

Assessing the Impact of EDTA Chelating Effect on some Macro- and Microminerals in Prussian Carp (*Carassius Gibelio*) Tissues

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Abstract

Chelators are used in chemical analysis, in medical applications, as water softeners, as decontamination agents on radioactive surfaces and they are ingredients in many commercial products such as shampoos and food preservatives. Such a synthetic chelator is EDTA (ethylenediaminetetraacetic acid). It is considered one of the tools that promises to control the heavy metal pollution in aquaculture. EDTA attaches itself to heavy metals and carries the metals from the fish body. EDTA can also slow free-radical activity produced by heavy metals in the body. Because its ability to sequester metal ions, we tried to estimate the potential risks of a chronic exposure to EDTA on tissue mobilization of some metals which have an essential role in realization of different cell functions in Prussian carp specimens. Ca, Mg, Fe, Zn, Mn and Cu, were the mineral elements we have targeted in this study. It was found that these minerals have a trend of their tissues distribution and concentration in the body of the control specimens (higher or lower related to other similar works) and EDTA presence in water led to a significant decreasing of their level in all tissues analyzed in a dose-dependent manner.

Keywords: EDTA, fish, macroelements, microelements.

1. Introduction

A variety of chemicals and drugs are used in aquaculture for purposes such as sediment and water management, enhancement of natural aquatic productivity, transport of live organisms, feed formulation, manipulation and enhancement of reproduction, growth promotion, health

management, processing and adding value to the final product [1]. They include those associated with structural materials, soil and water treatments, antibacterial agents, other terapeutants, pesticides, feed additives, anaesthetics, and hormones [2]. From multitude of chemicals and drugs, disodium ethylenediaminetetraacetic acid (EDTA) is routinely used in aquaculture as a broad-spectrum “cure-all” in preventing metal toxicity to aquatic organisms. On the other hand, it is routinely used to keep certain essential metals in solution when needed for phytoplankton culture

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[3] or removing hardness and softening the hatching pond water [4].

Despite the large number of areas where EDTA has found application (household sector, industry, agriculture, including human and veterinary medicine), EDTA itself or its metabolites may cause adverse effects targeting aquatic compartment [5, 6, 7].

So because of its ability to sequester metal ions, we tried to estimate the potential risks of a chronic exposure to EDTA on tissue mobilization of some metals which have an essential role in realization of different cell functions in Prussian carp specimens. Fe, Zn, Mn, Cu, Ca and Mg, were the mineral elements we have targeted in this study.

2. Materials and methods

One year old healthy Prussian carp were used as biological material. They were collected from Chişoda private Fishfarm – (Timiş county) and transported to the physiology laboratory of the Faculty of Animal Sciences and Biotechnologies Timişoara, România.

Individuals with a body weight of 35-40 g were selected by gravimetric measurements and then they were acclimated two weeks to laboratory conditions, removing the suspected unhealthy subjects. Fishes were housed during a 30 days experimental period in a 60 l capacity glass aquariums (20 fishes/aquarium) provided with aeration system and they were fed twice a day with commercial dry pellets containing 35% protein.

The physico-chemical parameters of the laboratory water were daily measured with a Hanna HI 9145 oxygen-meter with water resisting microprocessor (water temperature and dissolved oxygen) and a Germany TERMATEST kit (pH, NO₂⁻, NO₃⁻, hardness of water).

Three doses of the tested product (EDTA) were administered as follows (table 1):

Table1. Experimental groups and their notation

S.No.	Groups	Notation
1	Control (EDTA free water)	C
2	0.05 g EDTA/l	EDTA1
3	0.1 g EDTA/l	EDTA2
4	0.15 g EDTA/l	EDTA3

The water was replaced twice a week with an equal volume of stored dechlorinated water containing an appropriate concentration of EDTA.

A CONTRAA 300 analytik Jena atomic absorption spectrometer was used to determine, Fe, Cu, Zn, Mn, Ca and Mg concentration in fish tissue samples (muscle, liver, kidney, gills, skin, heart, ovaries, testis, brain, intestine) and the results were given as mg kg⁻¹ wet weight (w.w.).

Data were analyzed statistically using an ANOVA two factors without replication test, having in view two factors: the tissue and adopted treatment schema. The analysis of variance shows significant differences not only between applied treatment schemes but between fish tissues as well.

3. Results and discussion

Macrominerals (calcium, phosphorus, magnesium, sodium, potassium, chlorine) and trace elements (iron, copper, zinc, manganese, selenium, iodine, fluorine, chromium) are mineral elements recognized as essential for body functions in fish. Their main functions include the formation of skeletal structure, electron transfer, regulation of acid-base balance, and osmoregulation. Minerals are also important components of hormones and enzymes, and they activate enzymes. Complex biochemical mechanisms control and regulate the uptake, storage, and excretion of various inorganic elements, allowing fish to live in a dynamic equilibrium with their environment [8, 9].

Iron has an active part in oxidation/reduction reactions and electron transport associated with cellular respiration. It is found in complexes bound to proteins such as haem compounds (hemoglobin and myoglobin), in enzymes such as microsomal cytochromes, catalase, peroxidase etc., and in nonhaem compounds such as transferrin, ferritin and iron-containing flavoproteins - ferredoxins, dehydrogenases. Haemoglobin occurs in erythrocytes while transferrin is found in plasma; the latter one is the principal carrier of iron in blood. Although the gill membrane absorbs iron to a certain extent, the intestinal mucosa is considered to be the major site [9, 10].

Iron concentrations found by us in gills (75.89 mg kg⁻¹ w.w.) and intestine (85.04 mg kg⁻¹ w.w.) of the control group support this statement (figure 1).

Among the organs of the control group, liver (101.18 mg kg⁻¹ w.w.), skin (104.47 mg kg⁻¹ w.w.), kidney (377.66 mg kg⁻¹ w.w.) were found to contain large amounts of iron indicating the importance of these organs as storage or excretion places.

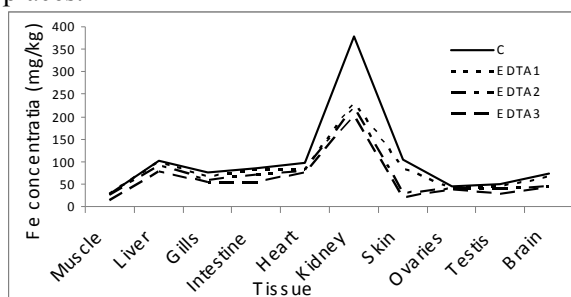


Figure 1. Graphic representation of Fe tissue concentrations in control and experimental groups

Some authors in stead [11] reported considerably lower level of this mineral in ciprinids or other freshwater fish soft tissues. Iron levels decreased gradually in all tissues considered when EDTA was added to the water, significant differences being recorded for dose of 0.15 g EDTA/l.

Zinc is an essential trace element in living organisms, being involved in nucleic acid synthesis [9, 12] and it serves as a specific cofactor of several metalloenzymes, including dehydrogenases, aldolases, peptidases, and phosphatases. In addition, Zinc is connected with prostaglandin metabolism [9]. The gills and gastrointestinal tract are involved in the uptake of this element and gills in play a major role in Zn excretion [13].

The highest concentration of Zn recorded the kidney (138.99 mg kg⁻¹ w.w.), heart (83.64 mg kg⁻¹ w.w.), intestine (73.80 mg kg⁻¹ w.w.), testis (46.96 mg kg⁻¹ w.w.), skin (44.40 mg kg⁻¹ w.w.) and gills (43.05 mg kg⁻¹ w.w.) and the lowest ones, the muscle (13.33 mg kg⁻¹ w.w.), liver (16.77 mg kg⁻¹ w.w.), brain (28.60 mg kg⁻¹ w.w.) and ovaries (35.65 mg kg⁻¹ w.w.) (figure 2).

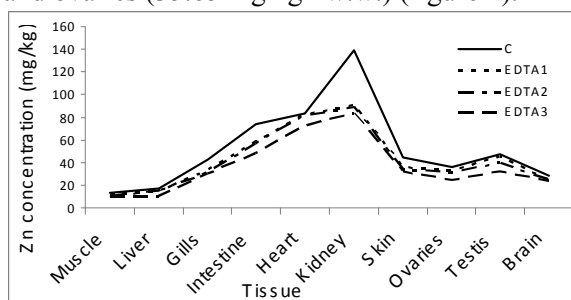


Figure 2. Graphic representation of Zn tissue concentrations in control and experimental groups

Tissue zinc concentration showed a significant decreasing as a result of EDTA exposure, kidney, gills and skin being actively involved in Zn excretion. In these organs Zn has reached values of 83.87 mg kg⁻¹ w.w., 30.43 mg kg⁻¹ w.w. and 32.45 mg kg⁻¹ w.w. respectively in the third experimental variant.

Copper is a constituent of many enzymes and is essential for their activities. It is associated with cytochrome *c* oxidase of the electron transport chain in the cell. Other cuproenzymes found in fish tissues include superoxide dismutase, tyrosinase, lysyl oxidase, ceruloplasmin, and dopamine β-hydroxylase [14]. One of the few investigations on copper metabolism in fish by Syed and Coombs [15] revealed similarities to mammals in the distribution of copper and copper-dependent enzymes. Copper levels are high in the eyes where it is found along with melanins, bound to protein. Organs such as liver, brain and heart also contain comparatively large amounts of copper. A copper-protein complex, ceruloplasmin, exhibiting oxidative activity, occurs in blood plasma.

In our experiment, high levels of Cu were found in liver (14.79 mg kg⁻¹ w.w.), kidney (10.77 mg kg⁻¹ w.w.) and heart (6.27 mg kg⁻¹ w.w.), very close levels in brain (3.42 mg kg⁻¹ w.w.), testis (3.13 mg kg⁻¹ w.w.) and ovaries (3.58 mg kg⁻¹ w.w.), while muscle (1.59 mg kg⁻¹ w.w.) and gills (1.30 mg kg⁻¹ w.w.) had the lower ones (figure 3).

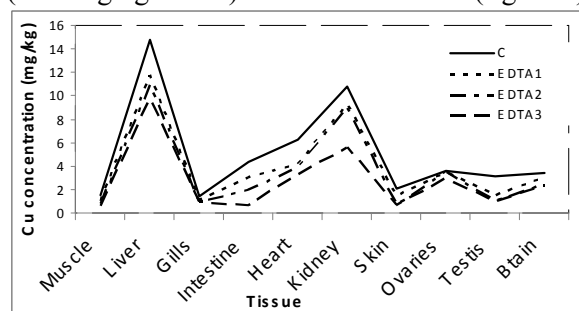


Figure 3. Graphic representation of Cu tissue concentrations in control and experimental groups

Also in this case the tissues of experimental groups lose copper, especially the kidney (5.54 mg kg⁻¹ w.w.) and especially at the dose of 0.15 g EDTA/l although, bile is the main site for secretion of copper in teleost fish [16] followed by gills.

Manganese is widely distributed in fish and animal tissue. The mitochondria have a greater concentration of manganese than cytoplasm or

other cell organelles. It is necessary for the normal functioning of brain and for proper lipid and carbohydrate metabolism. The mineral has two roles: first as a cofactor that activates metal-enzyme complexes; and second as an integral part of metalloenzymes. Manganese activates specific enzymes such as glycosyltransferase and non-specific enzymes such as kinases, transferases, hydrolases and decarboxylases [9].

Although the uptake of manganese from water at gills level is less efficiently absorbed than from feed al intestinal level, our data suggests otherwise: gills where manganese is in high concentration (13.27 mg kg⁻¹ w.w.) seem to be the major route of Mn entry in fish body not the intestinal epithelia where this mineral registered only 1.97 mg kg⁻¹ w.w. (figure 4).

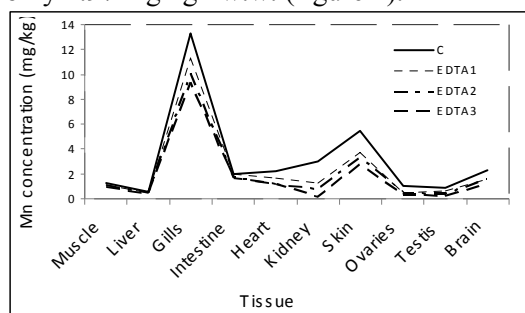


Figure 4. Graphic representation of Mn tissue concentrations in control and experimental groups

Quantitative distribution of manganese among other tissue samples shows a high concentration of manganese in skin (5.42. mg kg⁻¹ w.w.). But fish exposure to EDTA waterborne led to a general tissue depletion especially in kidney (from 2.99 mg kg⁻¹ w.w. in control group to 0.26 mg kg⁻¹ w.w. in EDTA3 group), gill (from 13.27 mg kg⁻¹ w.w. in control group to 9.31 mg kg⁻¹ w.w. in EDTA3 group), and skin (from 5.42 mg kg⁻¹ w.w. in control group to 2.76 mg kg⁻¹ w.w. in EDTA3 group).

Magnesium is an essential cofactor in many enzymatic reactions in intermediary metabolism. These enzymes include phosphokinases, thiokinases, phosphatases, pyrophosphatases, and amino acyl synthetases. Magnesium plays an important role in the respiratory adaptation of freshwater fish [17]. It is also required in skeletal tissue metabolism, osmoregulation, and neuromuscular transmission.

Magnesium level in the examined organs ranged from 160.37 mg kg⁻¹ w.w. in liver to 667.55 mg kg⁻¹ w.w. in kidney. Besides kidney, magnesium is

very well represented in gills (506.15 mg kg⁻¹ w.w.), testis (541.99 mg kg⁻¹ w.w.), skin (442.28 mg kg⁻¹ w.w.) and heart (439 mg kg⁻¹ w.w.) (figure 5).

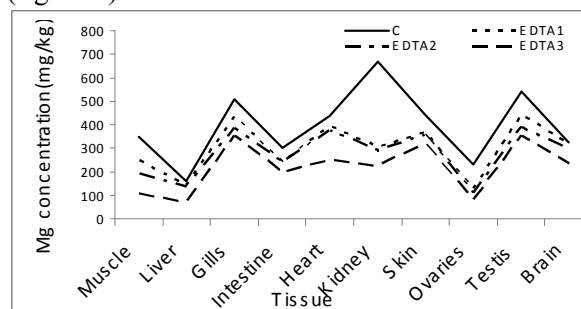


Figure 5. Graphic representation of Mg tissue concentrations in control and experimental groups

All tissues were affected by the EDTA addition to the water, kidney suffering finally the greatest loss of magnesium (440.89 mg kg⁻¹ w.w.).

Calcium

In addition to its structural functions, calcium plays an important role in muscle contraction, blood clot formation, nerve impulse transmission, the maintenance of cell integrity and acid-base equilibrium, and activation of several important enzymes [10]. The uptake of calcium occurs through gills, fins and oral epithelia, however gills are considered the most important site for calcium regulation. This is proven by its high content in the gills of the control group (934.43 mg kg⁻¹ w.w.) (figure 6).

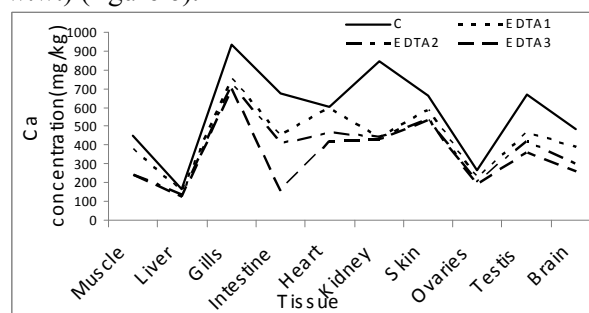


Figure 6. Graphic representation of Ca tissue concentrations in control and experimental groups

Like to the other bioelements, Ca shows a massive EDTA dose-dependent mobilization. This time the intestine (from 675.84 mg kg⁻¹ w.w. to 151.82 mg kg⁻¹ w.w.) followed by kidney (from 844 mg kg⁻¹ w.w. to 431 mg kg⁻¹ w.w.) registered a significant decrease of their calcium concentration at dose of 0.15 g EDTA/l.

4. Conclusions

1. To conclude, trace elements considered in this study showed some trend of distribution in tissues of the control group.
2. EDTA chelates divalent and trivalent metal cations and its addition to water led to their tissues depletion by complexation.
3. Histopathological studies are further required to determine whether EDTA used for different purposes in aquaculture disrupts mineral homeostasis and cellular specific activities and whether mineral replacement therapy both to correct deficiencies and keep up with losses due to chelation is imperative.

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