



The effects of EDTA on zinc accumulation in tissues of *Cyprinus carpio* (Linnaeus 1758)

Fahri Karayakar*,  Fikret Seen , Bedii Cicik 

Faculty of Fisheries, Mersin University, Mersin, Turkey

Abstract

The present study aimed to determine the zinc accumulation levels in the gill, liver, and muscle tissues of *Cyprinus carpio* (Linnaeus, 1758) under the effect of zinc (2.0 and 4.0 ppm) and zinc + EDTA (2.0 + 4.0 and 4.0 + 8.0 ppm) for 7, 15, and 30 days and the effects of EDTA on zinc accumulation in tissues. Tissue zinc levels were determined by inductively coupled plasma-mass spectrometry (ICP-MS). No mortality occurred in fish under the exposure times and concentrations with solutions containing zinc only and zinc combined with EDTA. At the beginning of the metal exposure, various behavioral changes were observed in the fish, and those behaviors returned to normal with longer exposure time. The exposure to both zinc alone and zinc combined with EDTA significantly increased metal accumulation in the tissues and organs ($P < 0.05$) as compared to the control, and a gill > liver > muscle accumulation relationship was found among the tissues. The results obtained showed that EDTA reduced zinc accumulation in the gill, liver, and muscle tissues when compared to the effect of zinc only.

Keywords:

Cyprinus carpio, zinc, EDTA, tissue, bioaccumulation

Article history:

Received 04 August 2020, Accepted 19 January 2021, Available online 25 January 2021

Introduction

Pollutants change the physicochemical properties, as well as the biological components, of the aquatic environment, therefore adversely affecting the amount of fish stock as well as species diversity (Ebrahimi & Taherianfard, 2011). Heavy metals are an essential group of chemicals that contribute to pollution in aquatic environments. Heavy metals such as Pb, Cd, Hg, and As have no biological function in living things, and metals such as Cu, Zn, Cr, Fe, Ni, Co, Mn, and Se are trace elements needed in low concentrations for metabolic events. When those trace elements exceed the optimum threshold level, they can be dangerous and toxic for all living things (Shukla et al., 2007).

* Corresponding Author: Fahri KARAYAKAR, E-mail: fkarayakar@mersin.edu.tr

Although aquatic ecosystems have various physicochemical mechanisms to reduce or eliminate the effects of toxic substances, these toxic substances can cause changes in the characteristics of aquatic ecosystems including growth, development, behavior, and reproduction or even death (Benedict & Ayotunde, 2008).

Zinc is widely used in various industrial fields, especially in the construction and automotive industries (Eisler, 2000). It is the most abundant metallic element in the body of most vertebrates after iron, as well as being an essential element for the metabolism of essential organic compounds for the functions of the immune and nervous systems (Hogstrand, 2012). More than 200 enzymes, including carbonic anhydrase, alkaline phosphatase, alcohol dehydrogenase, acid phosphatase, lactate dehydrogenase, carboxypeptidase, and superoxide dismutase, require zinc as a cofactor (Eisler, 2000).

Chelators are widely used in chemical analysis and in medical applications. They are also components of many commercial products such as water softeners, decontamination agents on radioactive surfaces, detergents, cosmetics, and food preservatives. Ethylenediaminetetraacetic acid (EDTA) is one of those chelators and is widely used as a potent complexing agent for metals such as Cu, Pb, Zn, and Fe (III) in industrial processes such as paper production, electrode coating, and photography (Nowack, 1996). EDTA, as it complexes with various heavy metals and strongly binds them, is widely used in the health care sector as an antidote in acute and chronic metal poisoning (Palaniappan et al., 2010). Accumulation of toxic metals in the bodies of aquatic organisms results when the rate of uptake into tissues and organs exceeds the rate of removal (De Boeck et al., 2004). Accumulation is closely related to the metabolic activity of tissues and organs, and heavy metals have been found to accumulate at high concentrations in fish in metabolically active tissues and organs such as the liver, gill, kidney, and spleen (Heath, 1995; VanDyk et al., 2007). Although the muscle tissue in fish is not an active tissue in terms of metal accumulation, it is important in terms of transport of metals through the food chain and of human health, as it constitutes the main consumable part of the fish (Palaniappan & Muthulingam, 2016).

Although there are many studies on heavy metal accumulation and its toxic effects on fish, studies on the effects of complexing agents on heavy metal accumulation and toxicity are very limited. The effects of EDTA on zinc accumulation in the gill, liver, and muscle tissues of *C. carpio* (common carp), a freshwater fish, were investigated in this study.

Materials and Method

C. carpio specimens with a weight of 30.56 ± 1.82 g and a length of 12.47 ± 0.76 cm were used as the material in the study. The experiments were carried out in the Basic Sciences Research Laboratory of Mersin University Faculty of Fisheries, which has a 24 ± 1 °C constant temperature and 12 hours of dark/12 hours of light photoperiod.

In the research, zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), which can be dissolved easily in water, and EDTA ($\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_8$) were used as the complexing agents. Both a literature review and preliminary studies in the laboratory were used to determine the sublethal concentrations of 2.0 and 4.0 ppm for the mentioned species to be used in the experiments (Abdel-Tawwab et al., 2013). Five glass aquariums, each $40 \times 100 \times 40$ cm in size, were used to contain the determined concentrations. To the first and second the aquariums, 120 L of solutions of zinc at determined concentrations (1.0 and 2.0 ppm) were added. The third and fourth aquariums contained mixtures

with the solution of the same concentrations of zinc in the same volume, along with the solution of the complexing compound at concentrations corresponding to double the zinc concentration. The fifth aquarium contained 120 L of zinc-free tap water and constituted the control group. The experiments were carried out in three repetitions, two fish in each repetition, taking into account the 7-, 15-, and 30- day periods determined for the experiment; 18 fish were placed in each aquarium, and 90 fish in total were used in the study.

During the experiments, the fish were fed once per day at the same hour with pellet fish feed (Pinar, Pellet No: 2) at 2% of their total biomass, and the aquariums were provided with a central ventilation system. Some physicochemical properties of water in experimental aquariums are shown below.

Temperature: 23 ± 1 °C ; Dissolved Oxygen: 6.16 ± 0.51 ppm O₂ ; pH: 8.09 ± 0.05

Total Hardness: 262.7 ± 6.15 ppm CaCO₃ ; Total Alkalinity: 354.7 ± 1.18 ppm CaCO₃

According to the semi - static test principle, the test solutions were changed every two days by making appropriate dilutions from the stock solution, and the medium was renewed, because the concentrations of test solutions in aquariums can change over time due to adsorption, precipitation, and evaporation. Fish removed from aquariums at the end of the determined 7-, 15-, and 30- day periods were anesthetized with phenoxy - ethanol (1 mL/L) (Morgan et al., 1997), washed with tap water to remove metal residues from body surfaces, and then prepared for dissection by drying with drying paper.

The liver, gill, and muscle tissue samples used in the metal analysis were dissected separately from each of the fish and dried for 72 hours in an oven set at 105 °C until constant weight was reached. After dry weights were determined, the tissue samples were transferred to test tubes, and 2 mL nitric acid (HNO₃, 65%, s.g. 1.40, Merck) and 1 mL perchloric acid (HClO₄, 60%, s.g. 1.53, Merck) were added. They were then heated for eight hours on a hotplate set at 120 °C. After the heating process, the samples were transferred to polyethylene tubes, their total volumes were completed to 10 ml with deionized water, and they were made ready for analysis (Muramoto, 1980). The zinc content of the tissue samples was determined by inductively coupled plasma-mass spectrometry (ICP-MS). Control samples were prepared from IAEA - 407 (International Atomic Energy Agency) fish tissue homogenate. Detailed results on the amount of zinc element obtained from the reference material and LOD-LOQ values are given in Table 1. Variance analysis and Student Newman Keul's (SNK) test were used in the statistical analysis of the experimental data.

Table 1. Validation parameters of the analytical method.

| Trace elements | LOD (ng g ⁻¹) | LOQ (ng g ⁻¹) | R ² | Certificated Values Concentration (mg kg ⁻¹) | Certificated Values 95% Confidence Interval (mg kg ⁻¹) |
|----------------|---------------------------|---------------------------|----------------|--|--|
| Zinc | 2.63 | 8.35 | 0.9999 | 67.1 | 66.3-67.9 |

IAEA - 407 was used as reference material. LOD = Limit of Detection, LOQ = Limit of Quantification

Results

No mortality was observed in fish from the exposure to the experimental concentrations of zinc for 7-, 15-, and 30- days. Various behavioral changes at the beginning of the zinc exposure, such as indifference to food, moving to the aquarium surface, immobility on the aquarium floor, sudden displacement, and increased operculum movement, were observed in the fish, but it was observed that those behaviors returned to normal with prolonged exposure time.

Zinc accumulation levels in the liver, gill, and muscle tissues of *C. carpio* under the effect of exposure to zinc only and to zinc and EDTA concentrations for the experiment's 7-, 15-, and 30- day periods are shown in Tables 2 - 4. Exposure to the zinc and zinc + EDTA mixtures for seven days increased the zinc accumulation in the liver, gill, and muscle tissues significantly in comparison to the control ($P < 0.05$), and the trend of the increase was parallel to the increase in the concentration in the medium. The maximum 7- day accumulation with zinc only and with zinc together with the chelator was in the gill tissue, and the lowest accumulation was in the muscle tissue. The effect of zinc together with EDTA decreased the zinc accumulation in all tissues examined when compared to the effect of zinc only (Table 2).

Table 2. Zinc accumulation in the tissues of *C. carpio* ($\mu\text{g Zn/g d.w.}$) under the effect of zinc only and of zinc with EDTA for 7 days.

| Concentration | Tissues | | | | | |
|-----------------------|--------------------|----|---------------------|----|-------------------|----|
| | Liver | | Gill | | Muscle | |
| | $X \pm Sx$ | * | $X \pm Sx$ | * | $X \pm Sx$ | * |
| Control | 85.28 ± 2.73 | as | 121.80 ± 2.40 | bs | 45.24 ± 2.85 | cs |
| 2 ppm Zn | 368.67 ± 5.93 | at | 1588.82 ± 8.23 | bt | 98.69 ± 2.62 | ct |
| 4 ppm Zn | 538.56 ± 9.59 | ax | 2116.62 ± 38.15 | bx | 132.12 ± 3.81 | cx |
| 2 ppm Zn + 4 ppm EDTA | 271.57 ± 12.51 | ay | 1461.20 ± 22.19 | by | 91.74 ± 4.79 | ct |
| 4 ppm Zn + 8 ppm EDTA | 415.53 ± 17.96 | az | 1662.20 ± 35.82 | bt | 111.38 ± 4.96 | cy |

$X \pm Sx$ = Arithmetic mean \pm Standard error ; * SNK; a, b, c were used to determine the differences between tissues while s, t, x, y, z were used to determine the differences between concentrations. Different letters indicate statistical differences at $P < 0.05$.

The studied concentrations of zinc - only and zinc + EDTA mixtures for 15 days also increased the metal accumulation in the liver, gill, and muscle tissues. This increase was 3, 6, and 7 times more in muscle, liver, and gill tissues, respectively, under exposure to the highest concentration administered. Exposure zinc with EDTA reduced zinc accumulation in the tissues when compared to exposure to zinc only (Table 3).

Table 3. Zinc accumulation in the tissues of *C. carpio* ($\mu\text{g Zn/g d.w.}$) under the effect of zinc only and of zinc together with EDTA for 15 days.

| Concentration | Tissues | | | | | |
|-----------------------|-------------------|----|--------------------|----|-------------------|----|
| | Liver | | Gill | | Muscle | |
| | $X \pm Sx$ | * | $X \pm Sx$ | * | $X \pm Sx$ | * |
| Control | 81.82 \pm 2.96 | as | 117.16 \pm 2.57 | bs | 49.95 \pm 2.64 | cs |
| 2 ppm Zn | 451.80 \pm 4.87 | at | 784.92 \pm 12.69 | bt | 119.71 \pm 5.23 | ct |
| 4 ppm Zn | 482.36 \pm 7.66 | ax | 810.72 \pm 9.26 | bt | 141.59 \pm 3.97 | cx |
| 2 ppm Zn + 4 ppm EDTA | 586.89 \pm 6.44 | ay | 739.93 \pm 5.82 | bx | 95.85 \pm 3.73 | cy |
| 4 ppm Zn + 8 ppm EDTA | 457.72 \pm 5.87 | at | 632.33 \pm 6.94 | by | 114.73 \pm 3.94 | ct |

$X \pm Sx$ = Arithmetic mean \pm Standard error ; * SNK; a, b, c were used to determine the differences between tissues while s, t, x, y were used to determine the differences between concentrations. Different letters indicate statistical differences at $P < 0.05$.

At the end of the 30- day exposure period, the determined concentrations of zinc-only and zinc + EDTA mixtures increased the zinc accumulation in the tissues and organs examined relative to the control and in parallel with the increase in the environmental concentration. While the highest accumulation from exposure to both zinc - only and zinc + EDTA mixtures was in the gill tissue, the effect of the mixture reduced metal accumulation in all three tissues by approximately 30% when compared to the effect of zinc only (Table 4).

Table 4. Zinc accumulation in the tissues of *C. carpio* ($\mu\text{g Zn/g d.w.}$) under the effect of zinc only and of zinc together with EDTA for 30 days

| Concentration | Tissues | | | | | |
|-----------------------|--------------------|----|---------------------|----|-------------------|----|
| | Liver | | Gill | | Muscle | |
| | $X \pm Sx$ | * | $X \pm Sx$ | * | $X \pm Sx$ | * |
| Control | 84.18 \pm 3.87 | as | 123.80 \pm 2.93 | bs | 52.10 \pm 1.81 | cs |
| 2 ppm Zn | 842.81 \pm 7.51 | at | 1773.02 \pm 14.28 | bt | 152.60 \pm 4.84 | ct |
| 4 ppm Zn | 1289.60 \pm 5.15 | ax | 1964.52 \pm 11.13 | bx | 170.31 \pm 2.88 | cx |
| 2 ppm Zn + 4 ppm EDTA | 671.78 \pm 8.97 | ay | 1125.01 \pm 10.82 | by | 106.98 \pm 6.96 | cy |
| 4 ppm Zn + 8 ppm EDTA | 897.36 \pm 7.98 | az | 1368.60 \pm 5.67 | bz | 113.27 \pm 4.52 | cy |

$X \pm Sx$ = Arithmetic mean \pm Standard error ; * SNK; a, b, c were used to determine the differences between tissues while s, t, x, y, z were used to determine the differences between concentrations. Different letters indicate statistical differences at $P < 0.05$.

The following relationship was determined among the tissues in terms of accumulation under the effect of all the determined durations and concentrations of zinc-only and zinc + EDTA mixtures.

$$\text{Gill} > \text{Liver} > \text{Muscle}$$

Discussion

The effects of heavy metals on mortality in aquatic organisms vary and depend on the species, metal, concentration of the metal, and duration of exposure. In a study conducted with *Labeo rohita*, it was found that 96 hour LC50 values of Fe, Zn, Pb, Ni, and Mn were different and that the difference also varied with age (Abdullah et al., 2007). No mortality was observed under the effect

of 0.5 ppm concentrations of cadmium and zinc for 30 days in *Oreochromis niloticus* and *C. carpio* (Yesilbudak, 2009). In the present study conducted with *C. carpio*, no mortality was observed in fish under exposure to 2.0 and 4.0 ppm concentrations of zinc only and to zinc together with EDTA for 7-, 15-, and 30- days. That was attributed to the fact that the concentrations were probably not lethal for the mentioned species for the determined periods.

Aquatic organisms react to stress factors such as hunger, predator pressure, pollution, and changes in the physicochemical properties of water by changing their behaviors, such as respiration, swimming, growth, feeding, and reproduction (Lu et al., 2017). In the present study, at the beginning of the exposure to the zinc - only and zinc + EDTA mixtures, various behavioral changes, such as indifference to food, moving to the aquarium surface, immobility on the aquarium floor, sudden displacement, and increased operculum movement, were observed, and those behaviors returned to normal with prolonged exposure time. The changes observed in the behavior of fish under the effect of metal can be explained as adaptation to changing environmental conditions.

Heavy metal accumulation varies in aquatic organisms, depending on the tissue type. In studies conducted under laboratory conditions with *Tilapia sparrmanii* (Preez et al., 1993), *Channa punctatus* (Shukla et al., 2007), and *L. rohita* (Palaniappan et al., 2010), zinc was found to accumulate in the liver more than in other tissues and organs under exposure to sublethal concentrations. In studies conducted with *Lethrinus lentjan* (Al-Yousuf et al., 2000) and *Gobio gobio* (Bervoets & Blust, 2003) under natural conditions, it was found that the level of zinc in the liver was higher than in other tissues and organs. In the present study conducted with *C. carpio*, accumulation in the liver was second highest after that of gill tissue after exposure to both zinc - only and zinc + EDTA mixtures. High levels of accumulation in the liver may result from the liver being the major detoxification organ and the primary synthesis area of metal - binding proteins such as metallothionein and glutathione, as well as from the retention of zinc in the liver by being bound to those proteins (Shukla et al., 2007).

In fish, gills, in addition to respiration, are important organs that function in osmoregulation, acid - base balance, and removal of nitrogenous metabolic wastes from the body (Palaniappan et al., 2010). Gills are the main target organs of pollutants and toxic substances, because they have a very large surface area due to their filament structure and because they interact directly with the environment (Heath, 1995; van Dyk et al., 2007). In a study conducted with *C. punctatus*, it was determined that the highest zinc accumulation concentration was 18.62 mg/L, determined in the gill tissue, whereas the lowest accumulation was in the muscle tissue, and there was a gill > liver > kidney > blood > muscle accumulation relationship among the tissues (Shukla et al., 2007). In the present study, a similar relationship was found among tissues, as the highest accumulation was in gill tissue under the effect of the zinc - only and zinc + EDTA mixtures, whereas the lowest accumulation was in muscle tissue. The high accumulation in the gill tissue can be attributed to the large surface area of the gill tissue due to its lamellar structure and to its direct interaction with the medium (Chavan & Muley, 2014).

As muscular tissue constitutes the main consumable part of aquatic organisms, it is important for the transmission of metal through the food chain and for the health of all trophic levels. It was determined that the concentrations of Cu and Zn in *O. niloticus* at 5.0 ppm and Cd at 1.0 ppm for 24, 48, and 96 hours accumulated at the lowest levels in the muscle tissue for all three

metals (Tunçsoy & Erdem, 2014). In *C. carpio*, the metal accumulation in muscle tissue was low at the beginning of the exposure to 53- and 443- ppb concentrations of Cd; however, after 127 days, the accumulation in muscle tissue increased significantly when compared to that of the control (DeConto Cinier et al., 1999). In the present study on *C. carpio*, it was determined that the lowest zinc accumulation was in the muscle tissue under the effect of the exposure times and concentrations for both zinc - only and zinc + EDTA mixtures and that this was associated with the metabolic activity of the muscle tissue or with the short duration of the exposure.

The accumulations and toxic effects of heavy metals in aquatic organisms vary, depending on the organic and inorganic substances in the environment. In a study examining the effects of complexing agents on metal accumulation in *C. carpio*, it was determined that EDTA nitrilotriacetic acid (NTA), and diethylene triamine penta acetic acid (DTPA) reduced metal accumulation in tissues and organs as compared to the sole effects of Zn, Pb, Cd, and Cu and that the decrease was associated with intake inhibition by those complexing agents (Muramoto, 1980). It was found that D-Penicillamine (DPA) and EDTA in *L. rohita* (Palaniappan et al., 2010) and EDTA in *Carassius gibelio* (Nicula et al., 2011) reduced the accumulation of zinc in tissues and organs and that DPA was more effective in reducing accumulation in *L. rohita* than was EDTA. In this study carried out with *C. carpio*, EDTA concentrations corresponding to double the concentrations of zinc exposure were found to decrease the zinc accumulation level in the gill, liver, and muscle tissues during the 7-, 15-, and 30- day exposure periods. The decrease in the level of metal accumulation under exposure to the zinc + EDTA mixtures can be associated with the chelator binding the metal, increasing its molecular size, and preventing it from being taken up by the fish (Nicula et al., 2011).

As a result, EDTA reduced zinc accumulation in the tissues of *C. carpio*. Therefore, it is recommended that EDTA can be used to reduce zinc toxicity in freshwater sources.

Funding Source

Mersin University Scientific Research Projects Unit for providing financial support for the project, BAP - 2017 - 1- TP2 - 2178.

Author Contributions

All author contributions are equal for the preparation research in the manuscript.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

Conflict of Interest

The authors declare that they have no competing interests. All applicable international, national, and institutional guidelines for the care and use of animals were followed. Local ethics committee approval was received.

References

- Abdel-Tawwab, M., Mousaad, M.N.M., Sharafeldin, K.M. & Ismaiel, N.E.M. (2013). Changes in Growth and Biochemical Status of Common Carp, *Cyprinus carpio* L. Exposed to Water-Born Zinc Toxicity for Different Periods. *International Aquatic Research*, 5(11), 1-9. doi:10.1186/2008-6970-5-11
- Abdullah, S., Javed, M. & Javid, A. (2007). Studies on Acute Toxicity of Metals to the Fish (*Labeo rohita*). *International Journal of Agriculture & Biology*, 9(2), 333-337.
- Al-Yousuf, M.H., El-Shahawi, M.S. & Al-Ghais, S.M. (2000). Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex. *The Science of the Total Environment*, 256, 87-94. doi: 10.1016/s0048-9697(99)00363-0
- Benedict, O.O. & Ayotunde, E.O. (2008). Toxicity of Lead to Freshwater Invertebrates, (Water Fleas; *Daphnia magna* and *Cyclop sp.*) in Fish Ponds in a Tropical Floodplain. *Water Air Soil & Pollution*, 192, 39-46. doi:10.1007/s11270-008-9632-0
- Bervoets, L. & Blust, R. (2003). Metal Concentrations in Water, Sediment and Gudgeon (*Gobio gobio*) from a Pollution Gradient: Relationship with Fish Condition Factor. *Environmental Pollution*, 126, 9-19. doi:10.1016/s0269-7491(03)00173-8
- Chavan, V.R. & Muley, D.V. (2014). Effect of Heavy Metals on Liver and Gill of Fish *Cirrhinus mrigala*. *International Journal of Current Microbiology and Applied Sciences*, 3(5), 277-288.
- De Boeck, G., Meeus, W., Coen, W.D. & Blust, R. (2004). Tissue-Specific Cu Bioaccumulation Patterns and Differences in Sensitivity to Waterborne Cu in Three Freshwater Fish: Rainbow Trout (*Oncorhynchus mykiss*), Common Carp (*Cyprinus carpio*), and Gibel Carp (*Carassius auratus gibelio*). *Aquatic Toxicology*, 70, 179-188. doi:10.1016/j.aquatox.2004.07.001
- DeConto Cinier, C., Petit-Ramel, M., Faure, R., Garin, D. & Bouvet, Y. (1999). Kinetics of Cadmium Accumulation and Elimination in Carp *Cyprinus carpio* Tissues. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 122, 345-352. doi:10.1016/S0742-8413(98)10132-9
- Ebrahimi, M. & Taherianfard, M. (2011). The Effects of Heavy Metals Exposure on Reproductive Systems of Cyprinid Fish from Kor River. *Iranian Journal of Fisheries Sciences*, 10(1), 13-24.
- Eisler, R. (2000). *Handbook of Chemical Risk Assessment Health Hazards to Humans, Plants and Animals*. 1 st ed. Boca Raton, London, New York, D.C: Lewis Publishers.
- Heath, A.G. (1995). *Water Pollution and Fish Physiology*. 2nd ed. New York, NY, USA: CRC Press.
- Hogstrand, C. (2012). *Zinc, Homeostasis and Toxicology of Essential Metals*. In: Wood, C.M., Farrell, A.P. & Brauner, C.J. (editor). *Homeostasis and Toxicology of Essential Metals*. 1 st ed. San Diego, CA, USA: Elsevier, pp. 135-200.

- Lu, N., Sun, S., Song, W. & Jia, R. (2017). Behavioural Toxicity in Zebrafish (*Danio rerio*) Exposed to Waterborne Zinc and Chromium (VI). *Chemistry and Ecology*, 33(8), 725-738. doi:10.1080/02757540.2017.1373767
- Morgan, J.D., Sakamoto, T., Grau, E.G. & Iwama, G.K. (1997). Physiological and Respiratory Responses of the Mozambique Tilapia (*Oreochromis mossambicus*) to Salinity Acclimation. *Comparative Biochemistry and Physiology - A Physiology*, 117(3), 391-398. doi:10.1016/S0300-9629(96)00261-7
- Muramoto, S. (1980). Effects of Complexans (EDTA, NTA and DTPA) on the Exposure to High Concentrations of Cadmium, Copper, Zinc and Lead. *Bulletin of Environmental Contamination and Toxicology*, 25, 941-946. doi: 10.1007/BF01985635
- Nicula, M., Gergen, I., Harmanescu, M., Banatean-Dunea, I., Marcu, A., et al. (2011). Assessing the Impact of EDTA Chelating Effect on Some Macro and Microminerals in Prussian Carp (*Carassius gibelio*) Tissues. *Scientific Papers Animal Science and Biotechnologies*, 44 (2), 40-44.
- Nowack, B. (1996). Behavior of EDTA in Groundwater a Study of the Surface Reactions of Metal - EDTA Complexes. PhD, Swiss Federal Institute of Technology, Zürich, Switzerland.
- Palaniappan, P.L.R.M., Nishanth, T. & Renju, V.B. (2010). Bioconcentration of Zinc and its Effect on the Biochemical Constituents of the Gill Tissues of *Labeo rohita*: An FT-IR Study. *Infrared Physics & Technology*, 53, 103-111. doi:10.1016/j.infrared.2009.10.003
- Palaniappan, R. & Muthulingam, M. (2016). Impact of Heavy Metal, Chromium on Protein Metabolism in Brain and Muscle of Freshwater Fish, *Channa striatus* (Bloch). *International Journal of Current Microbiology and Applied Sciences*, 5(7), 638-647. doi:10.20546/ijcmas.2016.507.072
- Preez, H.H., Rensburg, E. & Vuren, J.H.J. (1993). Preliminary Laboratory Investigation of the Bioconcentration of Zinc and Iron in Selected Tissues of the Banded Tilapia, *Tilapia sparmanii* (Cichlidae). *Bulletin of Environmental Contamination and Toxicology*, 50, 674-681. doi: 10.1007/BF00194661
- Shukla, V., Dhankhar, M., Prakash, J. & Sastry, K.V. (2007). Bioaccumulation of Zn, Cu and Cd in *Channa punctatus*. *Journal of Environmental Biology*, 28(2), 395-397.
- Tunçsoy, M. & Erdem, C. (2014). Accumulation of Copper, Zinc and Cadmium in Liver, Gill and Muscle Tissues of *Oreochromis niloticus* Exposed to These Metals. *Fresenius Environmental Bulletin*, 23(5), 1143-1149.
- Van Dyk, J.C., Pieterse, G.M. & Van Vuren, J.H.J. (2007). Histological Changes in the Liver of *Oreochromis mossambicus* (Cichlidae) After Exposure to Cadmium and Zinc. *Ecotoxicology and Environmental Safety*, 66, 432-440. doi:10.1016/j.ecoenv.2005.10.012
- Yesilbudak, B. (2009). Zinc and Cadmium Accumulation in Gill, Liver, Kidney and Muscle Tissues of *Cyprinus carpio* and *Oreochromis niloticus*. MSc, Cukurova University, Adana, Turkey.