PHYSIOLOGICAL AND HISTOPATHOLOGICAL EFFECT OF LEAD CHLORIDE ON KIDNEY OF *GAMBUSIA AFFINIS*

Amal A. Al-Kshab
Lecturer
Coll. Edu. for Pure Sci ., University Mosul.
amal.biology@uomosul.edu.iq

ABSTRACT

This study was aimed to evaluate the effect of sublethal concentrations of lead chloride on physiological and histopathological studies in *Gambusia affinis* . A total of 500 fish were collected from the bank of the river east of Mosul city. The fish in the aquarium were divided into five groups (control, acute exposure(20,25mg/l) and chronic exposure(5,10mg/l)). The bioaccumulation of lead in acute and chronic periods of exposure showed a significant difference in values between control and treatment. The greatest level was seen in the kidney of fish treated with lead dichloride at 10 mg/l for 30 days. The level of antioxidant Glutathione has a significant decrease in different kidneys of fish treated with $pbcl_2$ exposed to acute and chronic concentrations. The lowest decrease was seen in kidneys of fish treated with $Pbcl_2$ at 10 mg/l for a month. At the same time, a significant increase in lipid peroxidation (malondialdehyde) level was seen in all kidney treated with $PbCl_2$. Histological study of the kidney showed varying degrees of pathological lesions. The glomeruli in the kidney shrank, and degeneration of Bowman's capsule. The Pb accumulation increases with increasing concentration and period of exposure. Both acute and chronic effects caused a change in the level of antioxidants and histopathological changes in kidney of the fish. Histopathological study may be a useful indicator for determining the extent of aquatic contamination. It could be concluded that the kidney changes caused by lead exposures in fish may serve as a biomarker for the contamination of sub-lethal levels of heavy metals and other anthropogenic contaminants.

Keywords: toxicity, heavy metals, sub lethal concentration, acute exposure, chronic exposure

التاثير الفسلجي والمرضي النسجي لكلوريد الرصاص في كلى *Gambusia affinis*

امال عبدالاله الخشاب

مدرس

كلية التربية للعلوم الصرفة– جامعة الموصل

المستخلص

هدفت الدراسة إلى تقييم تاثير التراكيز تحت القاتلة لـ $PbCl_2$ على التأثيرات الفسلجية والمرضية النسجية في كلى اسماك *Gambusia affinis*. استخدمت 500 سمكة تم جمعها من ضفة النهر شرق الموصل. وزعت الاسماك في الاحواض في خمسة مجاميع(السيطرة، مجموعة التعريض الحاد 20و25 ملغم/لترو مجموعة التعريض المزمن 5و10 ملغم/لتر). أظهر التراكم الأحيائي للرصاص في فترات التعرض الحادة والمزمنة اختلافًا كبيرًا في القيم بين مجموعتي السيطرة والمعاملة. وجد أكبر معدل للتراكم الحيوي في كلية الأسماك المعاملة بثنائي كلوريد الرصاص بمعدل 10 ملغم / لتر لمدة 30 يومًا. حدث انخفاض في مستوى مضاد الأكسدة الكلوتاثايون في كلى الأسماك المعاملة بـ $PbCl_2$ بسبب تاثيرات التعرض الحاد والمزمن. وان اقوى انخفاض للكلوتاثايون وجد في كلى الأسماك المعاملة بـ $PbCl_2$ بتركيز 10 ملغم / لتر لمدة شهر. في الوقت نفسه، لوحظت زيادة كبيرة في مستوى بيروكسيد الدهون (مالون ثنائي الديهايد) في جميع الاسماك المعاملة بـ $PbCl_2$. أظهرت التغيرات النسيجية المرضية للكلية درجات متفاوتة من الآفات المرضية. تضمنت انكماش الكبيبات الكلوية، وتمزق محفظة بومان. يزداد تراكم المعادن مع زيادة التركيز وفترة التعرض. تسبب كل من التأثيرات الحادة والمزمنة في حدوث تغيير في مستوى مضادات الأكسدة والتغيرات النسيجية المرضية في بعض أعضاء الاسماك قيد الدراسة. قد تكون الأختبارات النسيجية مؤشر لتحديد مدى التلوث المائي. يمكن الاستنتاج أن التغيرات الكلوية الناتجة عن تلوث الأسماك بالرصاص قد تكون بمثابة مؤشر حيوي لتلوث المستويات شبه المميتة من المعادن الثقيلة وغيرها من الملوثات البشرية.

الكلمات المفتاحية: السمية، المعادن الثقيلة، التراكيز تحت القاتلة، التعريض الحاد، التعريض المزمن

Received:14/11/2021 Accepted:2/3/2022

INTRODUCTION

Fish are an important indicator in aquatic environments, especially when evaluating the lethal hazards of human use (2, 22). Pollutants picked up by an organism are disseminated to various organs of the fish due to their biochemical diversity (23). Fish tissues such as muscle, liver, gonads, and gills have been used in heavy metal in many ways (37). Metals are unrelenting contaminants, and they aggregate in groundwater, water, sedimentary rocks, and figsynthetic chains (32). Heavy metal ingestion by submerged species may have a long-term effect on ecosystem nutrient cycling. Heavy metals may also have a major impact on the of growth rate of major carp (15,33). For ensuring the quality of food and protection, it is vital to keep track of all possible ecosystem contaminations and their effects on food webs (6 ,27). Also, Trace metals in low concentrations may cause residual stress., which does not cause the individual fish to die but does cause the fish's size and weight to decrease (35). Lead (Pb), among other heavy metals, is one of the most toxic pollutants in the marine setting. Lead emissions come from a variety of sources, including lead ore mining and smelting, industrial effluents, fertilizers, pesticides, and sewage waste (4,30). The Pb can enter a fish's body through a number of pathways, including the skin, gills, and respiratory tract (21). Heavy metal is characterized by its ability to accumulate in the environment and the difficulty of eliminating its effects. Therefore, these toxic substances are harmful to the aquatic ecosystem and to humans; When it passes up the food chain, it comes into contact with it (7). Mosquito fish is a small fish native to the southern United States that possesses a polytrophic system, in which feeding varies among detritus, planktons, and small invertebrates , It is one of the most common types of high temperatures (11).

MATERIALS AND METHODS

The study aimed to investigate the accumulation level of lead in the kidney, determination the GSH activity measuring the lipid peroxidation, and and studying histopathological changes following exposure to $PbCl_2$ in *Gambusia affinis*.

A. Specimens Preparation

A total of 500 fish, *G. affinis* (average weighed 0.2 g, length 2.0 cm) were collected from the coasts of Tigris in Mosul city. Fish were acclimated for at least two weeks in laboratory conditions. The animals were held in glass aquaria of tap water that had been dechlorinated. A total of 20 fishes were put in dechlorinated tap water as a control group, and twenty fish were put in the glass aquariums (30x30x40 cm); concentrations of $PbCl_2$ 5,10 mg/l for long term exposure (15 days and month) and two concentrations lead chloride 25,20 mg/l for a short exposure (24 hours and four days), with three replicates for each treatment.

B. Determination of the level $PbCl_2$ accumulation, GSH, and MDA

The concentration of $PbCl_2$ accumulated in the kidneys was measured using of Vosylien (35). Estimation GSH level using alman method (14). Method was relied upon (34) to determination of MDA level.

C. histopathology study

Following the technique of Suvarna (31), tissue samples were subsequently processed to study histopathological changes. A microtome was used to cut tissue into 5 mm sections (Histo-line MR 2258). Ribbons of tissue slices made of paraffin wax were soaked in hot water (45 C°) for a while before being put on a slide containing adhesive materials such as albumen and glycerine. Tissue-filled slides were baked overnight at 37 C°. The slides were placed in each for three minutes in xylene using clearing material. Immerse the slide for 1–2 minutes at a time in ethanol alcohol. Hematoxylin and Eosin were used to stain the slides for 4–6 minutes. Under a light microscope, the slides were mounted with Canada balsam and coverslips, figgraphed, and histological alterations were compared to control histology slides at magnification (400X).

D. Statistical analysis

Graphpad prism 5 was used to analyze data in this study. Different letters indicate statistically relevant discrepancies between different therapies and controls. Analysis of variance in the same row have significantly different values (Tukey test, $P< 0.05$).

RESULTS AND DISCUSSION

The result of the current study is clarified the occurrence of a significant increase in the amount of lead accumulating in the kidneys and that the accumulation in the period of chronic exposure is more than in the acute exposure. The largest bioaccumulation of $PbCl_2$ was seen in the fish treated with 10 mg/dl (Table 1)(Fig.1,2).

Table 1. Bioaccumulation of $PbCl_2$ in kidneys of fishes(mean±SD), level of GSH and MDA

test	c	20mg/l 24h	20mg/l 96h	25 mg/l 24h	25mg/l 96h	5mg/l 15days	5mg/l 30days	10mg/l 15days	10mg/l 30days
Accum. µg/g dry weight	0.054±0.01 a	0.092±0.002 ab	0.152±0.02 ac	0.172±0.1 ad	0.200±0.1 ae	0.214±0.002 af	0.290±0.005 ag	0.456±0.1 h	0.571±0.2 i
GSH nmol/g	2724±20 a	2615±.62 b	2487±10 c	2366±33 d	2115±12 e	1010±10 f	985±10 g	869±15 h	762±22 i
MDA nmol/g	150±20 a	180±10 ab	195±16 c	230±20 d	285±10 e	325±12 f	350±20 g	376±22 h	390±15 i

• Major variations at $P<0.05$ are indicated by a different letters.

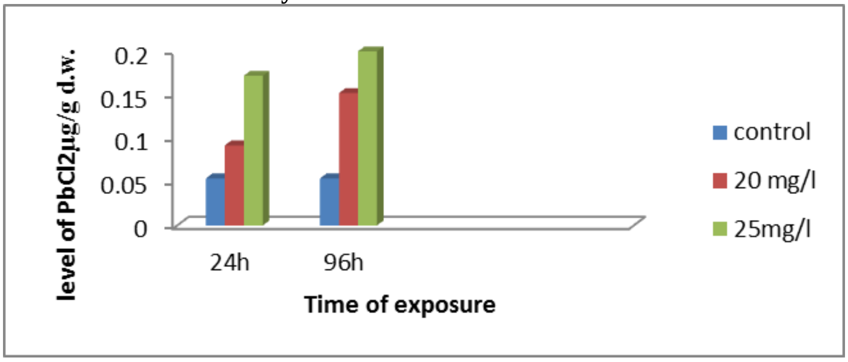

Figure1. Accumulation of lead on kidney of *G. affinis* in Acute exposure

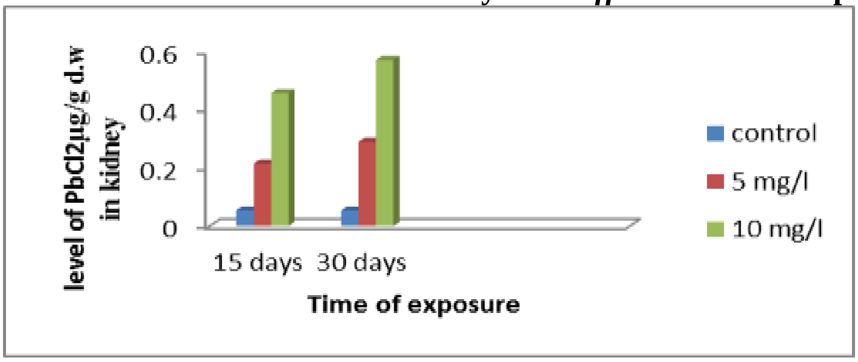

Figure2. Bioaccumulation of $PbCl_2$ in kidney of *G. affinis* in chronic exposure

Activity of antioxidants (GSH) significantly decreased in different concentration in both acute and chronic exposure of fish to $PbCl_2$(Table 1)(Fig 3,4).

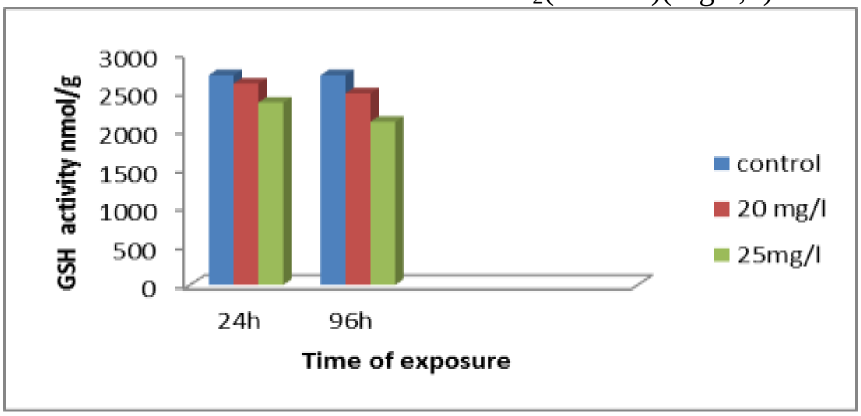

Figure3. GSH level activity in kidney of *G. affinis* in acute exposure

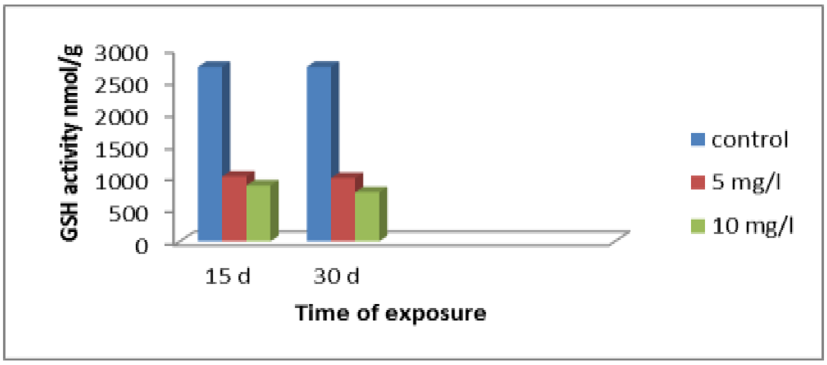

Figure 4.GSH level in kidney activity of *G. affinis* in chronic exposure

Result of the current study indicated a significant increase in MDA level in all concentration for all periods (Table 1)(Fig 5,6).

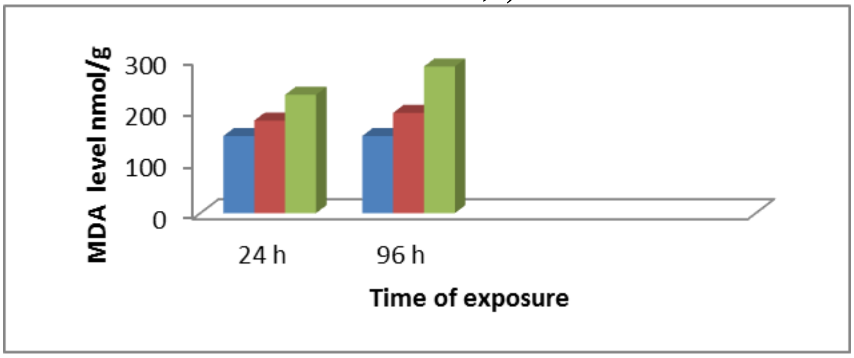

Figure 5. Acute effects of lead exposure on MDA level in kidney of *G. affinis*

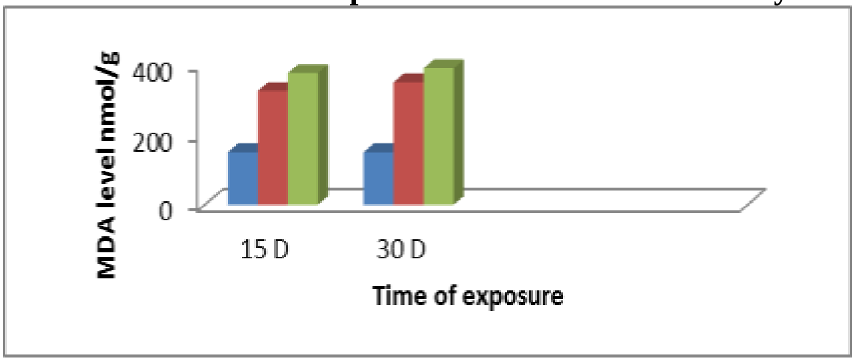

Figure 6. Chronic effects of lead exposure on MDA level in kidney of *G. affinis*

Lead causes oxidative damage at higher concentrations, which can impact the cell membrane directly. The balance of tissue and cellular components between the production and extraction of ROS (reactive oxygen species), which causes injury to the membrane, DNA, and enzymes, are involved in the process of lead-induced oxidative stress (28). Glutathione is an antioxidant-rich in the Sulfhydryl group. It gives it a distinctive electron-giving property and thus gives an electron to the active oxygen species / free radicals, which easily oxidizes GSSG. This rapid use of both GST and GSH leads to a decrease in their levels (18). Glutathione binds to the metal lead. And thus, it works to protect cells from its negative effects(17). Earlier studies have clearly shown that exposure Depending on the species, GSH levels in organisms have risen or fallen as a result of organic pollutants. Types of exposure, duration of exposure, and dosage (10). Oxidative stress causes damage to cell components by active oxygen species arising from the influence of heavy metals. To avoid pathological consequences as a result of the interaction between biological molecules and active oxygen species, glutathione is used by cells for detoxification The liver is one of the most important tissues that makes GSH, and it releases it into other tissues such as the gills, brain, muscles, and kidneys. The gills are the organs most exposed to water contaminated with heavy metals, which can penetrate their delicate epithelial cells, causing low levels of glutathione , In case of acute oxidative stress, the toxic effects of pollutants overwhelm the antioxidant defenses of fish(29). MDA is a degradation of the peroxidation of the lipid. It is also an important biomarker of the toxins in fish that exposure to toxins (5, 38). Abd El-satar et al (1) reported that the effect of Pb(CH3COO)2 on catfish (Clarias gariepinus). They noticed a considerable rise in the amount of MDA in the serum of these fish. A study by Nourian et al (24) who found increased the concentration of MDA in serum of Cyprinus carpio after exposure to lead.

Histological study

The kidney involved in excretion activities in control fish is made up of a large number of nephrons. A renal corpuscle and a coiled uriniferous tubule make up each nephron. The glomerulus and Bowman's capsule made up the corpuscle (fig.7A,B). The histological examination of renal tissue of treated fish at the concentration of 20mg/l for 24h showed swelling of epithelial cells lining renal tubules, leading to lumen stenosis at renal tubules and infiltration of inflammatory cells (fig.7C). At a concentration of 25mg/l for 24h, there was an infiltration of inflammatory cells in the interstitial tissues, degeneration, necrosis, and sloughing of epithelial cells with interstitial hemorrhage (fig.7D). The histological changes of renal tissue of treated fish at a concentration of 20mg/l for 96h showed severe changes characterized by severe infiltration of inflammatory cells in the interstitial tissue (fig.7E). Whereas, at a concentration of 25mg/l for 96h, there were sloughing of epithelial cells lining the renal tubules with severe hemorrhage in the interstitial tissue and necrosis (fig.7F). While the group of fish treated with 5mg/l for 15 days showed severe diffused interstitial infiltration of inflammatory cells (interstitial nephritis) (fig.7G). At 10mg/l for the same time, it founded acute necrosis of epithelial cells lining the tubules of kidney, infiltration of leucocyte cells, and hemorrhage (fig.7H). After 30 days, it showed focal inflammatory cells infiltration between the tubules and necrosis at a concentration of 5mg/l (fig.7I). Whereas fish exposure to the concentration of 10mg/l for a month, showed increased at the cellularity of glomerular tufts, which led to Bowman's space stenosis, necrosis of lining renal epithelial tubules, degeneration, and severe hemorrhage in the interstitial tissue (fig.7J).

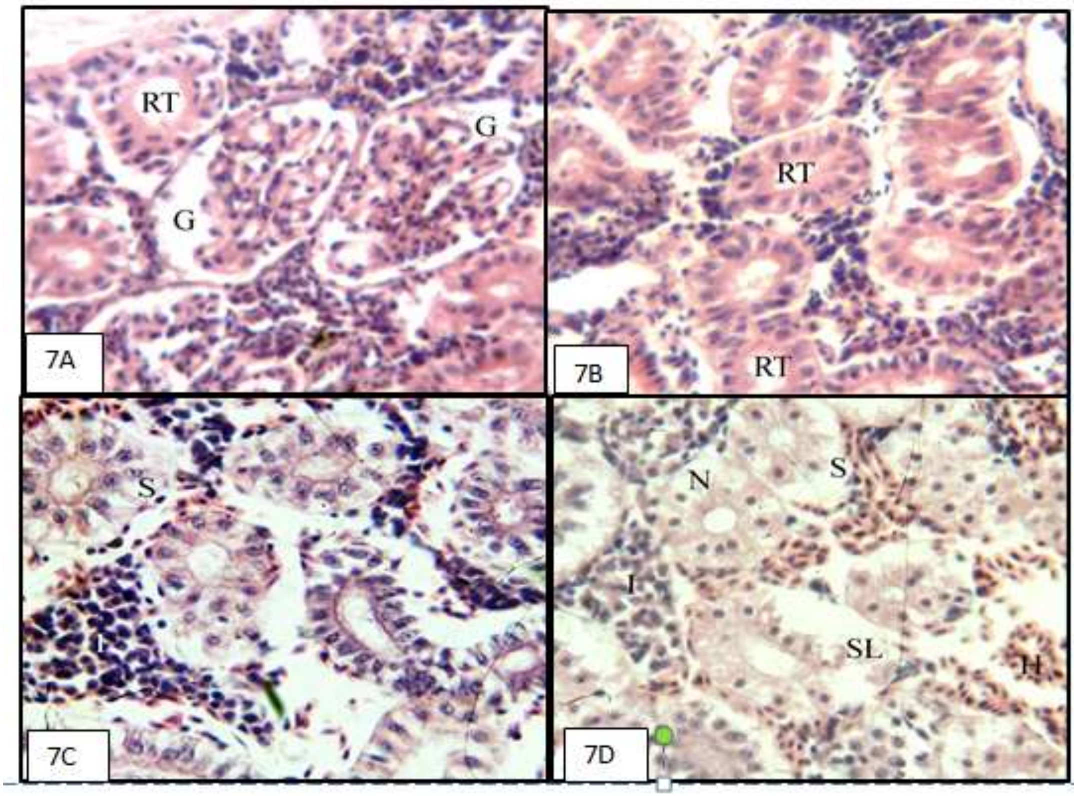

Fig (7A): the structure of normal kidney in the *G. affinis* ;
Fig (7B): Structure of the kidney in the *G. affinis*,400X
Fig (7C): Histological changes of the kidney in the *G. affinis* at 20mg/l $PbCl_2$ for 24h
Fig (7D): Histological changes of the kidney in the *G. affinis* at 25mg/l $PbCl_2$ for 24h.
Abbreviations: G= Glomerulus; RT= Renal tubules; I= Infiltration of inflammatory cells; S= Swelling; N= Necrosis; SL= Sloughing.

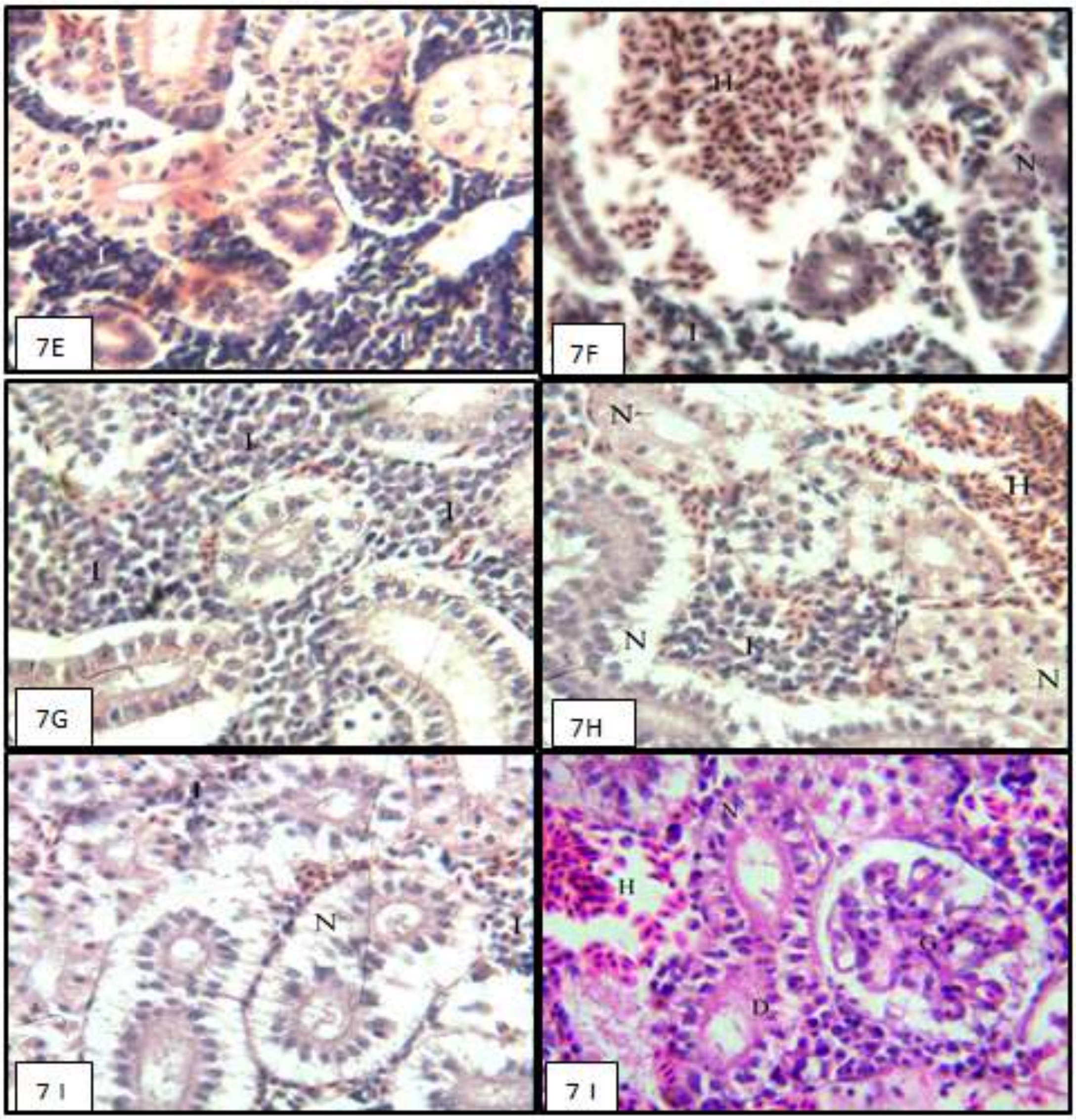

Fig (7E). Histological changes of the kidney in the *G. affinis* at 25mg/l $PbCl_2$ for 24h; Fig (7F).Histological changes of the kidney in the *G. affinis* at 25mg/l $PbCl_2$ for 96h; Fig (7G). Histological changes of the kidney in the *G. affinis* at 5mg/l $PbCl_2$ for 15d; Fig (7H). Histological changes of the kidney in the *G. affinis* at 10mg/l $PbCl_2$ for 15d; Fig (7 I). Histological changes of the kidney in the *G. affinis* at 5mg/l $PbCl_2$ for 30d; Fig (7J). Histological changes of the kidney in the *G. affinis* at 10mg/l $PbCl_2$ for 30d. Abbreviations: G= Glomerulus; I= Infiltration of inflammatory cells; N= Necrosis; H= hemorrhage; D= Degeneration, 400X

The kidney is among the most body tissue for metal pollution detoxifying and removal. (13) , Azmat *et al* (9) explained that The kidney was a good metal pollution indicator. Heavy metals accumulate in the aquatic environment, and in the bodies of fish, it is depends on the properties of the minerals and on environmental conditions , The toxic effect usually occurs when the rate of absorption exceeds the mechanism of metabolism, storage and detoxification (26). Previous studies have identified the accumulation of $PbCl_2$, induced by antioxidants and histological changes in kidneys of fish that exposed to the toxins (12,14,36). The study by Mirmazloomi et al (20) also showed that exposing *Cyprinus carpio* fish to lead acetate caused an increase in the level of MDA in the liver, kidney, and brain of these fish. Experimental fish kidney sections revealed necrosis in renal tubular cells and a weakened glomerulus in the current sample. These findings were in agreement with Al-Balawi et al (3), who studied effects of lead acetate on histopathology of kidney of *Clarias gariepinus* and reported That glomerulus of *C. gariepinus* showed the most significant improvement. There was glomerular expansion, which resulted in Bowman's space being reduced. Tubular necrosis was the most common alteration in the tubules. These results were consistent with mahboob (19) who made the observation tubular hemorrhage, necrosis of renal tubular cells, weakened glomerulus, collecting duct injury, and Congestion of the blood. Mirmazloomi et al (20) reported the identified abnormalities in the kidney reflected glomerulus tissue degeneration, necrosis, and tubular lumen occlusion. Paul et al (25) who investigate Assessment for quality of water and risk in a freshwater fish after exposure to $Pb(NO_3)_2$, an important source of water contamination Extreme necrotic and degenerative effects in the kidney tubules, as well as edema between Bowman's capsules and glomeruli atrophy, were observed in the kidneys of treated fish. Lead is consumed and distributed across the body, including the liver, kidneys, gills, and heart (8).

CONCLUSIONS

In light of the results of this research, it is concluded that lead chloride is moderately toxic to mosquitofish *Gambusia affinis*. Its bioaccumulation in the kidney and induced oxidative stress. Histopathological studies revealed changes in the structural integrity of the cells of the kidney. The current study findings indicate that *G. affinis* is a potential biological indicator of environmental pollution and may be appropriate species for assessing water quality.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the University of Mosul/College of Education for Pure Sciences for providing facilities that aided in the improvement of the quality of this work.

REFERENCES

1. Abd El-satar, S. ,E. Nasr, A. Khaled, and E Hossam.2019. Hemotoxic and genotoxic effects of lead acetate and chlorpyrifose on freshwater cat fish (*Calarias gariepinus*). Sloventan Veterinary Research., 56 (22): 681–691. DOI 10.26873/SVR-807-2019

2. Al-Aboudi, H. J., A. J. Al-Rudainy,. and A. A. M. Maktoof. 2022. Accumulation of lead and cadmium in tissues of *Cyprinus carpio* collected from cages of al-gharraf river /thi qar / Iraq, Iraqi Journal of Agricultural Sciences, 53(4):819–824.
https://doi.org/10.36103/ijas.v53i4.1594

3. Al-Balawi, H. F. , A. S. Al-Akel, F. Al-Misned, , E. Suliman , K. Al-Ghanim, S. Mahboob, and Z. Ahmad. 2013. Effects of Sub-lethal Exposure of Lead Acetate on Histopathology of Gills, Liver, Kidney and Muscle and its Accumulation in these Organs of Clarias gariepinus. Brazilian Archives Biology and Technology .,56(2): pp. 293-302.
https://doi.org/10.1590/S1516-89132013000200015

4. Alengebawy A ., S. Abdelkhalek, S. Qureshi, and M. Wang. 2021. Heavy metals and pesticides toxicity in agricultural soil and plants: ecological risks and human health implications. Toxics. , 9(3): 42. doi: 10.3390/toxics9030042

5. Al-Niemi R .S. and A. Al-Kshab. 2023. Physiological effects of lead, cadmium and their mixture on some organs of *Alburnus mossulensis* fish. Iraqi Journal of Agricultural Sciences, 54(3):638- 646.
https://doi.org/10.36103/ijas.v54i3.1741

6. Andreji, J., I. Stanai, P. Massanyi, and M. Valent. 2006. Accumulation of some metals in muscles of five fish species from lower nitra river. Journal of Environmental Science and Health., 41: 2607-2622. doi: 10.1080/10934520600928003

7. Arojojoye,O. A. , A. A. Oyagbemi, and J. M. Afolabi.2018. Toxicological Assessment of Heavy Metal Bioaccumulation and Oxidative Stress Biomarkers In Clarias gariepinus from Igbokoda River of South Western Nigeria. Bulletin of Environmental Contamination and Toxicology., 100:765-771.
doi: 10.1007/s00128-018-2341-5

8. ATSDR .2005. Draft toxicological profile for lead. Atlanta: US Department of Health and Human Services., 54: 102-225

9. Azmat, H. ,and M. Javed. 2011. Acute toxicity of chromium to Catla catla, Labeo rohita and Cirrhina mrigala under laboratory conditions. International Journal of Agriculture and Biology, 13, 961-965

10. Cheng, D.R., K. Cao, T.T. Wang, X.S. Zhang, M.B. Feng, and H. Liu.2019. Evaluation of the oxidative stress in liver of crucian carp (*Carassius auratus*) induced by 3,4,40 -tri-CDE, 2-MeO-30 ,4,40 -tri-CDE and 2-HO-30, 4,40 -tri-CDE. Journal Environmental Science and Health., 26, 5164–5175

11. Cheng, y. , W. Xiong, J. Tao, D. He, K. Chen, and Y. Chen. 2018. life – history traits of the invasive mosquito fish (*Gambusia affinis* Baird and Girard, 1853) in the central Yangtze River, china. Invasives net., 7 (3): 309-318. https://doi.org/10.3391/bir.2018.7.3.13

12. Choi, E., D. Alsop, and J.Y. Wilson.2018. The effects of chronic acetaminophen exposure on the kidney, gill and liver in rainbow trout (*Oncorhynchus mykiss*). Aquatic toxicology. 198,20-29.
DOI: 10.1016/j.aquatox.2018.02.007

13. Dallinger R, F, Prosi H, Segner and H. Back Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. Oecologia. .1987. Aug;73(1):91-98. doi: 10.1007/BF00376982. PMID: 28311410

14. Gobe, G. and D. Crane. 2010. Mitochondria, reactive oxygen species and cadmium toxicity in the kidney. Toxicology Letter .,198, 49-55.
doi: 10.1016/j.toxlet.2010.04.013

15. Hayat, S., M. Javed and S. Razzaq. 2007. Growth performance of metal stressed major carps viz. Catla catla, Labeo rohita *and* Cirrhina mrigala *reared* under semi-intensive culture system. Pakistan Veterinary Journal . 27(1): 8-12

16. James, R. C. , D. R. Goodman, and R. D. Harbison. 1982. Hepatic glurathione and Hepatotoxicity,changes induced by selected macrortcs. Journal Pharmacology Therapy.,221: 708-714

17. Jan, A., A. Ali, and Q. Haq. 2011. Glutathione as an antioxidant in inorganic mercury induced nephrotoxicity. Journal Postgraduate Medical, 57: 72-77.
doi: 10.4103/0022-3859.74298

18. Javed,M., I. Ahmad, N. Usmani, M. Ahmad.2016. Bioaccumulation, oxidative stress and genotoxicity in fish (*Channa punctatus*) exposed to a thermal power plant effluent. Journal Ecotoxicology and Environmental Safety., 127:163–169. https://doi.org/10.1016/j.ecoenv.2016.01.007

19. Mahboob, S. , K. Al-Ghanim, H.F Al-Balawi,., F. Al-Misned, and Z. Ahmed.2019. Toxicological effects of heavy metals on histological alterations in various organs in Nile tilapia (*Oreochromis niloticus*) from freshwater reservoir. Journal of King Saud University – Science.
https://doi.org/10.1016/j.jksus.2019.07.004

20. Mirmazloomi, S. , D. Shahsavani, and H. Baghshan .2015. Studies on the protective effects of ascorbic acid and thiamine on lead-induced lipid and protein oxidation as well as enzymatic alterations in some tissues of *Cyprinus carpio*. Journal of Comparative Clinical Pathology.24(1). Comp Clin Pathol. http://dx.doi.org/10.1007/s00580-015-2065-4

21. Muneer J., A. AlObaidb, K. Ur Rehman and K. Erinled. 2022. Appraisal of toxic metals in water, bottom sediments and fish of fresh water lake. Journal of King Saud University - Science Volume 34, Issue1, 101685.
https://doi.org/10.1016/j.jksus.2021.101685

22. Mustafa, S.A. ,A. J. Al-Rudainy, and S.M. Al-Samawi. 2020. Histopathology and level of bioaccumulation of some heavy metals in fish, Carasobarbus luteus and *Cyprinus carpio* caught from Tigris River, Baghdad. Iraqi Journal of Agricultural Sciences, 51(2): 698-704. https://doi.org/10.36103/ijas.v51i2.997

23. Mustafa, S.A., A. J. Al-Rudainy and J.K. Al-Faragi . 2019. Assessment of hydrogen peroxide on histopathology and survival rate in common carp, *Cyprinus carpio* L. infected with Saprolegniasis. Iraqi Journal of Agricultural Sciences,50(2):697-704. https://doi.org/10.36103/ijas.v2i50.669

24. Nourian, K. Shahsavani, D. and H. Baghshani.2018. Effects of lead (Pb) exposure on some blood biochemical indices in Cyprinus carpio: potential alleviative effects of thiamine. Comparative Clinical Pathology. https://doi.org/10.1007/s00580-018-2814-2

25. Paul S., A. Mandal , P. Bhattacharjee, S . Chakraborty, R. Paul ,and B. Mukhopadhyay.2019. Evaluation of water quality and toxicity after exposure of lead nitrate in fresh water fish, major source of water pollution. Egyptian Journal of Aquatic Research.45(4):345-351 .Egyptian-journal-of-aquatic-research
https://doi.org/10.1016/j.ejar.2019.09.001

26. Rajeshkumar, S. and X. Li. 2018. Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China. Toxicol Reports 5: 288-295. DOI: 10.1016/ j.toxrep .01.007

27. Rashid, F. H., A. A. Taha and N. J. Hameed. 2019. Study of toxic heavy metal

removal by different chitosan/hyacinths plant composite. Iraqi Journal of Agricultural Sciences, 50(5): 1416-1424. https://doi.org/10.36103/ijas.v50i5.809

28. Rautray, A K and D. Swarup .2011. Oxidative Stress in Lead and Cadmium Toxicity and Its Amelioration. *Veterinary* Medicine International , (5):457327. doi: 10.4061/2011/457327

29. Sukhovskaya, I.V., E.V Borvinskaya,., L.P. Smirnov, and A. A. Kochneva .2017. Role of glutathione in functioning of the system of antioxidant protection in fish (review). Inland Water. Biol 10, 97–102. https://doi.org/10.1134/S1995082917010187

30. Sultan, M. S. ,M. Z. Thani , H. S. Khalaf and A. J. Salim. 2018. Determination of some heavy metals in solid waste from haevy water treatment station in Baghdad, Iraqi Journal of Agricultural Sciences, 49(3):500-505. https://doi.org/10.36103/ijas.v49i3.122

31. Suvarna, S. K., C. Layton, and J. D. Bancroft. 2019. Bancroft's theory and practice of histological techinques. 8 th. Elesvier. DOI https://doi.org/10.1016/C2015-0-00143-5

32. Swaileh KM,and and R. Sansur .2006. Monitoring urban heavy metal pollution using the house sparrow (*Passer domesticus*). *Journal Environmental Monitoring*, 8: 209-213. doi: 10.1039/b510635d

33. Vinodhini, R. and M. Narayanan.2008. Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). International Journal Environmental Science & Technology, 5: 179-182. https://doi.org/10.1007/BF03326011

34. Volken,E. , G. Nurperi ,and B.Ahmet.2001. N-acetyl cystine reduces cerebral lipid peroxidation in a rat model of infanttile hydrocephalus. Journal Neurol. *Sci.*, Issue1302-1310

35. Vosylien, M Z., and A. Jankaite.2006. Effect of heavy metal model mixture on rainbow trout biological parameters. Ekologija. 4: 12-17.

36. Warchatowska-S´liwaa, E., M. Niklin´skab, A. Go¨rlichc, P. Michailovad, ,and E. Pyzac. 2005. Heavy metal accumulation, heat shock protein expression and cytogenetic changes in *Tetrix tenuicornis* (L.) (Tetrigidae, Orthoptera) from polluted areas. Environmental Pollution., 133: 373–381. http://dx.doi.org/10.1016/j.envpol.2004.05.013

37. Wei H, F. Liu , L. Vilizzi , L E.Wood, Y . Hu1 and H.Gordon. 2022. Environmental related variation in growth and life-history traits of non-native sailfin catfishes (Pterygoplichthys spp.) across river basins of South China. Aquatic Invasions , Volume 17. https://www.researchgate.net/publication/357117343

38. Zhao, F., Q. Xiang, , Y. Zhou, , X. Xu, X. Qiu, ,and Y. Yu. 2017. Evaluation of the toxicity of herbicide topramezone to, chlorella vulgaris: Oxidative stress, cell morphology and figsynthetic activity. *Ecotoxicology* Environmental Safety., 143, 129–135. doi: 10.1016/j.ecoenv.2017.05.022.