AACL BIOFLUX

Aquaculture, Aquarium, Conservation & Legislation International Journal of the Bioflux Society

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

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Abstract. Due to their interesting physico-chemical properties (i.e. cation-exchange capacity, adsorption, catalysis and dehydration), natural zeolites are useful in very different fields: agriculture, animal husbandry, chemical and pharmaceutical industry, environmental protection (water, air and soil depollution). Ion-exchange capacity of zeolites consists in their ability to retain divalent cations such as Ca^{2+}, Mg^{2+}, Cu^{2+}, Cd^{2+}, Fe^{2+}, Zn^{2+} from a contact solution, yielding to the solution an equivalent amount of an own cation (Na^{+}). In this paper we have exploited this property of a zeolite (namely clinoptilolite) in an attempt to reduce Cd bioaccumulation in tissues of Prussian carp specimens under induced intoxication with cadmium acetate and Cd antagonism as against some essential micro- and macroelements - Fe^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+} and Mg^{2+} at the same species.
Key Words: chroinic cadmium intoxication, zeolite, fresh water fish, mineral micronutrients, mineral macronutrients.

Résumé. En raison des propriétés physico-chimiques intéressantes (par exemple, la capacité d'échange cationique, l'adsorption, la catalyse et la déshydratation) zéolithes naturelles peuvent être utilisées dans des domaines très différents: agriculture, élevage, industries chimiques et pharmaceutiques, la protection de l'environnement (depollution de l'eau, de l'air et de sol). Capacité d'échange d'ions de zéolithes, c'est la capacité de conserver divalents cations tels que Ca^{2+}, Mg^{2+}, Cu^{2+}, Cd^{2+}, Fe^{2+}, Zn^{2+} à partir d'un contact avec la solution, ce qui donne la solution d'un montant équivalent d'un cation propres (Na^{+}). Dans cet article nous avons exploité cette propriété de clinoptilolite dans une tentative de réduire la bioaccumulation du Cd dans les échantillons du corps de l'argent carassin exposés intoxication volontaire par l'acétate, le cadmium et l'antagonisme des Cd à des micro-et macroelementele essentiel de la même espèce: Fe^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+} et Mg^{2+}.
Mots-Clés: intoxication par le cadmium chronique, zéolite, poissons d'eau douce, oligo-éléments minéraux, minéraux macronutriments.

Rezumat. Datorită proprietăţilor lor fizico-chimice interesante (respectiv capacitate de schimb cationic, adsorbţie, cataliză şi dehidratare) zeoliţii naturali îşi găsesc utilizarea în domenii extrem de diferite: agricultură, zootehnie, industria chimică şi farmaceutică, protecţia mediului (acţiuni de depoluare a apei, aerului şi solului). Capacitatea de schimb ionic a zeoliţilor constă în posibilitatea de a reţine cationi divalenţi cum ar fi Ca^{2+}, Mg^{2+}, Cu^{2+}, Cd^{2+}, Fe^{2+}, Zn^{2+} dintr-o soluţie de contact, cedând soluţiei o cantitate echivalentă dintr-un cation propriu (Na^{+}). În lucrarea de faţă am exploatat această proprietate a clinoptilolitului în încercarea de a reduce nivelul bioacumulării cadmiului în organismul exemplarelor de caras argintiu expuse intoxicaţiei induse cu acetat de cadmiu şi a antagonismului cadmiului faţă de unele micro- şi macroelemente esenţiale la aceeaşi specie: Fe^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+} şi Mg^{2+}.
Cuvinte cheie: intoxicaţie cronică cu cadmiu, zeolit, peşti de apă dulce, micronutrienţi minerali, macronutrienţi minerali.

Introduction. Clionoptilolite is the most well known natural zeolite, we can find it especially in rocks with volcanic origins, in concentrations of 60-90%. Near by clinoptilolite this volcanic tuffs (also called zeolitic tuffs) contain feldspars, clays, glas and quartz. There is in Romania an important reserve of volcanic tuffs with an average contain of 70% clinoptilolite. Natural zeolit showed high performances on ammonia ion (a frequent pollutant found in waste waters and waters from animal husbandry farms), and metalic ions (from industrial waters) removal (Briggs & Smith 1996; Grozea et al 2002; Tenciu et al 2008).

Zeolites are used in industry, agriculture, environment protection and even in medicine. Managed as feed additives in farm animals feeding – pigs, ruminants, farm birds and fish – zeolites determined the growth of body weigth, improving of feed conversion ratio and keeping animals healthy.

The aim of the paper was pointing of the toxic tissular valency of cadmium in *Carassius gibelio* (Bloch, 1782) under induced chronic intoxication with $Cd(CH_3COO)_2 \times 2H_2O$, and we focused also on investigation of clionoptilolite effects as ion-exchanging agent on reduction of the cadmium bioaccumulation and its antagonism as against some essential micro- and macroelements at the same species.

Material and Method. Choissing 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 Ghiroda' Fishfarm – (Timiș county) and transported to the physiology laboratory of the Faculty of Animal Sciences and Biotechnologies Timișoara, Romania. We opted in favour 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 atested by the papers of the last years is to work with culture fish (carp, perch, tilapia, Prussian carp), because they are easy to acces.

Individuals with a body weight of 35-40g were selected by gravimetric measurements and then they were acclimated two weeks to laboratory conditions, removing the suspected unhealty subjects. Fish were housed in a 120 L capacity glass aquariums (20 fishes/aquarium) provided with aeration system.

The physico-chemical parameters of the laboratory water (during a 21 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 10 ppm, and its water circulation was supported by two AC 9904 air pumps. The sublethal treatment dose (25% of LC_{50}) was calculated from percentage mortalities of fish as described by Veena & Chacko (1997).

Three doses of the tested product (clinoptilolite from Mârşid quarry – Zalău county) was administered as follows in Table 1.

Table 1

Experimental groups and their notation

S.No.	*Groups*	*Notation*
1	Control (metal free water)	C
2	Cadmium (10 ppm)	Cd
3	Cadmium (10 ppm) + 0.5 g Zeolit/L	CdZ1
4	Cadmium (10 ppm) + 2.0 g Zeolit/L	CdZ2
5	Cadmium (10 ppm) + 4.0 g Zeolit/L	CdZ3

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

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

Data were analyzed statistically using an ANOVA two factors without replication test, having in view two factors: the tissue and adopted treatment schema.

Results and Discussion. Toxicity of the chemical elements depends on more factors, the most important being concentration of a certain element in the organism and its action period. Prolonged exposure at toxic elements action produces their accumulation in organism tissues and humours.

In water Cd administration has as a result important increasing of its concentration in all samples belonging to intoxicated group. In accordance with data from Table 2, the exposure of Prussian carp individuals to a sublethal dose of 10 ppm Cd leads to its bioaccumulation in different proportions in the all analyzed tissues.

Table 2

Cd tissue level (mg/kg wet weight)

Tissue	*C*	*Cd*	*Cd Z1*	*Cd Z2*	*Cd Z3*
Gills	ND*	24.71	21.42	20.69	11.38
Intestine	ND	47.01	41.60	33.01	29.39
Liver	ND	11.03	10.00	9.07	7.82
Kidney	ND	51.52	48.76	45.20	42.00
Muscles	ND	0.15	0.11	0.05	0.04
Skin	ND	2.01	1.99	1.97	1.65
Brain	ND	1.10	0.90	0.62	0.46
Ovaries	ND	1.34	0.90	0.54	0.25
Cord	ND	5.34	4.27	2.53	1.68

Source of variation	*p*
between tissues	$p<0.001$
between doses	$p<0.001$

*undetectable

Thus, intestine, liver, kidneys, gills and cord, being organs with intensive metabolic activity, revealed the highest bioaccumulation levels, while in muscles, ovaries, skin and brain was found the smallest ones. The high metal concentrations found in gills (24.71 mg/kg) and intestine (47.01 mg/kg) especially consecutive to the Cd contamination is owing to the fact that these two organs are the most important access ways in fish body, gills being not only in direct contact with the water but also an organ whose structure facilitates the toxic compounds entrance, while intestine and digestive tract, generally, allow heavy metal absorbtion as a consequence of fodder, water or contaminated sediments consumption.

Cd bioaccumulation keeps at the high levels in kidneys (51.52 mg/kg), and a large increasing also has been found in liver (11.03 mg/kg). The liver is considered the main organ for metals storage and detoxification (Avenant & Marx 2000). Additionally liver detains an important role in contaminants deposition, their redistribution, detoxification or transformation, acting as an active situs of pathogenetic effects induced by them (Evans et al 1993). Consequently the fish hepatic tissue is often recommended as an indicator of pollution level in fish basins (Al Yousuf et al 2000).

If the gills assure entrance of heavy metals into fish body, it seems that the kidney is responsible for exit control of them, contributing at detoxification, reson why it was found a serious concentration of Cd at this level. Also, McGeer et al (2000) consider, that kidney is the major site of Cd accumulation in rainbow trout *Oncoryncus mykiss*. Cd accumulation in kidney is facilitated by its transporter protein – a metallothionein (see details in Iwama et al 1998, 1999; Petrescu-Mag & Petrescu-Mag 2010) – with who it forms a complex which is retained, while protein is quickly degraded and not reabsorbed, allowing Cd accumulation. Instead, Cd bioaccumulation levels are similar in brain (1.10 mg/kg) and ovaries (1.34 mg/kg). In regard to gonads, testis were not collected, because we did not find any male between studied individus (juvenile cyprinid females developed intersexuality).

Zeolite addition in dose of 0.5 g/L, 2.0 g/L and 4.0 g/L respectively in water allowed reduction of metal bioaccumulation, the magnitude of this process being related to the administered dose. Thus, in gills, Cd concentration decreased at 24.71 mg/kg in treatment scheme with 0.5 g zeolite/L, at 21.42 mg/kg in treatment scheme with 2.0 g zeolite/L and at 11.38 mg/kg in treatment scheme with 4.0 g zeolite/L; an 4.0 g/L zeolite addition in liver determinated a fall of metal accumulation, its concentration reaching the level of 7.82 mg/kg.

Intestine developed the same tendency of reduction of Cd tissue retention (33.01 mg/kg in treatment scheme with 2.0 g zeolite/L and 29.39 mg/kg in treatment scheme where zeolite was incorporated in dose of 4.0 g/L.

Zeolite addition also reduced Cd accumulation rate at the kidney level, treatment schemes with 2.0 g zeolite/L (45.20 mg/kg Cd) and 4.0 g zeolite/L (42.00 mg/kg Cd) proving to be the most efficient.

Between organs where Cd accumulation although present, registered lower levels of retentions, the muscle was tissue that, under zeolite action, Cd concentration reached the maximum permissible level (0.05 mg/kg) for the treatment scheme with 2.0 g zeolite/L and it was placed even under this maximum value (0.04 mg/kg) for treatment scheme with 4.0 g zeolite/L.

In respect to tissue Cd bioaccumulation, the results of the present study (Table 2), show that: the intestine, liver, gills, and the kidneys belonging to Cd intoxicated fish, retain the metal in a higher concentration ($p<0.001$), than the tissues collected from the subjects receiving a Cd-zeolite mixture in water. Also, zeolite addition to the contaminated environment reduces significantly ($p<0.001$) its level in water relative to treatment scheme without ionic-exchanging agent. The explanation of this fact is related to the interesting physical and chemical properties of zeolites, and theirs ion-exchange capacity and adsorbtion. Thus Cd and zeolite could interact in the aqueous medium as follows: zeolite has extra framework ions (Na^{+}), and framework ions (Al^{3+} and Si^{4+}), which are easily exchangeable and non-exchangeable respectively. The ionic radius of Na^{+}, Al^{3+} and Si^{4+} are 0.95, 0.50, and 0.54 Å; ionic radius of Cd is 0.97 Å (Huheey 1983, cited by James & Sampath 1999) that appropriated to the Na^{+} radius in zeolite, reason why both of them could be easily exchanged with each other, than Al^{3+} and Si^{4+} ions. There is possible that Cd^{2+} ions binding Na^{+} extra framework ions from zeolite and thus Cd^{2+} will be immobilized. Cd-zeolite complex thus formed can be stored in sediments on the one hand, or get into the fish organism without accumulating on the other hand, finding it in the faeces. Cd toxic tissue load is reduced in this way and also its toxicity.

The individuals of Cd intoxicated group presented low levels of Fe tissue concentration in any assayed organ (Table 3). If in kidneys, liver, intestine and cord of subjects belonging to the control group, was found the highest Fe concentration (168.59 mg/kg, 85.51mg/kg, 77.82 mg/kg and 81.78 mg/kg respectively), consecutively to toxic metal exposure, the Fe level was significantly low, reaching 85.25 mg/kg, 64.14 mg/kg, 44.93 mg/kg and 50.32 mg/kg respectively.

In this case, Cd exert an inhibitory effect on Fe uptake and metabolism by its possible binding with ferritin in the intestinal mucosa and transferring (has a role in the iron transport), in blood circulation respectively, proteins that appear to have a high affinity for Cd. As such, liver Fe storage is lower, and also Fe concentration in posthepatic blood flow.

Further heme production necessary for erythrocytary hemoglobine synthesis will be impaired and heme synthesis anemia is installed, as Shalaby (2007) and Karuppasamy et al (2005) found. Otherwise, in humans, anemia appearance is one of associated Cd intoxication symptoms (Peraza et al 1998).

Table 3

Fe tissue level (mg/kg wet weight)

Tissue	*C*	*Cd*	*CdZ1*	*CdZ2*	*CdZ3*
Gills	43.15	17.96	24.18	30.47	33.04
Intestine	77.82	44.93	66.12	69.31	77.27
Liver	85.51	64.14	69.11	72.06	80.54
Kidney	168.59	85.25	127.24	131.34	140.90
Muscles	5.36	1.47	1.89	2.22	4.28
Skin	15.61	8.98	10.85	13.48	14.52
Brain	57.37	20.36	27.57	30.15	44.98
Ovaries	51.44	27.18	32.24	34.46	38.82
Cord	81.78	50.32	56.00	62.75	67.40
Source of variation					*p*
between tissues					$p<0.001$
between doses					$p<0.001$

Zeolite administration in Cd contaminated environment reduces the antagonism exerted by itself against Fe proportionally with increasing added dose so that, at 4.0 g zeolite/L, iron level in fish tissues to return to values recorded in the control group: 140.90 mg/kg in kidney, 80.54 mg/kg in liver, 77.27 mg/kg in the intestine and 67.40 mg/kg in cord.

Zn tissue content found in high concentrations in kidney (68.45 mg/kg), cord (66.20 mg/kg), intestine (62.24 mg/kg) and ovaries (46.28 mg/kg) of control animals, decreased significant ($p<0.001$) following exposure to Cd poisoning at 48.62 mg/kg in kidney, 10.05 mg/kg in cord, 45.08 mg/kg in intestine, and 27.18 mg/kg in ovaries (see Table 4).

Table 4

Zn tissue level (mg/kg wet weight)

Tissue	*C*	*Cd*	*CdZ1*	*CdZ2*	*CdZ3*
Gills	27.17	17.14	22.49	23.48	25.96
Intestine	62.24	45.08	46.41	50.78	56.33
Liver	22.37	14.33	14.59	15.87	16.08
Kidney	68.45	48.62	53.87	57.67	63.22
Muscles	7.20	4.99	5.11	6.95	7,01
Skin	22.13	14.70	15.36	17.92	21.64
Brain	17.07	13.25	14.82	14.95	15.18
Ovaries	46.28	27.71	28.69	31.77	35.47
Cord	66.20	10.05	43.9	47.96	54.59
Source of variation					*p*
between tissues					$p<0.001$
between doses					$p<0.001$

Zinc is widespread in the animal organism, its presence being essential for catalytic activity of more than 170 enzymes.

Probably one of the most recognized and best metal-metal interactions studied in animal bodies is the one established between Cd and Zn, some similarities existing between the two metals: both Zn and Cd are members of the IIB group of metals and both tend to form tetrahedral complexes. However, apparently, the two metals would have similar interactions in animal body: duodenum and jejunum are the main sites of Cd absorption, while Zn is absorbed in the jejunum and ileum preponderantly. Additionaly, Cd shows an inhibitory effect on Zn containing enzymes such as carboxypeptidase and alpha-mannosidase. Also, Cd occupies the Zn binding site on metallothionein, reduces in vivo kidney leucinaminopeptidase activity that could be responsible for proteinuria, or competes with Zn to occupy binding sites on the enzymes involved in gametogenesis.

Cd presence in high concentrations in the intestine leads to the reduction of Zn absorbtion and its bioavailability implicitly. Also, Cd could disturb Zn metabolism, liver involving actively in this regulation: if Zn input decreases, hepatocytes metallothionein synthesis decreases too; as a result less Zn will be accumulated in hepatocytes. Hence, the Cd description as a Zn antimetabolite. Zeolite administered additionally in water with cadmium acetate, immobilizes Cd, thus decreasing Cd antagonistic effect against Zn.

Accordingly Zn tissue level gradually increases with the added zeolite dose, the increases being significant, even if only for muscle, gills and skin the dose of 4.0 g zeolite/L allows to Zn accumulating very close to concentrations registered in control group.

Similar to the situation encountered for Zn, Cd reduces Cu level in all tissues of animals exposed to its action: from 4.27 mg/kg to 1.96 mg/kg in the intestine, from 7.51 mg/kg to 2.73 mg/kg in liver, from 8.09 mg/kg to 3.00 mg/kg in cord, kidney being the most affected (from 23.54 mg/kg to 8.42 mg/kg) (Table 5).

Table 5

Cu tissue level (mg/kg wet weight)

Tissue	*C*	*Cd*	*CdZ1*	*CdZ2*	*CdZ3*
Gills	2.61	0.90	1.32	1.46	2.10
Intestine	4.27	1.96	2.56	2.95	3.19
Liver	7.51	2.73	4.29	5.02	6.48
Kidney	23.54	8.42	13.18	18.31	20.09
Muscles	3.74	0.55	0.84	1.03	2.00
Skin	3.77	1.34	1.74	2.16	3.05
Brain	3.11	1.61	1.66	1.97	2.37
Ovaries	2.13	0.84	1.12	1.71	2.07
Cord	8.09	3.00	4.40	5.92	7.12
Source of variation					*p*
between tissues					$p<0.001$
between doses					$p<0.001$

Cd interferes with Cu for binding to metallothionein, which is responsible for homeostatic control of Zn and Cu. Cd binding to metallothionein is however a fortuitous process due to its chemical similarities to trace elements and not to the high affinity of protein for Cd (affinity for Cu is higher than one for Cd). Most likely Cd disturbs Cu metabolism by decreasing ceruloplasmin concentration – a major protein responsible for Cu carrying in posthepatic blood flow.

In tissues taken from animals which received different concentrations of a Cd-zeolite mixture in water, Cu tissue levels decrease has been less intensely in group where the combination 10 ppm Cd – zeolite 4.0 g/L was used.

Ca was found in high concentration in skin (3015.69 mg/kg), gills (6222.14 mg/kg), intestine (1684.97 mg/kg), cord (2817.59 mg/kg) and kidney (1675.72 mg/kg) sampled from control group tissues (Table 6). A significant decrease ($p<0.001$) of tissue Ca occurred in specimens exposed to chronic Cd poisoning, which proves the antagonistic relationship between the two elements.

Similarly to all other trace elements having entrance-control, a carrier protein takes Ca from the intestinal mucosa and whose synthesis depends on vitamin D. Cd affects homeostatic control of Ca tissue level, by competition for the same binding site on carrier protein. It also increases renal calcium excretion because of its toxic effect on renal tubules generally accompanied by disruption of Ca reabsorption.

Cd-Ca interaction was not recognized until the event Itai-Itai syndrome in a population of women in Japan, whose diet is rich in rice with a low Ca content. To them, Cd was deposited in bone tissue, interfering with the calcification, decalcification and bone remodeling processes (Peraza 1998). As a result of these abnormal mineralization, risk of osteomalacia and osteoporosis installation increased.

Table 6

Ca tissue level (mg/kg wet weight)

Tissue	*C*	*Cd*	*CdZ1*	*CdZ2*	*CdZ3*
Gills	6222.14	3636.19	3908.00	4678.05	5996.24
Intestine	1684.97	429.75	482.48	600.00	882.34
Liver	396.36	199.50	201.17	338.39	371.22
Kidney	1675.2	860.20	752.39	907,72	1311.36
Muscles	445.72	315.52	329.40	335.52	443.96
Skin	3015.69	1523.09	1873.88	2517.33	2782.79
Brain	975.25	485.39	530.01	716.01	810.07
Ovaries	269.71	31.67	157.84	190.23	210.29
Cord	2817.59	1082.00	1181.90	1265.78	1467.03

Source of variation	*p*
between tissues	$p<0.001$
between doses	$p<0.001$

Increasing zeolite concentrations in experimental groups determined the attenuation of Cd antagonistic effect vis-à-vis absorption and tissue Ca bioavailability especially for the last treatment scheme when zeolite was added in dose of 4.0 g/L.

Manganese is a bioelement present in all tissues of animal organism. In tissues of control group Mg was found in greater quantities in skin (445.85 mg/kg), gills (948.42 mg/kg), kidney (488.18 mg/kg), intestine (573.45 mg/kg), ovaries (418.77 mg/kg), while in Cd intoxicated subjects to register a severe decrease ($p<0.001$) of its concentration in all analyzed tissues (Table 7).

Table 7

Mg tissue level (mg/kg wet weight)

Tissue	*C*	*Cd*	*CdZ1*	*CdZ2*	*CdZ3*
Gills	948.42	158.28	184.21	657.04	706.29
Intestine	573.45	319.66	352.00	374.20	389.15
Liver	232.17	65.18	74.91	146.60	195.03
Kidney	488.18	100.96	132.10	174.63	230.36
Muscles	306.16	220.25	226.62	230.15	249.82
Skin	445.95	328.64	341.00	345.39	370.28
Brain	291.46	192.33	210.13	240.64	270.22
Ovaries	418.77	294.65	303.51	310.20	408.95
Cord	205.35	96.30	105.92	112.96	134.28

Source of variation	*p*
between tissues	$p<0.001$
between doses	$p<0.001$

As it happened in the case of Ca the antagonistic effect of Cd to Mg is translated by alteration of absorption, transport and it seems of tubular reabsorption processes. And because Mg, similarly to Zn is a cofactor in hundreds of enzymatic reactions, it is possible that the activity of enzymes working with this element to be severely altered as a consequence of the decrease of its tissue bioavailability. But, zeolite used as ion-exchange agent, immobilizes Cd^{++} and thus its inhibitory action on Mg is significantly reduced.

Conclusions. The data we obtained allow us to develop the following conclusions: zeolite forms a complex with Cd that reduces Cd bioaccumulation in fish's organism and diminishes in the same time Cd antagonistic effect against the some essential minerals as Fe^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+} or Mg^{2+}. Zeolite action intensity is dose-dependent.

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Received: 12 August 2010. Accepted: 03 September 2010. Published online: 04 September 2010.
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How to cite this article:
Nicula M., Bănăţean-Dunea I., Gergen I., Hărmănescu M., Simiz E., Pătruică S., Polen T., Marcu A, Lunca M., Szucs S., 2010 Effect of natural zeolite on reducing tissue bioaccumulation and cadmium antagonism related to some mineral micro- and macronutrients in Prussian carp (*Carassius gibelio*). AACL Bioflux **3**(3):171-179.