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Effect of EDTA on reducing tissue cadmium bioaccumulation and cadmium antagonism related to some mineral micro- and macronutrients in Prussian carp (*Carassius gibelio*)

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Abstract. EDTA is a chelating agent used deliberately in various fields (pulp and paper industry, detergents industry, food industry, medicine, biomedical labs) in order to sequester metal ions which have harmful effects in many processes as well as in obtaining of many products. Taking as starting point the EDTA property to form metal-EDTA complexes we decided to test its effectiveness in mobilizing of the contaminant metal ions, especially cadmium from tissue of Prussian carp specimens subjected to chronic poisoning with cadmium acetate. At the same time we investigated the EDTA ability to reduce the known cadmium antagonism exhibited vis-à-vis some essential macro-and microminerals.

Key Words: chronic cadmium intoxication, EDTA, fresh water fish, mineral micronutrients, mineral macronutrients.

Résumé. L'EDTA est un agent chélateur utilisés délibérément dans divers secteurs (industrie du papier et pâte à papier, industrie des détergents, industrie alimentaire, médecine, laboratoires laboratoires biomédicaux) afin de séquestration les ions métalliques qui ont des effets délétères dans de nombreux procédés industriels ainsi que dans l'obtention de plusieurs produits. Prenant comme point de départ la propriété de l'EDTA pour former des complexes métal-EDTA, nous avons décidé de tester son efficacité dans la mobilisation des ions métalliques contaminants, en particulier du cadmium dans les échantillons de tissus de carpe argentée soumis à l'intoxication chronique avec de l'acétate de cadmium. Dans le même temps, nous avons suivi la capacité de l'EDTA à réduire l'antagonisme de cadmium connu de certains macro-et microminéraux essentiels.

Mots-clés: intoxication chronique par le cadmium EDTA, poissons d'eau douce, oligo-éléments minéraux, macronutriments minéraux.

Rezumat. EDTA este un agent chelatant utilizat în mod deliberat în diverse domenii (industria hârtiei şi celulozei, industria detergenţilor, industria alimentară, medicină, laboratoare biomedicale) în scopul sechestrării ionilor metalici care au efecte dăunătoare în multe procese industriale ca şi în obţinerea multor produse. Având ca punct de plecare proprietatea EDTA de a forma complexe metal–EDTA ne-am propus sa testăm eficiența sa în mobilizarea ionilor metalici contaminanţi, în speţă a cadmiului din ţesuturile exemplarelor de caras argintiu supuse intoxicaţiei cronice cu acetat de cadmiu. In acelaşi timp, am urmărit capacitatea EDTA de a diminua antagonismul cunoscut al cadmiului faţă de unele macro- şi microminerale esenţiale.

Cuvinte cheie: intoxicaţie cronică cu cadmiu, EDTA, peşti de apă dulce, micronutrienţi minerali, macronutrienţi minerali.

Introduction. The problems of protecting and improving the environment on a planetary scale is one of the most acute and complex contemporary problems. Interrelations of the environment with the economy fields and all sides of social life leads to a mutual conditioning (Varga & Sabo 2009; Petrescu et al 2010). The impetuous economic and social development of human communities has induced an accelerated environmental change deeply disturbing the natural balance of the compensatory processes in the biosphere (Balan et al 2010).

Among different types of pollution the chemical one is more dangerous and obvious, affecting all the components of the biosphere. Chemical compounds penetration in the body can have acute or chronic biological effects that depend on many factors (concentration, route of entry, health status, genetic factors etc, Trif et al 2010ab; Dumitrescu et al 2010; Petrovici et al 2010abc). Hazard degree for environment of the chemicals compounds is represented by their toxicity, ability of pollution sources, retention in the environment, synergic effects, as well the possibilities of contamination and spread of contaminants (Chiroma et al 2007; Fleșeriu et al 2010).

Increased environmental pollution reflects on the aquatic ecosystems activity. Radioactive, chemical or biological impurities, threaten the balance of these ecosystems. The presence of chemical contaminants in water can have very serious environmental consequences through restructurings of the biocoenosis, altering their integrity and consequently, of aquatic ecosystems.

Heavy metals are considered harmful pollutants for the aquatic creatures by themselves or through their toxic salts, which exhibit high stability (Podar 2010; Rahman et al 2010). Contamination of the surface water is made through discharge of wastewater from factories that use such substances in their production processes. The biological activity of these waters can be seriously compromised due to the destruction of a large number of microorganisms and to the inhibition of the methane fermentation process from sludge by the pollutants of this group.

Cadmium and its compounds, compared with other heavy metals, is relatively soluble in water. As such, it is easier to be mobilized, it has a greater bioavailability and tends to accumulate (Nicula et al 2010). It is quickly taken, especially by micro-organisms and mollusks, whose bioconcentration factors are thousands.

Furthermore, cadmium interacts with other essential elements in tissues of several species, showing an antagonistic effect against them.

As such, it requires finding scientific detoxification methods to improve the health of economic interest species in any environmental conditions (accidental or caused heavy metals discharges) which can induce severe biochemical changes in normal metabolism of fish.

Usually, the chemical procedures can remove toxic elements from industrial waste water and polluted environment, but they are expensive. However, there are some chemicals that are cheap and moreover, they are free from undesirable side effects. Thus, metal remobilisation using chelating agents enjoys attention.

EDTA is a widely used acronym for the chemical compound ethylenediaminetetraacetic acid (which has many other names). It is produced on a large scale with multiple domestic and industrial applications. EDTA is used as a chelating agent, thanks to its ability to "sequester" metal ions such as Ca^{2+} and Fe^{3+}. Synthetic compound like ethylenediaminetetraacetic acid (EDTA) is known to be an effective chelating agent of heavy metals (James et al 1998) and there are authors who claim that EDTA appears to be promising tool to control cadmium pollution in aquaculture (Shalaby 2007).

The present study was carried out to investigate the effect of EDTA on reducing tissue cadmium bioaccumulation and cadmium antagonism related to some mineral micro- and macronutrients in Prussian carp *Carassius gibelio* (Bloch, 1782).

Material and Method. Choosing the test organisms we had in view the accessibility and representativity criteria ecologically speaking, reason why we have orientated to *Carassius gibelio* (Prussian carp) from Cyprinidae family, Pisces class. One year old healthy fish of Prussian carp were collected from Chişoda private Fishfarm – (Timiș county) and transported to the physiology laboratory of the Faculty of Animal Sciences and Biotechnologies Timișoara, România. We opted in favor of this species because it is easy to purchase, individuals are big sized and they easily acclimate to the captivity conditions. They are representative for continental waters, covering a large ecological valence, from criofile organisms to euritherm and termophilic organisms, being highly euribiont. Although the notoriety standards envisage aquarium fish utilization (guppy, fathed minow etc) or small sized cyprinids from the genus *Phoxinus* (Popek et al 2008; Petrovici & Pacioglu 2010), actual tendency certified by the papers of the last years is to work with culture fish (carp, perch, tilapia, Prussian carp), because they are easy to access.

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. Fish were housed in a 60 L capacity glass aquariums (20 fishes/aquarium) provided with aeration system.

The physico-chemical parameters of the laboratory water (during a 30 days experimental period) were measured with a Hanna Hi 9145 oxygen-meter with water resisting microprocessor (water temperature and dissolved oxygen) and a Germany TERMATEST kit (pH, NO^-_2, NO^-_3, hardness of water).

Fishes were fed twice a day with commercial dry pellets containing 35% protein.

The investigated metal (Cd), was administered in concentrations of 5 ppm, and its water circulation was supported by two AC 9904 air pumps. The sublethal treatment dose (25% of LC50) was calculated from percentage mortalities of fish as described by Veena & Chacko (1997).

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

Table 1

Experimental groups and their notation

S.No.	*Groups*	*Notation*
1	Control (metal free water)	C
2	Cadmium (5 ppm)	Cd
3	Cadmium (5 ppm) + 0.05 g EDTA/L	Cd/EDTA1
4	Cadmium (5 ppm) + 0.1 g EDTA/L	Cd/EDTA2
5	Cadmium (5 ppm) + 0.15 g EDTA/L	Cd/EDTA3

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

A CONTRAA 300 analytik Jena atomic absorption spectrometer was used to determine Cd, Fe, Cu, Zn, 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 variance analysis shows significant differences not only between applied treatment schemes but between fish tissues.

Results and Discussion. Analyzes performed at the end of the experimental period, show significant increases in Cd concentration in all sampled tissues from the intoxicated group with cadmium acetate (Table 2). Thus, the highest concentrations of cadmium were found in gills, kidney, intestine, liver and heart of Prussian carp specimens while the smallest ones were observed in gonads, muscles, skin and brain.

The highest accumulation of Cd in the gills (9.05 mg kg^{-1} w.w.) is due to their intimate contact with contaminated environment, their structure (it has the thinnest epithelium of all the organs allowing metals penetration) and their importance as an effector of ionic and osmotic regulation (Mohamed 2008). Also intestine and generally digestive tract where Cd registered a bioaccumulation of 7.93 mg kg^{-1} w.w., seems to be another main route of the toxic metal uptake.

A significant proportion of the Cd body burden is stored in liver (7.43 mg kg^{-1} w.w.) probably bound to metallothionein (Muñoz-Olivas & Camara 2001). High level of Cd concentrated in the liver reflects liver role in heavy metals storage and detoxification (Avenant & Marx 2000). The high accumulation of Cd in the liver and gills observed in our study is in support of the work of Sehgal & Saxena (1986) on *Clarias gariepinus.*

Table 2

Cd tissue level (mg kg^{-1} wet weight)

Tissue	C	Cd	Cd/EDTA1	Cd/EDTA2	Cd/EDTA3
Gills	ND*	9.05	5.47	2.87	1.47
Intestine	ND	7.93	5.89	3.56	1.07
Liver	ND	7.43	2.88	1.79	0.8
Kidney	ND	11.46	4.36	3.62	2.81
Muscles	ND	0.31	0.26	0.04	0.02
Skin	ND	1.72	1.61	0.35	0.27
Brain	ND	1.95	0.67	0.49	0.35
Ovaries	ND	0.78	0.73	0.52	0.16
Testis	ND	3.79	2.9	2.33	0.54
Heart	ND	9.48	4.61	2.66	2.21
Source of variation					*p*
between tissues					$p<0.001$
between doses					$p<0.05$

* not detectable

Cadmium is very efficiently retained in the organism and normally only a very small quantity is daily excreted. The main route of excretion is via kidney. Excretion is low, less than 0.01% of the total body burden per day (Piscator 1979). Similarly to the liver, kidney is a critical organ in Cd detoxification as evidenced by its renal marked accumulation (11.46 mg kg^{-1} w.w.). Furthermore, kidney continues to accumulate Cd after exposure ceases, probably as a result of the Cd redistribution from large store in the liver (Sorensen 1991).

Cadmium circulates in the blood primarily bound to the red cells. It is evidently bound partly to hemoglobin and partly to metallothionein (Webb & Verscheyle 1976). Once in the blood vascular system, it binds to large proteins (e.g. albumin) for distribution to the target tissues, including heart. So its high bio-concentration in heart (9.48 mg kg^{-1} w.w.) is not surprising. Workers as Mohamed (2008), reported much higher level of Cd in heart tissue of *Oreochromis niloticus* (52.39 mg kg^{-1} d.w.) and *Lates niloticus* (46.31 mg kg^{-1} d.w.) from the selected khors of Lake Nasser.

Muscle was analyzed because of the implications it carries for human consumption and health risk. This is why the muscles and skin are included in bio-monitoring programs (Nussey et al 2000). But muscle Cd bioaccumulation is among the lowest (0.31 mg kg^{-1} w.w.) in analyzed tissues. Cadmium is accumulated primarily in major organ tissues of fish rather than in muscle. In general, residues in fish muscle cannot be related to concentrations in water (Moore & Ramamoorthy 1984). Lower Cd level in fish muscle may result from elevated concentrations of cystine and methionine compared with other protein. Absence of sulfhydryl groups in these sulfur-rich amino acids probably play a role in decreasing Cd biding in skeletal muscle.

Regarding the gonads, it appears that testicles can retain more Cd (3.79 mg kg^{-1} w.w.) than ovaries (0.78 mg kg^{-1} w.w.). Mohamed (2008) were found even more Cd concentrations in testicles of *Oreochromis niloticus* (21.35 mg kg^{-1} d.w.) and *Lates niloticus* (18.91 mg kg^{-1} d.w.). Generally, male gonads have higher contents of the non-essential metal as cadmium or lead (Huang et al 2003). Latkovskaya (2000) has explained that different concentrations of heavy metals between males and females are due to the specific nature of physiological processes among sexes and to the specificity of biochemical composition of tissues during the period of growth and gonad formation in fish.

The lower level of Cd detected in the skin (1.72 mg kg^{-1} w.w.) might indicate that this organ is a possible routes of excretion for Cd through the mucus layer of its outer surface.

As a partial conclusion, studied metal was more concentrated in the non-edible parts of the fish than the edible parts, muscle or skin.

EDTA addition to the polluted media in dose of 0.05 g L^{-1}, 0.15 g L^{-1} and 0.15 g L^{-1} gradually led to a reducing of Cd bioaccumulation in dose-dependent manner. Thus, Cd concentration was placed under maximum permissible level in muscle (0.02 mg kg^{-1} w.w.) when EDTA was introduced as chelating agent in the treatment scheme in dose of 0.15 g L^{-1}.

All these findings suggest the EDTA ability to chelate Cd ions, producing a stable complex that reduces on the one hand Cd uptake by tissues and on the other hand allows Cd removal from the fish body.

Effect of Cd and Cd-EDTA Mixture on the Tissue Level of some Essential Mineral. The highest Fe concentrations in tissues of control group (Table 3) ranged from 336.5 mg kg^{-1} w.w. in liver to 282.25 mg kg^{-1} w.w. in kidney, 197.27 mg kg^{-1} w.w. in testis, 154.85 mg kg^{-1} w.w. in intestine, 119.15 mg kg^{-1} w.w. in ovaries and 105.4 mg kg^{-1} w.w. in brain. The lowest ones were found in heart (73.29 mg kg^{-1}), muscle (18.58 mg kg^{-1} w.w.), skin (10.54 mg kg^{-1} w.w.), and gills (9.05 mg kg^{-1} w.w.). Some of these organs can concentrate even more Fe than we found. Thus, Mohamed (2008) reported values of 403.50 mg Fe kg^{-1} d.w. in liver, 631.25 mg Fe kg^{-1} d.w testis, 4446.00 mg Fe kg^{-1} d.w. intestine, 621.01 mg Fe kg^{-1} d.w. heart, 217.38 mg Fe kg^{-1} d.w. gills and 78.00 mg Fe kg^{-1} d.w muscle of *O. niloticus*.

Table 3

Fe tissue level (mg kg^{-1} wet weight)

Tissue	C	Cd	Cd/EDTA1	Cd/EDTA2	Cd/EDTA3
Gills	9.05	0.36	1.47	2.87	5.47
Intestine	154.85	55.01	95.18	127.76	130.1
Liver	336.5	96.59	253.35	332.77	333.68
Kidney	282.25	58.8	194.34	223.79	277.44
Muscles	18.58	8.96	10.28	11.24	17.71
Skin	10.54	4.84	6.83	8.82	9.73
Brain	105.4	26.04	47.72	47.80	58.17
Ovaries	119.15	54.41	59.74	75.66	87.83
Testis	197.27	37.96	84.08	113.81	194.72
Heart	73.29	2.41	32.46	38.89	60.85
Source of variation					*p*
between tissues					$p<0.001$
between doses					$p<0.001$

Fe levels significantly decreased in any assayed tissue of Cd intoxicated group (Table 3) suggesting that Cd interferes with Fe absorption and metabolism. Cd inhibitor effect on Fe absorbtion at the intestinal level can be explained by its bound to ferritin that is involved in the mucosal uptake and transfer of iron. The major blood iron carrier is the transferrin, a protein that bound a variety of metals in addition to iron including Cd as well. These two metal ions may compete for the same binding sites on the transferrin molecules. As such, liver Fe storage is lower, and also Fe concentration in posthepatic blood flow. Interaction of cadmium with iron in the plasma will impaired heme production necessary for erytrocytar hemoglobine synthesis and causes anemia as Moshtaghie et al (1994), Shalaby (2007) and Karuppasamy et al (2005) found.

Otherwise in humans, anemia appearance can be one of associated Cd intoxication symptom (Peraza et al 1998).

EDTA addition in Cd contaminated water, reduced toxic effect of Cd so Fe tissues levels are closer to those of the control group.

Zinc is a widespread essential micronutrient in the animal organism. Zinc is an important component of many vital enzymes having a catalytic, co-catalytic or structural role, as well as being a structural stabilizer for proteins, membrane and DNA-binding proteins (Zn-fingers) (Vallee & Falchuk 1993).

Zn is weakly accumulated in fish tissue, the highest Zn content (Table 4) being detected in the testis (191.81 mg kg^{-1} w.w.), intestine (190.26 mg kg^{-1} w.w.), cord (152.74 mg kg^{-1}) and kidney (128.87 mg kg^{-1} w.w.) of control group while the lowest in the muscles (15.91 mg kg^{-1} w.w.). Sun & Jeng (1998) reported a similar trend of Zn content in cyprinids muscle, kidney and liver and Mohamed (2008) for Zn content in *O. niloticus* and *L. niloticus* liver, gills and muscle.

Exposure to Cd led to disturbance in Zn absorption, distribution in the organism and excretion, the most affected being testis where its level registered only 24.84 mg kg^{-1} w.w. Indeed, high concentrations of Cd in the intestine led to the reduction of Zn absorption and its bioavailability implicitly. Also, Cd could disturb Zn metabolism, liver involving actively in this regulation; it is known that if Zn input decreases, hepatocytes metallotionein synthesis decreases too because Cd has a higher affinity for these enzymes than Zn has and displaces this micronutrient from the cysteine binding site; as a result, less Zn that in hepatocytes was accumulated. Hence the Cd description as a Zn antimetabolite.

Table 4

Zn tissue level (mg kg^{-1} wet weight)

Tissue	C	Cd	Cd/EDTA1	Cd/EDTA2	Cd/EDTA3
Gills	53.14	34.71	36.78	49.29	50.96
Intestine	190.26	98.35	117.25	135.02	144.62
Liver	35.52	18.75	30.20	32.27	33.25
Kidney	128.87	102.21	70.94	72.21	81.97
Muscles	15.91	10.35	12.07	12.18	12.2
Skin	83.15	36.37	38.8	45.18	57.35
Brain	39.13	21.34	23.78	25.86	36.53
Ovaries	93.88	53.27	72.05	79.32	53.27
Testis	191.81	24.84	42.68	66.63	91.84
Heart	152.74	10.47	38.3	45.75	53.81
Source of variation					*p*
between tissues					$p<0.001$
between doses					$p<0.001$

Cadmium and zinc (IIB transition elements) have a similar electronic configuration and valence state, possessing equal affinities for sulphur, nitrogen and oxygen ligands (Nieboer & Richardson 1980) and hence similar geochemical and environmental properties (Nan et al 2002).

It has been hypothesized that elements whose physical and chemical properties are similar will act antagonistically to each other biologically (Das et al 1997).

EDTA additionally administered in water as chelating agent diminishes the antagonic effect of Cd. Consequently Zn tissue increased with EDTA dose. A dose of 0.15 g EDTA/L allowed to Zn to return very close of its initial level in gills, liver, muscle and brain.

Cu, another indispensable mineral for animal organism, showed high concentrations (Table 5) in the heart (16.23 mg kg^{-1} w.w.), kidney (15.52 mg kg^{-1} w.w.), intestine (12.15 mg kg^{-1} w.w.) and testis (10.29 mg kg^{-1} w.w.) of the control group. Muscles (3.49 mg kg^{-1} w.w.) and skin (4.42. mg/kg w.w.) had lower level of its concentration.

Compared to other works, Öztürk et al (2009) have found very close values of Cu in the muscles and liver of *Cyprinus carpio*. Except the heart and muscle, much more Cu can accumulate the liver, gills, intestine and testis of *O. niloticus* and *L. niloticus* (Mohamed 2008).

Table 5

Cu tissue level (mg kg^{-1} wet weight)

Tissue	C	Cd	Cd/EDTA1	Cd/EDTA2	Cd/EDTA3
Gills	6.18	1.35	1.58	1.71	3.2
Intestine	12.15	3.8	4.26	4.35	4.83
Liver	8.84	4.32	4.49	4.72	5.24
Kidney	15.52	1.37	8.1	12.08	13.72
Muscles	3.49	1.02	1.74	1.88	3.44
Skin	4.42	1.15	1.24	2.04	3.58
Brain	8.2	2.92	3.9	4.17	6.78
Ovaries	6.62	3.16	3.41	3.88	4.08
Testis	10.29	6.31	6.58	8.95	9.31
Heart	16.23	5.08	5.1	5.27	7.78
Source of variation					*p*
between tissues					$p<0.001$
between doses					$p<0.001$

The presence of cadmium in water caused a severe decrease ($p<0.001$) of Cu biodisponibility in the all analyzed tissues (Table 5). Cu is another element interfering with Cd for binding to metallothionein but Cu has a higher affinity for metallothionien than Cd has. The most likely disturbance induced by Cd on the copper metabolism consists in decreasing of the ceruloplasmin concentration – a major protein responsible for Cu carrying throughout the circulatory system (Mills & Dalgarno 1972).

An EDTA addition to environment reduced the suppressor effect of Cd on the transport and tissue uptake of Cu, the most efficient being a dose of 0.15 mg EDTA/L especially in testis, skin, muscles and kidney.

Skin (7500.14 mg kg^{-1} w.w.), gills (4879.26 mg kg^{-1} w.w.), intestine (2715.78 mg kg^{-1} w.w.), heart (2325.39 mg kg^{-1} w.w.) and kidney (1269.16 mg kg^{-1} w.w.) were the organs with the highest Ca content in control group. Chronic cadmium exposure reduced significantly tissue Ca concentrations (Table 6) proving the antagonistic relation between the both metals.

When the cadmium enters in the human organism, cadmium (Cd^{2+}) is powerful competitor of calcium (Ca^{2+}) in biochemical processes (Zeneli et al 2010).

Cadmium occurs in a single ionic state Cd^{2+} and is not metabolized into other forms (Patrick 2003). Cadmium (Cd^{2+}) in its ionic state can displace calcium (Ca^{2+}) and interfere with homeostatic processes requiring calcium (Hardingham et al 1997).

Thus, alteration of human calcium metabolism by chronic cadmium exposure has been show with development of an osteomalacia syndrome in Japan known as Itai-Itai disease (Jones & Fowler 1980). In this case cadmium induced abnormal bone mineralization interfering with the calcification, decalcification and bone remodeling processes (Peraza et al 1998).

Also it is generally agreed that cadmium influences the intestinal absorption of calcium. Cadmium may exert its effect in at least two ways: 1) by direct alteration of the uptake and absorption properties of the intestinal mucosa; and/or 2) by inhibiting the hydroxylation of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol (Chertok et al 1981). The active form of cholecalciferol is 1,25- dihydroxycholecalciferol, and one function of this compound is the regulation of intestinal calcium absorption. The conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol occurs exclusively in the kidney and is catalyzed by the enzyme 25-hydroxycholecalciferol-1-

hydroxylase. Lorentzon & Larsson cited by Chertok et al (1981) have reported that 25-hydroxychole calciferol-1-hydroxylase activity decreases in rats that receive cadmium orally. Thus, it appears that cadmium can interfere with calcium transport by reducing the activity of 25-hydroxycholecalciferol-lhydroxylase, by reducing calcium binding to calcium-binding protein and by inhibiting the activity of alkaline phosphatase and calcium-stimulated ATPase.

Table 6

Ca tissue level (mg kg^{-1} wet weight)

Tissue	C	Cd	Cd/EDTA1	Cd/EDTA2	Cd/EDTA3
Gills	4879.26	3065.88	3435.24	408.56	4599.48
Intestine	2715.78	926.11	1082.34	1368.97	1792.33
Liver	643.41	280.3	314.47	363.31	643.41
Kidney	1269.16	299.69	751.74	893.7	1199.17
Muscles	716.67	580.42	598.84	680.80	701.84
Skin	7500.14	2790.29	2851.98	4323.86	5440.03
Brain	2307	745.28	904.81	1259.17	1924.07
Ovaries	424.48	60.14	276.68	290.74	379.52
Testis	855.36	47.66	370.45	541.73	749.59
Heart	2325.39	66.2	119.81	580.31	1596.87
Source of variation					*p*
between tissues					$p<0.01$
between doses					$p<0.001$

In addition, Cd increases renal calcium excretion because of its toxic effect on renal tubules generally accompanied by disruption of Ca reabsorption.

Increasing concentrations of EDTA added to the Cd contaminated water attenuated Cd antagonistic effect related to the calcium absorption and bioavailability (Table 7).

Magnesium had a different distribution between tissues of control group; Mg high levels were found in the testis (512.08 mg kg^{-1} w.w.), heart (244.72 mg kg^{-1} w.w.) and kidney (338.47 mg kg^{-1} w.w.) while the lowest ones were found in the liver (72.99 mg kg^{-1} w.w.) and muscle (67.71 mg kg^{-1} w.w.). All these values were drastically reduced ($p<0.001$) (Table 7) under the chronic cadmium poisoning.

Table 7

Mg tissue level (mg/kg wet weight)

Tissue	*C*	*Cd*	*Cd/EDTA1*	*Cd/EDTA2*	*Cd/EDTA3*
Gills	116.55	74.4	84.54	95.63	109.93
Intestine	126.94	86.35	89.82	106.05	121.84
Liver	72.99	48.93	65.3	65.85	66.39
Kidney	338.47	25.52	198.36	230.5	329.58
Muscles	67.71	48.55	49.3	54.17	64.17
Skin	136.02	88.82	91.42	102.28	114.24
Brain	123.84	11.61	102.87	111.6	122.33
Ovaries	124.09	72.68	73.69	100.57	106.23
Testis	512.08	106.22	208.19	218.58	233.3
Heart	244.72	180.62	182.18	185.96	225.8
Source of variation					*p*
between tissues					$p<0.001$
between doses					$p<0.001$

One of the important mechanisms of cadmium toxicity in human and animals is its interactions with bioelements, including magnesium. This has been proven by numerous authors. Thus (Kobylec-Zamlynska et al 1998) showed that under Cd exposure, a population of children developed hypomagnesemia (Kobylec-Zamlynska et al 1998). In rabbits, prolonged Cd intoxication induced significant decrease of blood Mg, which was associated with increased Mg elimination via urine (Soldatovic et al 1998). The in vitro antagonism between Cd and Mg has been reported on the human amniotic membrane at the maternal level sites (Durlach & Bara 2000).

EDTA used as chelating agent binds Cd^{2+} and thus its inhibitory action on Mg is significantly reduced (see Table 7).

Conclusions. The following conclusions can be developed from our data:
1. EDTA has the ability to reduce Cd bioaccumulation in fish's organism and diminishes in the same time the Cd antagonistic effect for some essential minerals as: Fe^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+} or Mg^{2+};
2. Magnitude of the EDTA action is dose-dependent;
3. Further studies of the possible risks related to the mobilization by EDTA of the vital body elements are recommended.

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