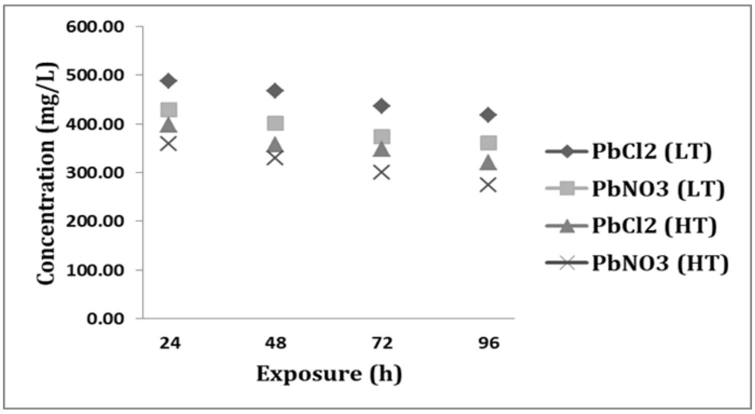

Figure 1 Average LC50 values obtained at different concentrations.

3.4 Estimation of ammonia at different concentrations of $PbCl_2$ and $PbNO_3$ in winter

The ammonia concentration in water at the average water temperature in winter was recorded as 7.2 ± 0.75°C at concentrations of 250, 300, 350, 400, 450 and 500 mg/L of $PbCl_2$. Ammonia levels of 0.17 ppm and 0.67 ppm were recorded at the concentration at 450 mg/L and 500 mg/L whereas with the toxicant $PbNO_3$ at the same concentrations of $PbCl_2$, the ammonia concentration of 0.33 ppm, 0.42 ppm, 0.83ppm at the concentration of 400, 450 and 500 mg/L were recorded as depicted in Figure 2.

3.5 Estimation of ammonia at different concentrations of PbCl2 and $PbNO_3$ in summer

The ammonia levels in summers with increase in temperature were elevated with water temperature of 29.66 ± 0.47°C. Ammonia concentration in water was observed as 0.33, 0.42, 0.83, 0.83 and 1.33 ppm at the concentration of 300, 350, 400, 450, 500 mg/L $PbCl_2$ respectively. As well ammonia excretion increased with $PbNO_3$ salt as compared to $PbCl_2$ at high temperature as 0.42, 0.83, 1.17, 1.33, 2.67 and 3.33 ppm at the concentration of 250, 300, 350,400, 450 and 500 mg/L respectively as depicted in Figure 2.

3.6 Evaluation of dissolved oxygen content at different concentrations of PbCl2 and $PbNO_3$ in winter (LT)

The dissolved oxygen content at the average water temperature of 7.2 ± 0.75°C was estimated as 7.50 ± 0.10mg/L, 7.23 ± 0.06 mg/L, 6.80 ± 0.10mg/L, 6.50 ± 0.10 mg/L and 5.90 ± 0.10mg/L and 5.40 ± 0.10 mg/L at different concentrations of $PbCl_2$ whereas the dissolved oxygen content with $PbNO_3$ was estimated as 7.40 ± 0.10 mg/L, 7.20 ± 0.17 mg/L, 6.80 ± 0.10 mg/L, 6.40 ± 0.10 mg/L and 5.30 ± 0.44 mg/L and 5.23±0.25 mg/L at different concentrations with no significant difference with the dissolved oxygen content of both salts of Lead toxins in winter as depicted in Figure 3.

3.7 Evaluation of dissolved oxygen content at different concentrations of $PbCl_2$ and $PbNO_3$ in summer (HT)

The dissolved oxygen content at the average water temperature of 29.66 ± 0.47°C with toxicant $PbCl_2$ was recorded as 6.70 ± 0.10 mg/L, 6 ± 0.10 mg/L, 5.43 ± 0.03mg/L, 5.10 ± 0.10mg/L, 4.80 ± 0.10 mg/L and 4.50 ± 0.10 mg/L at different concentrations of $PbCl_2$ .There was considerable reduction in the dissolved oxygen content with the toxicant $PbNO_3$ as 6.07 ± 0.12 mg/L, 5.79 ± 0.44 mg/L, 5.41 ± 0.08mg/L, 4.87 ± 0.15 mg/L, 4.70 ± 0.10 mg/L and 4.60 ± 0.06 mg/L at different concentrations of $PbNO_3$ in summer as depicted in Figure 3.

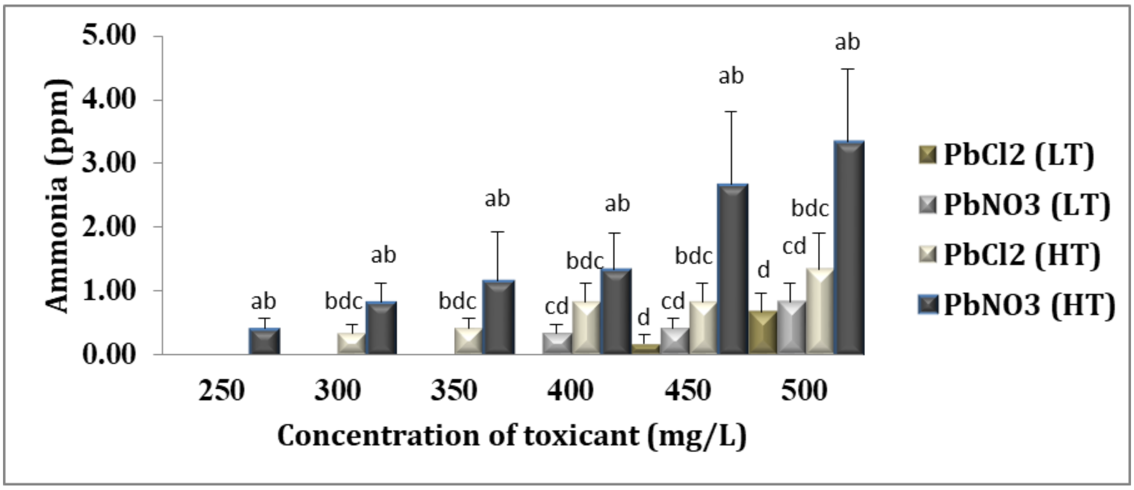

Figure 2 Ammonia levels at different concentrations after 96 h LC 50. Data are presented as mean and error bars with ± S.D. The Tukey's HSD (honestly significant difference) within ANOVA data provides pair wise comparisons. The f-ratio value is 6.48044. The p-value is .003051. The overall result is significant at p < .05. The different letters represent significant different results.

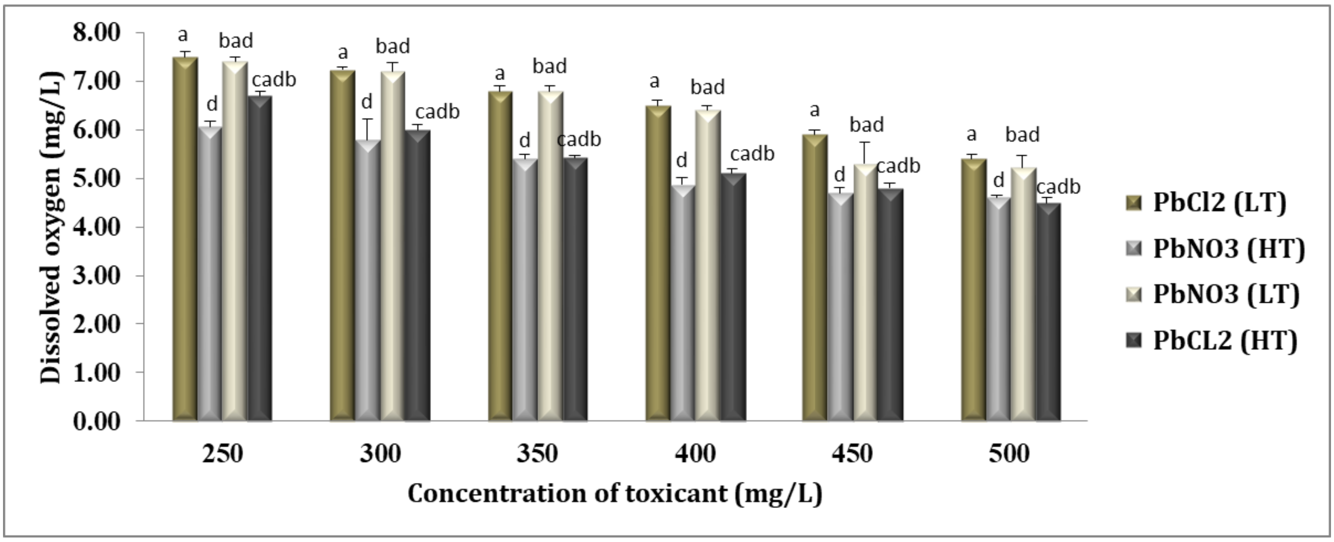

Figure 3 Dissolved oxygen level after 96 h at different concentrations with $PbCl_2$ and $PbNO_4$ in summer and winter. Data is presented as mean and error bars with ± S.D. The f-ratio value is 4.0899. The f-ratio value is 4.18856. The p-value is .018741. The overall result is significant at p < .05. The different letters represent significant different
result.

4. Discussion

Aquatic environment is polluted by toxic chemicals due to anthropogenic or natural activities because harmful chemicals especially heavy metals which tend to bio accumulate in fish tissues make the fish consumption risky (**Javed and Usmani, 2019**). Heavy metals affects the swimming performance as well causes alterations in the sensory perception of fish (**Prashanth *et al.*, 2011**). Lead is toxic to both fish and humans as it affects the hematology, bio accumulates in muscles, bones as well creates disorders in children with low I.Q, stillbirth and birth defects in children (**Lee *et al.*, 2019; Krshisagar *et al.,* 2015; Shah *et al.*, 2020; Wani *et al.*, 2015**). In this study, the toxicity of two salts of Lead was evaluated. The toxins $PbCl_2$ and $PbNO_3$ were accessed for the toxicity test in *Cyprinus carpio* in two different seasons of varied temperature conditions. The toxicity was evaluated in winter and summer. In this study at high temperature in summer, increased in the toxicity of both salts of Lead with behavioral and morphological changes in fish. The LC 50 values in summer at high temperature at five different concentrations of 250, 300, 350, 400, 450 and 500 mg/L $PbCl_2$ were 396.83 ± 1.38 mg/L, 359.92 ± 1.71 mg/L, 345.95 ± 1.80 mg/L, 321.67 ± 1.24 mg/L for 24, 48, 72 and 96 hours respectively. The LC 50 values were lower in $PbNO_3$ at the same concentrations as 357.93 ± 1.69 mg/L, 329.76 ± 0.83 mg/L, 300.47 ± 1.41 mg/L and 274.29 ± 2.06 mg/L for 24, 48, 72 and 96 hours respectively which were low as compared to winter season as 487.98 ± 1.17mg/L for 24 hours, 466.66 ± 1.31 mg/L at 48 hour for 72 hour LC 50 was observed as 436.11 ± 1.68 mg/L and 416.49 ± 1.61 mg/L for 96 hours respectively for $PbCL_2$ whereas the LC 50 values of $PbNO_3$ at the same concentrations were 427.76 ± 2.02 mg/L, 400.13 ± 1.01 mg/L, 373.42 ± 1.76 mg/L and 359.92 ± 1.36 mg/L for 24, 48, 72 and 96 hours respectively. In another study at high temperature and prolonged exposure of heavy metals to fish, mortalities were observed (**Khanna *et al.*, 2007**). Also with the increase in the temperature Lead accumulation rate was high in estuarine teleost fish *Gillichthys mirabilis* (**Somero *et al.*, 1977**). Relatively high temperatures can accelerate the toxicity of heavy metals leading to greater mortality of fish. The LC 50 of $PbNO_3$ in the *Pangasius hypothalamus* was found to be 83.10mg/L, comparatively high at high temperatures than the normal temperature which was 84.93mg/L (**Kumar *et al.*, 2018**). Also, thermal stress along with Lead (Pb) toxicity decreased survivability, affected antioxidant activity as well as body malformations in Zebrafish (*Danio rerio*) embryos (**Park *et al.*, 2020**). At a high temperature of 32°C, pesticide toxicity was increased in goldfish (*Carassius auratus)* with greater cellular damage to the liver and gills (**Jacquin *et al.*, 2019**). In another finding, it was also observed that the toxicity of pesticides endosulfan, chlorpyrifos, endosulfan, and phenol was increased with the increase in temperature in both cold water fish Rainbow trout and warm water fish silver perch with rainbow trout evincing greater toxicity at 30 °C whereas silver perch evincing greater toxicity between 30 to 35 °C for the short duration of 24 and 96 hours (**Patra *et al.*, 2015**).

In this study $PbNO_3$ was found to be more toxic than $PbCl_2$. The role of counter ions in the toxicity of Lead was also studied in the spring tail *Folsomia candida* in soil in which $PbNO_3$ was found to be more toxic than $PbCl_2$, which affected the survivability of *Folsomia candida* in soil (**Bongers *et al.*, 2004**). As well Lead with nitrate was more toxic than chloride in Lumbricid earthworm *Eisenia fetida* (**Peredney and Williams, 2000**). In our study at high temperature the toxicity of $PbCl_2$ and $PbNO_3$ increased with reduction in dissolved oxygen content and increase in ammonia levels with increase in the concentrations of toxicant. The dissolved oxygen content was recorded as 6.07 ± 0.10 mg/L, 6 ± 0.10 mg/L, 5.43 ± 0.03mg/L, 5.10 ± 0.10mg/L, 4.80 ± 0.10 mg/L and 4.50 ± 0.10 mg/L at different concentrations of PbCl2 .There was considerable reduction in the dissolved oxygen content with the toxicant PbNO3 as 6.07 ± 0.12 mg/L, 5.79 ± 0.44 mg/L, 5.41 ± 0.08mg/L, 4.87 ± 0.15 mg/L, 4.70 ± 0.10 mg/L and 4.60 ± 0.06 mg/L at different concentrations of PbNO3 in summer. Also in another study Increase in temperature caused stress in fish that resulted in the mortalities (**Hobbs and Mcdonald, 2010**). In rainbow trout (*Salmo gairdnerii)* the toxicity of Zinc sulphate caused reduction in dissolved oxygen content with increase in toxicity of Zinc sulphate (**Lloyd, 1960**). Increase in temperature caused increase in the oxygen consumption and increase in ammonia excretion in Artic charr (**Lyytikainen and Jobling, 2005**) also in *Alepes djidaba* increase in oxygen consumption and ammonia excretion was observed (**Krishnamoorthy *et al.*, 2008**). Increase in ammonia excretion with increase in the concentration of toxicant was observed by several workers (**Abdullah and Javed, 2006; Abdullah *et al.*, 2007; Boudjema *et al.,* 2016; Rai *et al.*, 2015**). In our study ammonia concentration in water during summer was observed as 0.33, 0.42, 0.83, 0.83 and 1.33 ppm at the concentration of 300, 350, 400, 450, 500 mg/L $PbCl_2$ respectively as well ammonia excretion increased with $PbNO_3$ salt as compared to $PbCl_2$ at high temperature as 0.42, 0.83, 1.17, 1.33, 2.67 and 3.33 ppm. The toxicity of $PbCl_2$ and $PbNO_3$ was high in summers at high temperatures which might be the result of combined stress due to the toxicity of $PbCl_2$ and $PbNO_3$ as well increase in oxygen demand and decrease in oxygen availability to respiratory tissues of fish and excess ammonia excretion enhancing toxicity of $PbCl_2$ and $PbNO_3$ resulting in lower LC 50 values in summers at high-temperature conditions. One of the important and less addressed roles of counter ions in any type of metal toxicity in fish toxicology was studied and for these two different salts of Lead was used in our experiment and it was observed that NO_3- counter ion was more toxic at both temperatures than Cl- ions.

5. Conclusion

The LC50 of $PbCl_2$ and $PbNO_3$ differ in common carp with $PbNO_3$ more toxic than $PbCl_2$; this might be due to the effect of counter ion NO_3^- which was found to be more toxic than Cl^- ions. The LC 50 of $PbCl_2$ and $PbNO_3$ in common carp varied seasonally in this experiment with greater toxicity in summers at high temperatures than in winters with low-temperature conditions as well enhanced toxicity in *Cyprinus carpio* might be due to combined metabolic stress created by high temperature conditions in summer which resulted in elevated toxicities of both Lead salts.

Acknowledgement

Highly thankful to the Head of the Department of Applied Aquaculture department, Barkatullah university for giving valuable suggestions during the research period.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Al- kshab, A.A., Fathi , O.Q., 2021. Determination of the lethal concentration of 50 % (LC50) of lead chloride and its accumulation in different organs of Gambusia affinis fish. *Iraqi Journal of Veterinary Sciences*. 35(2), 361-367. http://www.doi.org/10.33899/ijvs.2020.126853.1401

2. Abdullah, S., & Javed, M. 2006. Studies on acute toxicity of metals to the fish, Catla catla. *Pakistan Journal of Biological Sciences*. 9 (9), 1807-1811. http://dx.doi.org/10.3923/pjbs.2006.1807.1811
3. APHA., 1985. Standard methods for the examination of water and wastewater.
4. Abdullah, S. A. J. I. D., Javed, M. U. H. A. M. M. A. D., & Javid, A. R. S. H. A. D., 2007. Studies on acute toxicity of metals to the fish (Labeo rohita). *Int. J. Agr. Biol*. 9, 333-337.
5. Boudjema, K., Meknachi, A., Kourdali, S., Bounakous, N., & Badis, A., 2016. Effect of sublethal concentrations of heavy metals (cadmium, lead, and copper) on the soluble nitrogen and phosphorus excretion of marine brown mussel (Perna perna)(Linnaeus, 1758)(Mollusca; Bivalvia). *International Journal of Fisheries and Aquatic Studies*. 4(2), 455-462.
6. Bongers, M., Rusch, B., & Van Gestel, C. A., 2004. The effect of counterion and percolation on the toxicity of lead for the springtail Folsomia candida in soil. *Environmental Toxicology and Chemistry*: An International Journal, 23(1), 195-199.
7. Bruckner, J. V., (2000). Differences in sensitivity of children and adults to chemical toxicity: the NAS panel report. *Regulatory toxicology and pharmacology*: RTP. 31(3),280-285. https://doi.org/10.1006/rtph.2000.1393
8. Cai, Y and Summerfelt, R.C., 1992. Effects of temperature and size on oxygen consumption and ammonia excretion by walleye. *Aquaculture*. 104 (1-2), 127-138. https://doi.org/10.1016/0044-8486(92)90143-9
9. Christensen, E.A.F., Svendsen ,M.B.S., Steffensen, J.F., 2020. The combined effect of body size and temperature on oxygen consumption rates and the size-dependency of preferred temperature in European perch Perca fluviatilis. *Journal of Fish Biology*. 97, 794-803. https://doi.org/10.1111/jfb.14435
10. Finney, D. J., 1952. Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge university press, Cambridge.
11. Fry F.E.J., and Hart J.S., 1948. The Relation of Temperature to Oxygen Consumption in the Goldfish. Marine Biological Laboratory, *Biological Bulletin*. 94(1), 66-77. https://doi.org/10.2307/1538211
12. Froese, R., Pauly, D., 2002. Fish Base: a global information system on fishes. World Wide Web electronic publication: www. Fish Base. org., 000-000.
13. Hunter P., 2008. A toxic brew we cannot live without. EMBO reports. 9: 15-18. https://doi.org/10.1038/sj.embor.7401148
14. Hobbs, J. P., & McDonald, C.A., 2010. Increased seawater temperature and decreased dissolved oxygen triggers fish kill at the Cocos (Keeling) Islands, Indian Ocean. *Journal of Fish Biology*. 77(6), 1219-1229. https://doi.org/10.1111/j.1095-8649.2010.02726.x
15. Jacquin L., Gandar, A., Aguirre-Smith, M., Perrault, A., Hénaff, M.L., 2019. High temperature aggravates the effects of pesticides on goldfish. *Ecotoxicology and environmental safety*. 172, 55-264. https://doi.org/10.1016/j.ecoenv.2019.01.085
16. Javed, M., & Usmani, N., 2019. An overview of the adverse effects of heavy metal contamination on fish health. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 89, 389-403. https://doi.org/10.1007/s40011-017-0875-7
17. Khanna D.R., Sarkar, P. , Gautam, A., Bhutiani, R., 2007. Fish scales as bioindicator of water quality of river Ganga. *Environmental Monitoring and Assessment*. 134, 153-160. https://doi.org/10.1007/s10661-007-9606-5
18. Kshirsagar, M., Patil, J., Patil, A., Ghanwat, G., Sontakke, A., & Ayachit, R. K., 2015. Biochemical effects of lead exposure and toxicity on battery manufacturing workers of Western Maharashtra (India): with respect to liver and kidney function tests. Al Ameen J Med Sci. 8(2),107-114. http://ajms.alameenmedical.org/ArticlePDFs/6%20AJMS%20V8.N2.2015%20p%20107-114.pdf
19. Krishnamoorthy R., Syed Mohamed, H.E., Shahul Hameed, P., 2008.Temperature effect on behavior, oxygen consumption, ammonia excretion, and tolerance limit of the fish fingerlings of Alepes djidaba. *Journal of Environmental Science and Engineering*. 50(3),169-174.
20. Kumar N., Krishnani, K.K., Brahmane, M.P., 2018. Temperature induces lead toxicity in Pangasius hypophthalmus: an acute test, antioxidative status, and cellular metabolic stress. International *Journal of Environmental Science and Technology*. 15, 57-68. https://doi.org/10.1007/s13762-017-1364-5
21. Kim, J.H., Kang, J.C., 2015. The lead accumulation and hematological findings in juvenile rockfish Sebastes schlegelii exposed to the dietary lead (II) concentrations. *Ecotoxicology and Environmental Safety*. 115, 33-39. https://doi.org/10.1016/j.ecoenv.2015.02.009
22. Kim, H. J., Mahboob, S., Viayaraghavan, P., Al-Ghanim, K.A., Al-Misned, F., Kim, Y.O., Ahmed, Z., 2020. Determination of toxic effects of lead acetate on different sizes of zebrafish (Danio rerio) in soft and hard water. *Journal of King Saud University-Science*. 32(2), 1390-1394. https://doi.org/10.1016/j.jksus.2019.11.032
23. Lyytikainen, T., Jobling, M.,1998. The effect of temperature fluctuations on oxygen consumption and ammonia excretion of under yearling Lake Inari Arctic charr. *Journal of Fish Biology*. 52, 1186-1198. https://doi.org/10.1111/j.1095-8649.1998.tb00965.x
24. Lewis, W.M.J., Morris, D.P., 1986. Toxicity of nitrite to fish: a review. Transactions of the American Fisheries Society. 115, 183-195. https://doi.org/10.1577/1548-8659(1986)115%3C183:TONTF%3E2.0.CO;2
25. Lee, J.W., Hwang, H. U.K., Kang, J.C., Kang, Y.J., Kim, K.I., Kim, J.H., 2019. Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review. *Environmental toxicology and pharmacology*. 68, 101-108. https://doi.org/10.1016/j.etap.2019.03.010
26. Lloyd, R., 1960. The toxicity of zinc sulphate to rainbow trout. *Annals of Applied Biology*. 48(1), 84-94. https://doi.org/10.1111/j.1744-7348.1960.tb03507.x
27. Marr, J.C.A., Lipton, J., Cacela, D., Hansen, J.A., Bergman, H.L., Meyer, J.S., 1996. Relationship between copper exposure duration, tissue copper concentration, and rainbow trout growth. *Aquatic Toxicology*. 36(1-2), 17-30. https://doi.org/10.1016/S0166-445X(96)00801-6
28. Mebane, C.A., Dillon, F.S., Hennessy, D.P., 2012. Acute toxicity of cadmium, lead, zinc, and their mixtures to stream-resident fish and invertebrates. *Environmental Toxicology Chemistry*. 31(6), 1334-48. https://doi.org/10.1002/etc.1820
29. Mager, E.M., Grosell., M., 2011. Effects of acute and chronic waterborne lead exposure on the swimming performance and aerobic scope of fathead minnows (*Pimephales promelas*), *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 154(1), 7-13. https://doi.org/10.1016/j.cbpc.2011.03.002
30. Park, k., Han, E.J., Ahn, G., Kwak, I.S., 2020. Effects of thermal stress-induced lead (Pb) toxicity on apoptotic cell death, inflammatory response, oxidative defense, and DNA methylation in zebrafish (Danio rerio) embryos. *Aquatic Toxicology*. 224, 105479. https://doi.org/10.1016/j.aquatox.2020.105479
31. Paul, S., Mandal A., Bhattacharjee P., Chakraborty S., Paul R., 2019. Evaluation of water quality and toxicity after exposure of lead nitrate in freshwater fish, a major source of water pollution. The Egyptian *Journal of Aquatic Research*. 45(4), 345-351. https://doi.org/10.1016/j.ejar.2019.09.001
32. Patra, R.W., Chapman, J.C., Lim, R.P. , Gehrke, P.C., Sunderam, R.M., 2015. Interactions between water temperature and contaminant toxicity to freshwater fish. *Environmental toxicology and chemistry*. 34(8), 1809-1817. https://doi.org/10.1002/etc.2990
33. Prashanth M.S, Sayeswara H.A, Goudar M.A., 2011. Free cyanide induced physiological changes in the Freshwater fish, Poecilia reticulate. *J. Exp. Sci.* 2(2), 27-31. http://jexpsciences.com/.../6011
34. Portner, H.O., ,Farrell, A.P., 2008. Ecology. Physiology and climate change. *Science.* 322, 690-692. https://doi.org/10.1126/science.1163156
35. Prieto, A.I., Pichardo, S., Jos, A., Moreno, I., Cameán, A.M., 2007. Time-dependent oxidative stress responses after acute exposure to toxic cyanobacterial cells containing microcystins in tilapia fish (Oreochromis niloticus) under laboratory conditions *Aquatic Toxicology*. 84(3), 337-345. https://doi.org/10.1016/j.aquatox.2007.06.012
36. Peredney, C.L., Williams, L., 2000. Comparison of the toxicological effects of nitrate versus chloride metallic salts on Caenorhabditis elegans in soil. In Price FT, Brix KV, Lane NK, eds, Environmental Toxicology and Risk Assessment: Recent Achievements in Environmental Fate and Transport, American Society for Testing and Materials, Philidelphia, PA, 9, 256-268.
37. Reynolds, E.J., Smith, D.S., Chowdhary, M.J., Hoang, T.C., 2018. Chronic effects of lead exposure on top smelt fish (Atherinops affinis): Influence of salinity, organism age, and relative sensitivity to other marine species. *Environmental Toxicology and Chemistry*. 26. https://doi.org/10.1002/etc.4241

38. Rozman, K.K., Doull, J., 2000. Dose and time as variables of toxicity. *Toxicology* 144(1-3), 169-178. https://doi.org/10.1016/S0300-483X(99)00204-8
39. Rai, A. N., Ullah, A., Haider, J., 2015. Determination of acute toxicity of copper and cobalt for Tilapia nilotica. *Journal of Bioresource Management*. 2(1), 3. https://doi.org/10.35691/JBM.5102.0012
40. Shah, N., Khisroon, M., & Shah, S. S. A., 2020. Assessment of copper, chromium, and lead toxicity in fish (Ctenopharyngodon idella Valenciennes, 1844) through hematological biomarkers. *Environmental Science and Pollution Research*. 27(26), 33259-33269. https://doi.org/10.1007/s11356-020-09598-z
41. Scheuplein, R., Charnley, G., Dourson, M., 2002. Differential sensitivity of children and adults to chemical toxicity. I. Biological basis. *Regulatory toxicology and pharmacology*: RTP. 35(3), 429-447. https://doi.org/10.1006/rtph.2002.1558
42. Somero, G.N., Chow, T.J., Yancey, P.H., Snyder, C.B., 1977. Lead accumulation rates in tissues of the estuarine teleost fish, Gillichthys mirabilis: salinity and temperature effects. *Archives of environmental Contamination and Toxicology*. 6(1), 337-348. https://doi.org/10.1007/BF02097774
43. Schrader, G., Metge, K., Bahadir, M., 1998. Importance of salt ions in ecotoxicological tests with soil arthropods. *Applied Soil Ecology*. 7,189-193. https://doi.org/10.1016/S0929-1393(97)00035-8
44. Weeks-Santos, S., Cachot, J., Gourves Clerandeau, P.Y.C., Morin, B., Gonzalez, P., 2019. Sub-lethal effects of waterborne copper in early developmental stages of rainbow trout (*Oncorhynchus mykiss). Ecotoxicology Environal Safety*. 170, 778-788. https://doi.org/10.1016/j.ecoenv.2018.12.045
45. Wani, A. L., Ansari, M. O., Ahmad, M. F., Parveen, N., Siddique, H. R., & Shadab, G. H. A., 2019. Influence of zinc levels on the toxic manifestations of lead exposure among the occupationally exposed workers. *Environmental Science and Pollution Research*, 26, 33541-33554. https://doi.org/10.1007/s11356-019-06443-w
46. Xing, SY., Li, Z.H., Li, P., 2022. A Mini-review of the Toxicity of Pollutants to Fish Under Different Salinities. *Bulletin Environment Contamination Toxicology*. 108, 1001–1005. https://doi.org/10.1007/s00128-022-03528-0
47. Zheng, Z., Jin, C., Li, M., 2008. Effects of temperature and salinity on oxygen consumption and ammonia excretion of juvenile miiuy croaker, Miichthys miiuy (Basilewsky). *Aquaculture International*. 16, 581-589. https://doi.org/10.1007/s10499-008-9169-7