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Research Article

Assessment of aluminium chloride ($AlCl_3$) toxicity in histological changes in the kidney of *Cirrhinus mrigala*

Rekha Rani*
Aquatic Toxicology Lab, Department of Zoology, Indira Gandhi National Tribal University (A Central University), Amarkantak-484887 (MP), India
Heena Yadav
Aquatic Toxicology Lab, Department of Zoology, Indira Gandhi National Tribal University (A Central University), Amarkantak-484887 (MP), India

*Corresponding Author. E-mail: reet14@yahoo.co.in

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Abstract
Water is indispensable for sustaining life. The present study aimed to find out the lethal concentration (LC_{50}) of aluminium chloride ($AlCl_3$) and its histological alterations in the kidney of freshwater fish *Cirrhinus mrigala*. A study was based on an experiment design based on lethal concentration. The LC_{50} of aluminium chloride in fish was reported to be 0.087ml/L after 24, 48, 72 and 96 hours. To do further evaluation, the fish were exposed to $AlCl_3$ sublethal concentration $1/10^{th}$ of LC_{50}, *i.e.,* 0.0087ml/L, for 7, 14, 21 and 28 days. After exposure to aluminium chloride, changes in behaviour, including erratic swimming, heightened sensitivity to stimuli, irregular fin movements, congregating at the tank, loss of balance, aggressiveness and respiratory distress, were noticed in *C. mrigala*. The kidneys of control fish showed no significant changes, while in the treated group, morphological changes were observed in each experimental duration, including a decrease in the number and size of tubular cells, expansion of tubular lumen, degeneration and damage in epithelial cells, necrosis, expanded Bowman's space, blood cell around tubular and interstitial cells and glomerulus enlargement were observed in kidney tissues. This histological analysis showed that the degree of alterations in fish kidneys increased time-dependent. These histological changes are early warning signs of environmental health risks, showing that even low levels of heavy metal contamination can severely impact aquatic life and the entire food chain, with direct and indirect consequences for human lives and nature.

Keywords: Aluminium chloride, *Cirrhinus mrigala*, Heavy metal, Kidney, Toxicity, Water

INTRODUCTION

Water, as the fundamental essence of life, is irreplaceable for all living organisms, acting as a critical solvent, facilitating biochemical reactions, and regulating temperature. Water pollution does not only harm nature; it severely affects the environment, ecosystem and living health at risk (Gleick, 1993, Jamil *et al* 2023). Industrial activities, agriculture, and improper waste disposal contribute to water pollution, demanding immediate efforts to reduce its impact. Only a small portion of the nearly 40 million liters of wastewater that enter rivers and other water bodies daily are properly treated (World Economic Forum, 2019; Jamil *et al*., 2023).

Heavy metals are metalloids with a high relative atomic density, typically greater than 4g/cm³ or 5g/cm³. They include elements like lead, aluminium, mercury, cadmium, and arsenic. Aluminium is one of the heavy metals. It appears silvery-white and is considered as the third most abundant metallic element of the periodic table, compromising 8% of earth's crust after oxygen and silicon. It has widespread applications due to its desirable properties such as light weight, low density, corrosion resistance, thermal conductivity, malleability, ductility and high strength. They do not undergo degradation by microorganisms. Instead, they persist in the environment and accumulate in soils, sediments, and living organisms. It rarely exists in its pure form; instead, it is commonly found combined with other elements like chloride, hydroxide, silicate, sulfate, and phosphate, forming aluminium compounds like $AlCl_3$, $Al(OH)_3$, $3Al_2O_3.2SiO_2$, $Al_2(SO_4)_3$, $AlPo_4$ respectively. These aluminium compounds are used in the construction, automotive, and aircraft industries, in producing metal alloys, in the electric industry, in cooking utensils, and in food packaging. They are also used in water

treatment to act as coagulants, aiding in the reduction of organic matter, colour, turbidity, and microorganism levels. Overuse of fertilizers and pesticides containing heavy metals leads to soil contamination and runoff into water reservoirs (Kaur *et al*., 2021).

Waste discharges from manufacturing units and mining operations release heavy metals into water bodies, soil, and the atmosphere (Gautam *et al.,* 2016). The improper disposal of heavy metal-laden consumer goods contributes to landfill accumulation and contaminates water sources with electronic waste, worsening environmental degradation (Cuculić *et al*., 2020). Due to their high toxicity even in low concentration, long-lasting effects, and inability to biodegrade, they are a significant group of contaminants in aquatic environments, leading to mutagenicity, teratogenicity and carcinogenicity (Dasharathy *et al*., 2021) in animals. The geo-accumulation index values for metals in the Amlakhadi, A tributary of Narmada River, Gujrat was Al>Sr>Ti>Cr>Fe>Mn>V>Cu>Zn. (Azaz and Rajendrasing, 2020).

Aluminium chloride ($AlCl_3$) is one of the hazardous and hazardous aluminium compounds (Hazardous substance fact sheet 2008, New Jersey Department of Health). It can enter in aquatic ecosystem into aquatic ecosystems through various natural and human-induced processes. Aluminium chloride poses a hazard to aquatic life due to its capacity to release aluminium ions (Al^{+++}) into the water to form aluminium hydroxide and release hydrogen ions, decreasing pH and water acidification. Al ion can disrupt metabolic, enzymatic, reproductive, and genotoxic effects, including DNA strand breaks and alterations in gene expression (Klingelfus *et al.,* 2015).

The dissolved aluminium concentrations in drinking water typically fall within the 0.001 to 0.05 mg/litre range in waters with near-neutral pH values. However, in environments characterized by acidity or an abundance of organic matter, these concentrations can elevate substantially, reaching levels of 0.5 to 1 mg/litre (WHO, 1998). The permissible limit for aluminium in drinking water, according to the Bureau of Indian Standards (BIS ,2012) standards, is 0.2 mg/L and the recorded average concentration of aluminium in Hooghly River was 78,127 ± 11,187 µg/L. (Mitra *et al*., 2018). India is the second-largest producer of freshwater fish after Japan. Freshwater fish account for 55% of the total production of 6.57 million metric tons (MMT) and carp contribute more than 87% of India's total production. Fish is a nutrient-rich food source that contains protein, essential amino acids, healthy fats, and a variety of vitamins and minerals, and EPA, Omega 3 which is required to maintain healthy life and have anticancer activity (Manson *et al*., 2019). Fish often accumulate heavy metals in tissues as a result of bioaccumulation and they can easily be passed from fish to others by the food chain (Naz *et al*., 2023).

To assisting heavy metal reciprocation in water, freshwater carp *Cirrhinus mrigala* the Indian major belonging to the family Cyprinidae, is selected for this study. Due to worldwide distribution and fish contains high-quality protein, fat, and vitamins. Additionally, it is a good source of amino like leucine, valine, aspartic acid, glutamic acid, glycine, alanine and a good source of minerals like phosphorus, sodium, and calcium (Paul *et al*., 2016). Fish can tolerate 14°C and are easily cultivated in Asian countries (FAO, 2009), the chosen fish hold economic and ecological importance. Additionally, its ability to acclimatize in laboratory conditions makes it a good bioindicator. Fish kidneys are sensitive to chemical changes in the freshwater ecosystem because they are directly and continuously exposed to all dissolved substances in the water (Bilal *et al*.,2019). Therefore, the present study aimed to examine the histological alterations induced by aluminium chloride ($AlCl_3$)in the kidneys of the edible and commercially important freshwater fish *Cirrhinus mrigala.*

MATERIALS AND METHODS

Fish collection and sampling

For experimental purposes, 60 freshwater fish *C. mrigala* (known as the white carp), both male and female, of average size (15 to 25cm) and average weight (150 to 240 gram), was caught with the aid of local fishermen using seine nets (70×80m) with a mess size (35-40mm) and dugout canoes from the Johilla river, Amarkantak. Fish were transferred with the help of a net into the clean plastic bucket. Then, the fish were transferred from a bucket to a pond like structure created using tarpaulin of size (12×9m) in an autorickshaw to provide natural atmosphere to them and brought to Aquatic Toxicology Laboratory, Department of Zoology, Indira Gandhi National Tribal University, Amarkantak. In the laboratory, six well-ventilated aquariums (75×35×37.5cm) with a capacity of approximately 100L were cleaned with a 1% KMnO4 solution. The aquariums, labelled I to VI, were filled with tap water. The tap water temperature was 27±2°C, as determined by a laboratory thermometer, and the pH was measured to range from 7 to 7.2 using litmus paper. Fish were disinfected with a 1% potassium permanganate ($KMnO_4$) solution for 1- 2 minutes to prevent any potential dermal infections. The fish were then weighed using a digital hook weighing machine (GLUN)) and 10 fish were placed in each glass aquarium filled with 50L of tap water. The level of water filled was marked. Each aquarium was covered with lid for fish safety. The fish were not subjected to any additional stress, and aeration was provided using an air pump to aid in acclimatization.

Acclimatization and maintenance

The fish were acclimatized for 7 days in the laboratory. Throughout the acclimatization period, the fish were fed twice daily with approximately 4-5 grains of artificial food pellets (TAIYO), purchased from local market of Rajendrgram. The pellet contained crude protein, fat, fibre, and moisture. The fish were fed the same type of food and were kept in similar living conditions. Water was replaced as per requirement. As water evaporated, it was replenished to the marked level. To prevent fouling, faecal matter was regularly removed from the aquarium using a siphon pipe, and extreme care was taken to ensure that no outside influences contaminated the water. Feeding was stopped twenty-four hours before starting the experiment.

Test compound

Aluminium chloride ($AlCl_3$) was selected as the toxicant for this study due to its potential to induce metabolic effects. Five hundred grams of anhydrous $AlCl_3$, produced by Central Drug House (CDH), were acquired from Jabalpur.

Reaction with water – Aluminium chloride does not dissolve in water; instead, it creates a hissing sound when it comes into contact with water. During the reaction, the $[Cl]^{3-}$ ions are replaced by H_2O molecules, forming hexahydrate($[Al(H_2O)6]Cl_3$.)

The chemical equation representing this reaction is:

$$AlCl_3 + H_2O \rightarrow [Al(H_2O)_6]^{3+}{}_{(aq)} + 3Cl^-{}_{(aq)}$$

Experimental design

The experiment was divided into two parts-Determination of lethal concentration (LC_{50}). The LC_{50} of aluminium chloride was determined using the log dose/probit regression line approach (Finney *et al*., 1971). An experiment was conducted using 24 fishes in march 2024. Four experimental setups, labelled I, II, III, and IV, were established with varying dosages of aluminium chloride to calculate LC_{50} which is 0.025, 0.05, 0.1, and 0.2 mg/L (Azmat H. *et al*., 2011). The dosages were precisely measured using an electronic balance (Shimadzu ATZ-224) in the Central Laboratory, Department of Zoology, Indira Gandhi National tribal university.Then, the measured chemical was mixed with distilled water in beaker. Each system contained 25 L of well-aerated water in which the prepared solution of chemicals was poured. Using a random selection method, six carps were taken out from the stock to determine the LC_{50} and placed into each aquarium. Before exposure to the compound under investigation, feeding was ceased for 24 hours.

At intervals of 24, 48, 72, and 96 hours, the percentage mortality and survival of fish in the treated groups were recorded for each aquarium. Cessation of operculum movement indicating fish death. The number of dead fish in each aquarium was noted, and immediate removal followed using a fish net to prevent infection in aquarium.

Determination of histological alteration

A 28-day experiment was conducted involving 30 fish. Five glass aquariums labelled control, I, II, III, and IV were prepared for duration of 7, 14, 21 and 28 days. Each aquarium housed a different group of six fish, totalling five groups overall. The control group remained in its natural habitat, while the other four groups were subjected to treatment. Six fish were selected from the stock using a random number method to ensure randomness and placed into each aquarium. One day prior to the experiment, feeding ceased to standardize conditions.

For the experiment, a set of fish was exposed to aluminium chloride at a concentration of 1/10th of the calculated LC_{50} (Lavanya *et al.,* 2011). This concentration was accurately measured by using an electronic weighing balance. Approximately 2ml of experimental aquaria water is taken into a beaker, and the weighted test compound i.e., 0.217mg was mixed well with stirring rod and pour it into the same aquarium. Same process was followed in all experimental sets of aquaria, i.e. I, II, III & IV, except the control set. Water levels will replenish as required to maintain a volume of 25L even as water evaporated over time. For the experimental purpose, the treated group and one control fish were removed from their respective aquariums at the intervals of 7, 14, 21, and 28 days. Kidney samples of each fish were collected for histological analysis to assess any alterations. Behavioural changes were observed daily for 12 hours in both the control and treated groups throughout the experiment.

Dissection of fish

At the end of each experimental duration, 7, 14, 21, and 28 days, the *C. mrigala* fish were removed from the aquariums using a fish net and weighed using a digital hook weighing machine (GLUN). Then, the fish was euthanized through cervical dislocation by applying of gentle pressure to the head while holding the tail. The fish was then placed ventrally on a dissection tray. Using scissors, a midline incision was made along the ventral side of the experimental fish from the posterior to the anterior.Then, the thin parietal peritoneum and connective tissue over kidney were gently separated using the blade to expose the kidneys. Kidney was carefully taken out from the body cavity using forceps. The microtomy procedure was performed, and the slides were observed under a light microscope (QUASMO STAR-7) at 40X magnification. The same procedure was repeated after intervals of 7, 14, 21 and 28 days. Histological analysis was conducted based on the methods described by Porter and Joseph 1953,

Carleton, 1967 and Bancroft *et al*. 2002.

Ethical approval

The experiment was performed with the prior permission of the Institution Animal Ethics Committee(IAEC) approval 2004/GO/ReBi/S/18/CCSEA.

RESULTS AND DISCUSSION

LC_{50} was observed after exposing the fish to aluminium chloride at different concentrations: 0.025, 0.05, 0.1 and 0.2mg for 24, 48, 72, and 96 hours, respectively, in four separate aquariums labelled I, II, III and IV. The LC_{50} value was found to be 0.087ml/L. The sublethal concentration of LC_{50} ($1/10^{th}$) was calculated to be 0.0087ml/L.

The toxicity of aluminium chloride exhibits variability among species and even within strains of the same species. The lethal concentration of aluminium in various fish species was determined to be 1.53mg/L in *Rasbora sumatrana* by Othman *et al*. (2013) and 0.25mg/L for *Labeo rohita* by Banavathu *et al*. (2016). The lethal concentration (LC_{50}) of aluminium was found to be 0.5mg/L by Gebara *et al*. (2021). The $AlCl_3$ sublethal concentration (1/4th of LC_{50}) was 4.698 mg/L in *Catla catla.* For other heavy metals like copper the LC_{50} was noted as 1.25ppm for *C. catla* by Naz *et al*. (2023), while for mercury,it was 0.24ppm by Emiyarti *et al*. (2020). The lower LC_{50} value observed in the present investigation may be attributed to factors such as fish weight, age, water quality, and the purity of the test compound. The weight of the control and treated group before and after the treatment was the same.

Behavioural change

In the control set of aquaria, there were no behaviour changes observed in the fishes due to good water quality, while in the treated group, several behavioural changes were observed during the experimental duration, *i.e.,* 7, 14, 21 and 28 days due to aluminium chloride exposure. Significant changes in the behaviour of treated group fishes, such as erratic swimming, heightened sensitivity to stimuli, irregular fin movements, and congregating at the tank, whereas some fish were observed alone in another corner of aquarium and there was a loss of balance and more frequent resting. Some groups of fish showed lethargic symptoms,

and some showed aggressiveness. Moreover, there was a noticeable reduction in feeding activity during the initial 7-8 days of exposure. Fish also tried to jump out of the aquarium, possibly due to stress caused by aluminium chloride or gill modifications observed during dissection, which were associated with respiratory distress.

Behaviour results from environmental adaptations and enables the organism to modify internal and external stimuli to accommodate the changing water condition. Fish exposed to aluminium chloride showed a number of behavioural abnormalities as a toxicological response, like- erratic swimming, heightened sensitivity to stimuli, irregular fin movements, congregating at the tank bottom, loss of balance, and more frequent resting. Moreover, there was a noticeable reduction in feeding activity during the initial 7-8 days of exposure. Fish also displayed surface-seeking behaviour, which could suggest gill modifications observed during dissection, which were associated with respiratory distress. Same behavioural changes were also observed by Senger *et al*. (2011) in *Danio rerio* after exposure to aluminium chloride. The behavioural changes like erratic swimming, jerky body movements, rolling the body, convulsions, loss of equilibrium and mucous secretion over the body of *Clarias batrachus* were observed at the concentration of 1.5mg/L cadmium sulphate exposure Pundir (2016). Schjolden *et al*. (2017) observed a decrease in swimming activity in crucian carp (*Carassius carassius*) due to aluminium exposure.

Histological observation

$AlCl_3$ sublethal concentration ($1/10^{th}$) of LC_{50} 0.087ml/L was mixed in each aquarium water except control for the period of 7, 14, 21 and 28 days to evaluate the toxicity of aluminium chloride ($AlCl_3$) in fish while maintaining a controlled group for comparing histological alterations in the kidney of *C. mrigala*.The kidneys of the control fish showed normal structures in each experimental duration, *i.e.*, 7, 14, 21, and 28 days at 40X magnification. The control group of fish showed normal anatomy and histology of the kidney (Fig. 1).

The treated fish were exposed to aluminium chloride for 7, 14, 21 and 28 days and showed histological alterations, including damage at nephric and tubular levels observed under a microscope with a 40X power (Fig. 1 I, III, V, VII). After 7 days of aluminium chloride exposure, the fish kidney showed a decrease in the number and size of tubular cells and enlargement of tubular lumen (Fig. 1 II). Whereas after exposure of 14 days, interstitial cell starts degenerating, necrosis of the interstitial cell, expansion of the tubular lumen, bowman's sapce start increasing, and damage in epithelial cells of tubules (Fig. 1 IV). After 21 days of exposure blood cells were noticed in the periphery of tubular cells, an increase in the size of tubular lumen, necrosis of interstitial cells, tubular cell degeneration, and excessive bleeding spread surrounding the epithelium of tubular cells were noticed with an increase in bowman's space (Fig. 1 VI). At the end of 28 day exposure, necrosis of intersitial as well as tubular cell, dark minute granules in tubular and interstitial cells, explained tubular lumen, damage in tubular epithelium, increased bowman's space, cellular degeneration, glomerulus enlargement were observed in kidney tissues (Fig. 1 VIII).

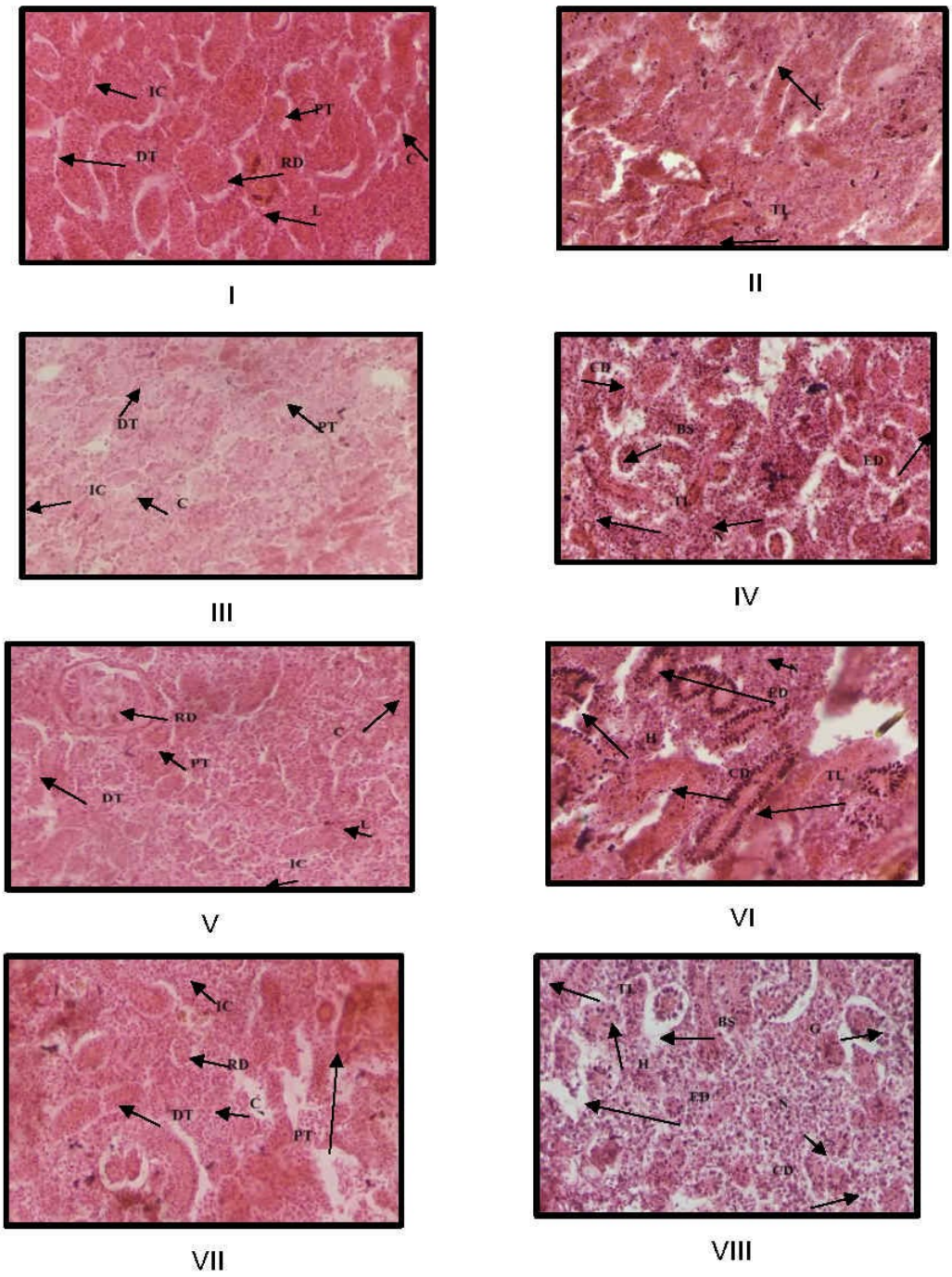

Fig. 1. *Transvers section of kidney of control as well as treated fish Cirrhinus mrigala observed after each experimental durations i.e., 7, 14, 21 and 28 days. (I) control at 7th day; (II) treated at 7th day; (III) control at 14th day; (VI) treated at 14th day; (V) control at 21st day; (VI) treated at 21st day; (VII) control at 28th day; (VIII) treated at 28th day of aluminium chloride exposure . Arrowheads are indicating- (PT) proximal tubule, (RD)Renal Corpuscle, (E)Distal tubule, (IT) interstitial cells, (TL) tubular lumen, (L) lymphocytes, (H) hemorrhage, (CD) cellular degenration, (ED) epithelium damage, (G) glomerulus enlargement, (BS) Bowman's space, (N) necrosis. Magnification 40X*

The findings of the histological alterations in the freshwater fish, *Cirrhinus mrigala*, exposed to a sublethal dose of aluminium chloride (0.087 ml/L) for 7, 14, 21, and 28 days indicate highly significant renal damage at various levels. During the sublethal treatment, the renal tubules exhibited various forms of cellular and structural alterations. These included a decrease in the number and size of renal tubular cells, which can lead to impaired renal function and affect the fish's ability to regulate water and electrolyte balance and maintain homeostasis. The presence of necrotic cells within the renal tubules highlights severe toxic injury, leading to cell death and further compromising kidney function (Fig.1,2) Additionally, an expanded tubular lumen sug-

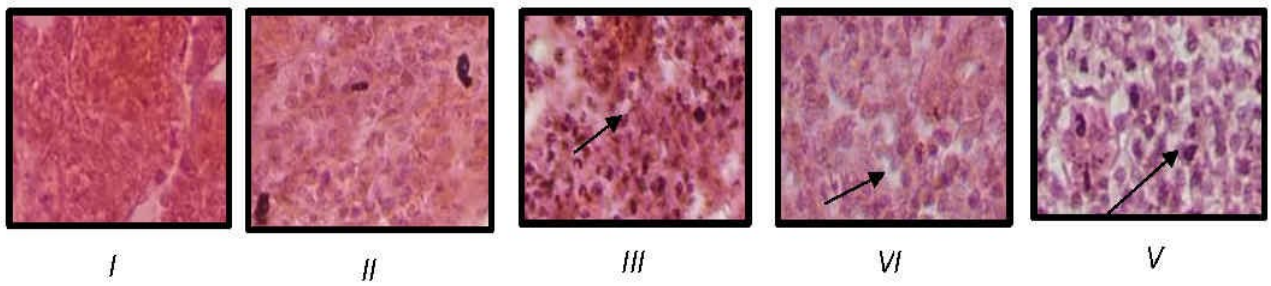

Fig. 2. *Necrosis in tubular cells of control and treated fish Cirrhinus mrigala during each experimental duration i.e., 7, 14, 21 and 28 days. (I) Control; (II) Treated on 7th day; (III) Treated on 14th day; (VI) treated on 21st day; (V) 28th day. Magnification 40 X.*

gests structural damage and potentially altered fluid dynamics within the tubules, which could interfere with normal filtration and reabsorption processes. A lymphocyte reduction was also observed, implying immunosuppression or a redirected immune response. Presence of erythrocytes in the interstitial fluid indicates vascular damage and increased permeability. Cellular degeneration and proximal tubule damage impair kidney function, potentially leading to systemic effects due to the accumulation of waste products. Furthermore, the accumulation of aluminium chloride within renal tissues was observed (Fig. 3). Significant changes were also noted in the renal corpuscles, suggesting hyperfiltration or increased blood flow through the glomerulus, potentially leading to glomerular damage and impaired filtration capacity. An enlarged Bowman's space was noticed, indicating glomerular damage and impaired filtration, contributing to protein loss and other essential molecules in the urine (Fig.1 and 4) These findings collectively demonstrated significant structural and functional abnormalities in the kidney's filtration units, which are crucial for maintaining homeostasis.

The histological changes in fish are a significant and compelling area to understand how environmental pollution impacts their organs' structural organisation.The kidney and brain were more sensitive to exposure to metals Galiciolli *et al*. (2023). Moreover, the nephrotoxic effects of aluminium oxide nanoparticles were confirmed in Oreochromis niloticus, as evidenced by Abdel -Khalek *et al*. (2021). Genotoxic, biochemical, hematological, and histological changes were observed in the kidneys of *Channa punctatus* exposed to mercury chloride, consistent with the findings of Trivedi *et al*. (2022). Al-Kshab (2023) noted necrosis of epithelial cells lining the kidney's tubules, infiltration of leucocyte cells, and hemorrhage in the kidney of lead chloride treated *Gambusia affinis*. This highlights the broader issue of heavy metal toxicity in aquatic organisms and the complex interplay of various metals on fish health. Additionally, sublethal mercury concentrations (0.24 ppm) in *Oreochromis niloticus* caused bleeding, nephritis, mononuclear cell infiltration, and tubular necrosis was evaluated by Emiyarti *et al*. (2020). The alterations observed in the kidneys of freshwater fish in the current study have been similarly documented in various fish species after exposure to different heavy metals. Furthermore, pyknotic nuclei, swollen proximal epithelial cells, necrotic nuclei, blood congestion, and hydropic swelling of tubules were observed in heavy metal-exposed Oreochromis niloticus by Kaoud and Dahshan, (2010) and same result found in *Clarias gariepinus* after exposed to lead acetate by Al-Balawi *et al*. (2013). Salman *et al*. (2017) . In *Clarias batrachus*, aluminium treatment led to the expansion of Bowman's capsule, loss of renal tubules, narrowing of tubular lumens, damage to hematopoietic cells, and clustering of hemosiderin pigments in the kidney observed by Raj *et al*. (2018), while Thophon *et al*. (2003) reported hydropic swelling, tubular cell vacuolation, numerous dark granule accumulations, tubular degeneration, and necrosis in *Lates calcarifer* exposed to cadmium. Gupta and Srivastava (2006) found loss of cellular integrity, glomerular vacuolization, disorganized blood capillaries, necrosis, and pyknotic nuclei in zinc-exposed *Channa punctatus*. Salman *et al*. (2017) found hydropic degeneration and renal tubular necrosis with nuclear pyknosis in lead acetate-exposed *C. carpio*. These findings align with the present study, underscoring the consistent impact of heavy metal exposure on renal health across different fish species.

Similarly, trace metals at environmentally relevant concentrations altered the biochemical and morphological characteristics of fish as observed by Pandey *et al*. (2008). These trace metals also depicted inducing histopathological changes in selected organs of *Cyprinus carpio* L. by Vinodhini and Narayana (2009). In the present study on the histological effects of heavy metal exposure in freshwater fish, observed various alterations in kidney tissues, consistent with existing observations. Same findings were observed in *Oreochromis niloticus*, *Channa punctatus, Cyprinus carpio, Labeo rohita, and Clarias batrachus, and* heavy metal exposure has been shown to induce hydropic swelling of renal tubules, pyknotic nuclei, and necrotic areas, along with swollen proximal epithelial cells with necrotic nuclei. Kaoud and Dahshan (2010). The exposure to

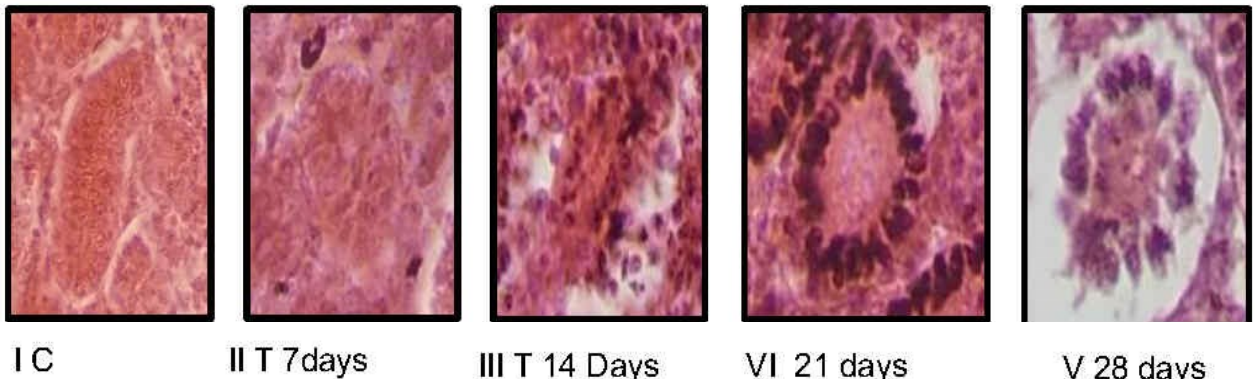

Fig. 3. *Histology Kidney- tubular Lumen of control and treated fish Cirrihinus mrigala*

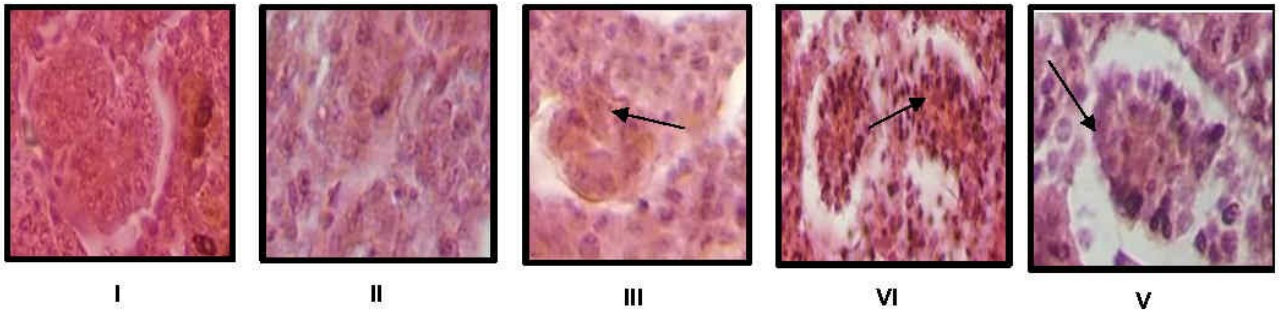

Fig.4. *Increase in bowman's space in control and treated fish Cirrhinus mrigala. (I) control; (II) treated at 7th day; (III) treated at 14th day; (VI) treated at 21st day; (V) treated at 28th day. Magnification 40X.*

lead and cadmium in *Anabas testudineus* resulted in necrosis, degenerated tubules, congestion, lymphocytic infiltration, and vacuolation in kidney tissue is evaluated by Ahmed *et al*. (2014), whereas the same finding was also observed by Bhatkar (2013) in nickel chloride exposure to *Labeo rohita* haemorrhage in the glomeruli, nephrosis, advanced vacuolation, tubular and interstitial tissue disintegration, and disruption of the tubular cells in the kidney that coincided with the present study. Similarly in *Channa striatus* and *Heteropneustes fossilis*, heavy metal exposure led to tubular necrosis, pyknosis, hemorrhage, and glomerular degeneration. Fatima and Usmani (2013). In *Wallago attu* and *Cirrhinus mrigala*, heavy metal exposure was associated with necrosis of the hematopoietic interstitial tissue, vascular degeneration of renal tubules, narrowing of the tubular lumen, glomerulonephritis, and renal tubular atrophy. Hussain *et al*. (2019). Similarly, chromium induces biochemical, genotoxic, and histopathological effects in the liver and kidney of goldfish (*Carassius auratus*), with necrosis of kidney tubular epithelial cells and tubules observed at higher chromium concentrations by Velma and Tchounwon (2010). These findings align with the present observations and support the idea that heavy metal exposure leads to significant renal histopathological changes in freshwater fish. The consistency of these alterations across various species highlights the ubiquitous and damaging impact of heavy metals on aquatic ecosystem, emphasizing the need for environmental monitoring and pollution control measures to protect aquatic ecosystems that directly and indirectly effecting the human.

Biological disarrangement, hypertrophy, and the presence of melanomacrophages were observed, with the severity of tissue distortion directly proportional to the aluminium concentration, as Hadi and Alwan (2012) reported. These alterations are indicative of the tissue's response to aluminium toxicity and suggest a dose-dependent relationship. Additionally, DNA damage was evident in the kidney tissue cells of *Rhamdia quelen* following trophic contamination with aluminium sulfate, confirming the genotoxic potential of aluminium exposure. Klingelfus *et al*. (2015). Similarly, sub-lethal concentrations of aluminium sulfate caused inflammatory changes in the interstitial tissues and partial shrinkage of convoluted tubules in the kidneys of *Clarias gariepinus* juveniles at higher exposure levels. Olurin *et al*. (2016). These findings underscore the histological impact of aluminium on renal tissues observed in the present study, highlighting the degenerative responses brought out by aluminium exposure. Furthermore, the findings of Singh *et al*. (2019) demonstrated that tissue injury and histological alterations increase in a time- and concentration-dependent manner. This suggests that prolonged exposure to aluminium exacerbates tissue damage, emphasizing the need for time-course studies to fully understand the progression of aluminium-induced toxicity.

Conclusion

The present study demonstrated that very low concentrations 0.0087ml/L of aluminium chloride can lead to severe effects in *C. mrigala*, showing altered behaviour, physiological responses and histology of fish organs. In acute exposure of Al for 7, 14, 21 and 28 days, the study observed drastic alteration in fish kidneys. This histological analysis showed that the degree of alterations in fish kidneys is increasing time-dependent. These histological changes are early warning signs of environmental health risks. The impact of heavy metals on fish histology helps in understanding ecological impact, bioaccumulation and biomagnification, toxicological mechanisms, human health risks, and regulatory implications of environmental pollution.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Abdel-khalek, A.A, Al-Quraishy, S. & Abdel,G. (2021). Evaluation of nephrotoxicity in Oreochromis niloticus after exposure to aluminium oxide nanoparticle: exposure and recovery study. *Bulletin of Environmental Contamination and Toxicology*, 108, 292-299.
2. Al-Balawi, H.F.A. & Al-Akel, A.S. Al-Misned, F. Suliman, E.M. Al-Ghanim, K.A. Mahboob, S. Ahmad Z. (2013). Effects of sublethal exposure of lead acetate on histopathology of gills, liver, kidney and muscle and its accumulation in these organs of *Clarias gariepinus*. *Braz. Arch. Biol. Technol*. 56 ,293-302.
3. Al-Kshab, A.A. (2023). Physiological and histopathological effect of lead chloride on kidney of *Gambusia affinis. Iraqi Journal of Agricultural Sciences*, 54(6), 1574-1582.
4. Ahmed, K. Elora, P. Islam, M. Aktar, S. Khan, & S. Habidullah. (2014), Lead and cadmium induced histopathological changes in gill, kidney and liver tissue of freshwater climbing perch *Anabas testudineus*. *Chemistry and Ecology*, 30(6), 532-540.
5. Azaz, S. & Rajendrasing, J. (2020). Assessment of heavy metal contamination of Amlakhadi: A tributary of Narmada River, Gujrat, India. *Int.l Res. J. of Environ. Sci.*, 9(3), 15-27.
6. Azmat, H. Javed & M. Jabeen, G. (2011). Acute toxicity of aluminium to the fish C*atla catla, Labeo rohita, Cirrhinus mrigala*. *Pak. Vet. J.,* 32(1), 85-87.
7. Banavathu, S.N. Rao, N. Ramavathu, N. & Mude, J.N. (2016).Acute toxicity of mercuric chloride ($Hgcl_2$) on survival and behavioural responses of freshwater fish *Labeo rohita* (Hamilton). *Intl. J.l of Fisheries and Aqua. Studies*, 4 (5), 323-327.
8. Bancroft, JD. & Gamble, M. (2002). Theory and Practice of Histological and Histochemical Techniques. 5th Edition, Butter Worths. 211-220.
9. Bilal, H. Maleeha, F.,Al-Ghanim, KA. & Shahib, M. (2019). Environmentally induced nephrotoxicity and histopathological alternations in *Wallago attu* and *Cirrhinus mrigla. Saudi J.l of Bio. Sci.*, 26(4), 752-757. *DOI: 10.1016/ j.sjbs.2019.02.003*
10. Carleton, HM. Druruy, RAB & Walli ngton, EA. (1967). Carleton's histological technique.
11. Cuculić, V. (2020). Heavy metals in electronic waste: Toxicity, recycling, and environmental impact. In Heavy Metals in the Environment: Microorganisms and Bioremediation. 177-194. *CRC Press.*
12. Galiciolli, M. Silva, J. F. Prodocimo, M. M. Laureano, H. Calado, S. Oliveira, C.S. & Guiloski, I.C. (2023).Toxicological effects of thimerosal and aluminium in the liver, kidney and brain of zebrafish (*Danio rerio*). *Metabolites*,13, 975, 1-17.
13. Gleick Peter, H. (1993).Water is Crisis, A Guide to the World's Fresh water Resource, Oxford University Press.
14. Dasharathy, S. Selvam, A. Basavaraju, A. Murugasen, V. Rohini, K. Murugan, R. & Ramachandra, S. (2021). Mutagenic, Carcinogenic, and Teratogenic Effect of Heavy Metals. *Evidence-Based Complementary and Alternative Medicine*, 1-11. https://doi.org/10.1155/2022/8011953
15. Emergency Response Guidebook (2020). U.S. Department of transportation pipeline and Hazardous materials safety administration, Guide 137 (43),210-211.
16. Emiyarti Nur, I. Astiti, Y. & Patadjali, R. (2020). Sublethal toxicity test of mercury (Hg) in the flesh and tissue of *Tilapia* (*Oreochromis Niloticus*). *Omni-Akuatika*, 16(2), 99-107.
17. European chemical agency (ECHA), 2024.
18. FAO (2009). *Cirrhinus mrigala*. Cultured aquatic species fact sheet.
19. Fatima, M. & Usmani, N. (2013). Histopathology and bioaccumulation of heavy metals (Cr, Ni and Pd) in fish (*Channa striatus and Heteropneustes fossilis*). *Pakistani Journal of Biological Science,* 16 (9), 412-420.
20. Finney, DJ. (1971). Probit analysis: A statistical treatment of the sigmoid response curve, second edition, Cambridge university press.
21. Gautam, P.K. Gautam, R.K.. Banerjee, S. Pandey, J.D. & Chattopadhyaya, MC. (2016). Heavy metals in the environment: fate, transport, toxicity and remediation technologies. *Toxicity and Remediation Techniques, Nova Sci Publishers*. 60, 101-30.
22. Gebara, R.C., Goncalves, Alho., Abreu, CB., Mansano, A., Moreira, R.A. Rocha, G.S. Gama Melao, M. (2021).Toxicity and Risk Assessment of zinc and alumnium mixtures to *Ceriodaphnia silvestrii*. *Environ. Toxicol. and Chem.*, 4, 2912-2922. DOI: 10.1002/etc.5162
23. Gleick, Peter H. (1993). Water is Crisis A Guide to the World's Fresh water Resource, Oxford University Press.
24. Gupta, P. & Srivastava, N. (2006). Effects of sublethal concentrations of zinc on histological changes and bioaccumulation of zinc by kidney of fish, *Channa punctatus* (Bloch). *J. Environ. Biol.,* 27: 211-215.
25. Hadi, A. & Alwan, S. (2012). Histopathological changes in gills, liver and kidney of freshwater fish *Tilapia zilli*. *International Journal of Pharmacy and Life Science*, (3), 2071-2081.
26. Hazardous Substance fact Sheet: Aluminium chloride (2008). New Jersey Department of Health.
27. Hussain, B. Maleeha, F. Al-Ghanim & Mahboob, S. (2019). Environmentally induced nephrotoxicity and histopathological alternations in *Wallago attu and Cirrhinus*

mrigala. Saudi Journal of biological science, 26(4), 752-757.
28. Jamil Emon, F. Rohani, MF. Sumaiya, N. Tuj Jannat, MF. Akter, Y. Shahjahan, M. Abdul Kari, Z. Tahiluddin, AB. & Goh, KW.(2023). Bioaccumulation and Bioremediation of Heavy Metals in Fishes-A Review. *Toxics.,* 6;11(6):510. doi: 10.3390/toxics11060510. PMID: 37368610; PMCID: PMC10302055.
29. Kaoud, H. & Dahshan, A. (2010). Bioaccumulation and histopathological alterations of the heavy metals in Oreochromis niloticus fish. *Nature and science*, 8(4), 147-156.
30. Kaur, R. (2021). Heavy metals in agricultural soils: Sources, pollution status, and remediation technologies. *In Environ. Poll. and Rem.*,271-296. https://doi.org/10.3390/agr onomy13061521.
31. Klingelfus, T. Costa, P. Scherer M. & Cestari, M. (2015).DNA damage in the kidney tissue cells of the fish *Rhamdia quelen* after trophic contamination with aluminium sulfate. *Genetics and Molecular Biology*, 38(4), 499-506.
32. Lavanya, S. Mathan, R. Chokkalingam, K. & Annamalai, M. (2010). Haematological, biochemical and ion regulatory responses of Indian major carp *Catla catla* during chronic sublethal exposure to inorganic arsenic. *Chemosphere,* 82, 977-985. DOI: 10.1016/j.chemosphere.2010.10.071.
33. Manson, J. Nancy, PH. Cook, R. Lee, I min. Christen, W. Bassuk, SS. Mora, S. Gibson, H. Albert, CM. Gordon, D. Copeland, T. D Agostino, D. Friedenberg, G. Ridge, C. Bubes, V. Giovannucci, E. Willett, WC. & Buring, JE. (2019). Marine n−3 Fatty acids and prevention of cardiovascular disease and cancer. *The New England Journal of Medicine*, 380(1), 23-32.
34. Mitra, S. Sarkar, S.K. Raja, P. Biswas, JK. & Murugan, K. (2018). Dissolved trace elements in Hooghly (Ganges) River Estuary, India: Risk assessment and implications for management. *Marine Poll. Bulletin*, 133, 402-414. https://doi.org/10.1016/j.marpolbul.2018.05.057
35. Naz, S. Chatha, M. Danabas, D. Khan, F. Xu, Y. Zhu & P. Shafique, L. (2023). Bioaccumulation pattern and health risk assessment of heavy metals in *Cirrhinus mrigala* at panjnad headworks, Bahawalpur. *Pakistan. Toxics.* 11(7), 596. https://doi.org/10.3390/toxics11070596.
36. Olurin, K.B. Mbaka, G.O. & Agbat, O.A. (2016).Histopathological effect of sub-lethal concentration of aluminium sulfate on *Clarias gariepinus* juveniles. *Brazilian Journal of Veterinary Research*, 36(7) ,574-580.
37. Othman, MS. Yakub, N. Ramle, Nur- A. & Abas, A. (2013). Comparative toxicity of eight metals on freshwater fish. *Toxic. and Industrial Health*,31(9), 1-10. DOI: 10.1177/0748233712472519.
38. Pandey, S. Parvez, S. Ansari, R. Ali, M. Kaur, M. Hayat, F. Ahmad, F. & Rasuddin, S. (2008). Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctata*. *Chemico-Biological Interactions* 174, 183-192.
39. Paul, B.N. Sridhar, N. Chanda, S. Saha, G.S. & Giri, S.S. (2016). Nutrition facts *Cirrhinus mrigala* (Mrigal). ICAR-Central Institute of Freshwater Aquaculture.
40. Porter, KR. & Joseph, Blum.(1953). A study in microtomy in electron microscopy, The anatomical record.
41. Pundir G. (2016). Behavioural manifestations in Teleost Clarias batrachus after exposure to heavy metal cadmium sulphate. Voyager *Journal of Science- Life Science* 8,1-5.
42. Raj, A. Panchal, R .& Rani, S. (2018).Histological changes in liver and kidney of Catfish *Clarias batrachus* exposed to aluminium. *International Journal of Zoology and Applied Biosciences,* 3(2), 220-223.
43. Salman, N. Rudainy, J. Faragi, K. & Mustafa, S. (2017). Histopathological alterations in gills, liver and kidney of common carp, *Cyprinus carpio* exposed to lead acetate. *Advances in Animal and Veterinary Sciences*, 5, 371-376.
44. Senger, M.R. Seibt, K.J. Ghisleni, G.C. Dias, R.D. Bogo, M.R. & Bonam, C.D. (2011). Aluminum exposure alters behavioral parameters and increases acetylcholinesterase activity in zebrafish (Danio rerio) brain. *Cell Biology and Toxicology*, 27, 199-205.
45. Schjolden, J. Antonio, B.S. Sorensen, J. & Nilsson, G.E. (2017). The high tolerance to aluminium in crucian carp (*Carassius carassius*) is associated with its ability to avoid hypoxia. *Journal POLS one*, 1-18.
46. Singh, A., Rana, K. & Sharma, K. (2019).Chromium, nickel and zinc induced histopathological alteration in the liver of Indian common carp *Labeo rohita. International Archive of Applied Sciences and Technology,* 10(2), 49-55.
47. Thophon, S. Kruatrachue, M. Upathan, ES. Pokethitiyook, P. Sahaphong, S. & Jarikhuan, S. (2003). Histopathological alterations of white seabass, Lates calcarifer in acute and subchronic cadmium exposure*. Environ. Poll.*121: 307-320.
48. Trivedi, S.P. Singh, S. Trivedi, A.& Kumar, M. (2022). Mercuric chloride induced oxidative stress, genotoxicity, haematological changes and histopathological Alterations in Fish *Channa punctatus. Journal of Fish Biology*, 1-31.
49. Velma, V. and Tchounwon, P. (2010). Chromium induced biochemical, genotoxic and histopathologic effects in liver and kidney of Goldfish, *Carassius auratus*. *Mutation Research*, 698(1-2), 43-51.
50. Vinodhini, R. & Narayanan, M. (2009). Heavy metal induced histopathological alterations in selected organs of the *Cyprinus carpio* L. (Common Carp). *Int. j. of environ. Res.* 3(1), 95-100.
51. World Economic Forum (2019). Water pollution is killing millions of Indians. Here's how technology and reliable data can change that. India economic summit. Tata Centre for Development at UChicago.
52. World Health Organization (1996). Guidelines for drinking-water quality. World Health Organization, Geneva
53. World Health Organization (1998). Guidelines for drinking-water quality 2nd edition. World Health Organization, Geneva.