The Effect of Active Principles of Cilantro and Spirulina Powder on Lead Antagonism to Copper and Chromium in *Carassius gibelio*

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Abstract

The goal of our work was to highlight the detoxifying potential of the active principles from lyophilized cilantro and spirulina in experimental contamination with lead, to *Carassius gibelio*, and their effect on lead antagonism to copper and chromium.

120 Prussian carps, weighing 22-25 g each were divided according to the following treatments for 21 days: C group (without treatment), E1 group (75 ppm Pb into water as $Pb(NO_3)_2x$ ½H_2O), E2 group (75 ppm Pb into water+2% lyophilized cilantro in feed), E3 group (75 ppm Pb into water+2% lyophilized spirulina in feed).

At the end of the experimental period, tissue samples (gills, muscles myotome– epaxial, heart, skin and scales, intestine, liver, brain, gonads, kidney) were collected after a starving for 12 hours, and fish euthanasia with clove oil.

Determination of Cu and Cr concentration in biological samples was performed using atomic absorption spectrophotometer AAS-VARIAN.

Pb addition into water in dose of 75 ppm, has resulted in Cu and Cr mobilization from fish tissues. Decreasing of Cu tissue level occurred less intensive in tissues sampled from groups receiving cilantro and spiriulina powder in feed, maximum efficiency in the counteracting the antagonism against Pb showing spirulina on the heart, liver, and kidney.

Cr was maintained at relatively low values, although, cilantro powder has induced in some wise the Pb complexing. In contrast, the freeze-dried spirulina brought the tissue level of Cr close to that of the control group or even has determined its more efficient takeover from the feed.

Keywords: copper; chromium; fish; lead intoxication; lyophilized cilantro; lyophilized spirulina

1. Introduction

Most living organisms accumulate and retain minerals from the environment; but their incorporation is very selective. Seven macro-minerals and 15 micro-minerals with a well-defined physiological role are known. Because their major roles in the organism [1, 2], these are named as bio elements.

Beside these, heavy metals are generally toxic and when the body cannot remove the surplus.

Unlike other metals, lead is not known to have biological role in animal organism, its tissue concentration leading to intoxication, and major organic dysfunction.

In the aquatic environment, ionic form of lead is very mobile and bioavailable [3]. Fish have the ability to retain some inorganic elements not only from their diet but also from the external environment - fresh water or sea water. As with

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other metals, Pb toxicity for fish depends on the species [4].

For cases of acute or chronic lead intoxication, EDTA infusions are considered the treatment of choice [5]. EDTA is a chemically synthesized substance used in chelation therapy. Natural herbs have shown the ability to act as chelates too. Cilantro knows as a popular culinary and medicinal herb. In animals, it decreased lead absorption [6] and enhanced its excretion in the kidneys. Another natural chelating agent facilitating lead removal is spirulina. Spirulina is a cyanobacterium classified as blue green algae. In aquaculture, spirulina is recommended as an alternative source of feed and as immune stimulant [7-13].

The purpose of our work was to:

- investigate the antagonistic interactions between lead and the micro elements copper and chromium in fish tissue exposed to experimental lead intoxication.
- access the efficacy of *Coriandrum sativum* (cilantro*)* and spirulina (*Arthrospira platensi)* powder on the decreasing the lead antagonism to copper and chromium.

2. Materials and methods

Materials and methods are the same with those presented in a previous paper [14], except the minerals which were investigated, in this case, copper and chromium.

3. Results and discussion

Pb administration (75 ppm in water) for 21 days resulted in the tissue mobilization of trace elements Cu and Cr which were recognize as essential micro nutrients for the development of biological functions in the organism [15, 16].

In our experiment, high levels of Cu in the tissues belonging to the control group were measured for heart (8.09 mg kg^{-1}), liver (4.27 mg kg^{-1}) and intestine (4.27 mg kg^{-1}). Lead chronic poisoning has drastically reduced the concentration of Cu in all fish tissues exposed to its action ($p<0.001$) (Table 1, Figure 1), the most affected being the muscles, with only 0.15 mg kg^{-1} and the ovary with only 0.12 mg kg^{-1} at the end of the experiment.

In tissues samples from the animals receiving cilantro and spirulina in feed, the decrease of Cu concentration was less intense, the maximum effectiveness in counteracting the Pb antagonism to Cu showing spirulina in the heart (7.04 mg kg^{-1}), liver (5.49 mg kg^{-1}), and kidney (3.67 mg kg-^{1}).

Copper is a constituent of many enzymes, playing an essential role in their activity. It is associated with cytochrome c oxidase involved in the intracellular transport of electrons. In fish tissues copper enzymes as superoxide dismutase, tyrosinase, lysyl oxidase, ceruloplasmin, and dopamine β-hydroxylase have been identified [17]. One of the few investigations of copper metabolism in fish, namely that of Syed and Coombs, (1982) [18] reveals similarities with mammals in terms of Cu distribution and enzymes dependent on it. Copper is the main element of proteins like hemocyanin or cyanodine that play a role similar to hemoglobin in oxygen transport in crustaceans [19].

Chromium is an essential mineral for the metabolic processes of the animal organism. Chromium promotes tissue glucose utilization in combination with insulin. It is a component of cromodulin, a protein that facilitates the binding of insulin to receptor sites is a constituent of the glucose tolerance factor (GTF) [20] and by its synergism with insulin promotes the cellular glucose uptake.

Table 1. Statistical indices and statistical segnificance regarding Cu tissue level

Tissue	Number	Sum	Average	Variance	SD
		Cu (mg kg^{-1} wet weight)			
Gill	4	6.25	1.56	0.53	0.63
Muscle	4	5.40	1.35	2.63	1.40
Skin+ scales	4	7.71	1.92	1.77	1.15
Intestine	4	10.38	2.59	1.94	1.20
Liver	4	13.05	3.26	4.31	1.79
Heart	4	22.82	5.70	10.04	2.74
Brain	4	9.20	2.30	1.30	0.98
Ovaries	4	6.42	1.60	1.04	0.88
Testis	4	5.76	1.44	0.72	0.73
Kidney	4	11.54	2.88	2.73	1.43
Group					
C	10	37.83	3.78	2.97	1.63
Pb	10	6.12	0.61	0.12	1.67
Pb+cilantro	10	25.19	2.51	2.87	1.77
Pb+spirulina	10	29.39	2.93	3.93	1.89
Source of variation					p
Between tissues					p<0.05
Between treatments					p<0.001

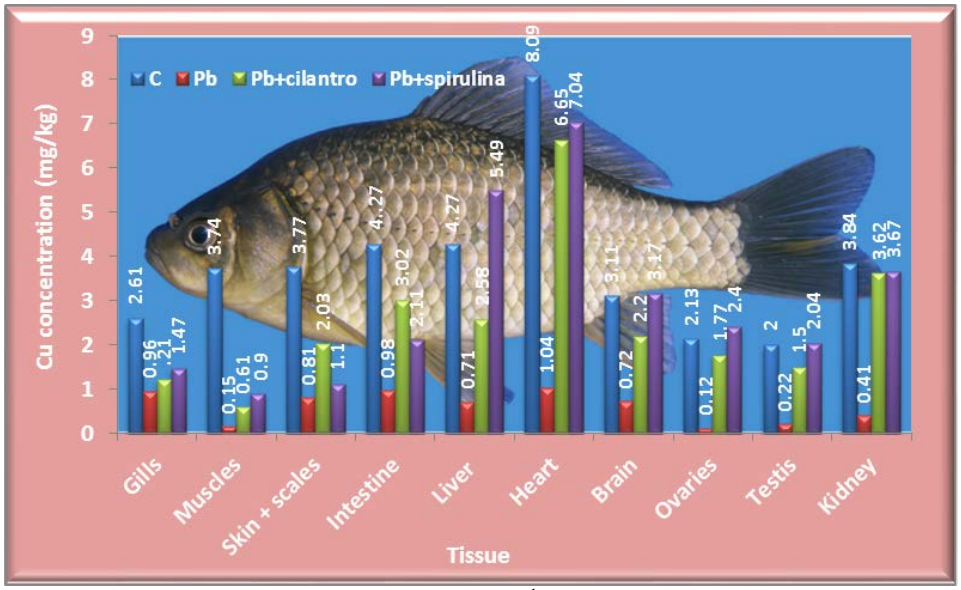

Figure 1. Graphical representation of Cu (mg kg^{-1}) in various tissues in *Carassius gibelio*

Cr reduces serum levels of total cholesterol. It is involved in the metabolism of lipoproteins, reducing the serum level of low-density lipoproteins (LDL) and causing elevated serum levels of high density lipoproteins (HDL) [21]; in this way promotes and maintains health; It is believed that Cr participates in the transport of amino acids in liver and heart cells. Indeed, this minerals was found in high concentrations in the heart (4.07 mg kg^{-1}), the liver (3.97 mg kg^{-1}), and the kidney of the control group (3.21 mg kg^{-1}) (Table 2, Figure 2). Pb exerts on this element an antagonistic action by substantially reducing its tissue bioavailability. The antagonistic effect of Pb is so intense that even if the addition of cilantro powder determines to some wise its complexation, Cr is maintained at relatively low levels. Conversely, spirulina powder brings the Cr tissue level very close to that of the control group or even determines its more efficient take-up from the feed, except for cardiac tissue (1.61 mg kg^{-1}) and ovary (0.79 mg kg^{-1}). As David L. Watts has shown [21], Pb exerts to chromium a direct antagonism either on a metabolic or absorptive level.

Table 2. Statistical indices and statistical segnificance regarding Cr tissue level

Tissue	Number	Sum	Average	Variance	SD
		Cr (mg kg^{-1} wet weight)			
Gill	4	3.56	0.89	0.05	0.20
Muscle	4	1.6	0.40	0.01	0.12
Skin+ scales	4	3.84	0.96	0.63	0.69
Intestine	4	5.21	1.30	1.27	0.97
Liver	4	9.70	2.40	4.22	1.77
Heart	4	7.63	1.90	2.28	1.31
Brain	4	7.94	1.98	2.03	1.23
Ovaries	4	2.73	0.68	0.27	0.45
Testis	4	4.7	1.17	0.35	0.51
Kidney	4	10.42	2.60	3.18	1.54
Group					
C	10	19.63	1.96	1.77	1.26
Pb	10	3.46	0.34	0.04	1.29
Pb+cilantro	10	14.31	1.43	0.70	1.23
Pb+spirulina	10	19.93	1.99	2.55	1.18
Source of variation					p
Between tissues					p<0.05
Between treatments					p<0.001

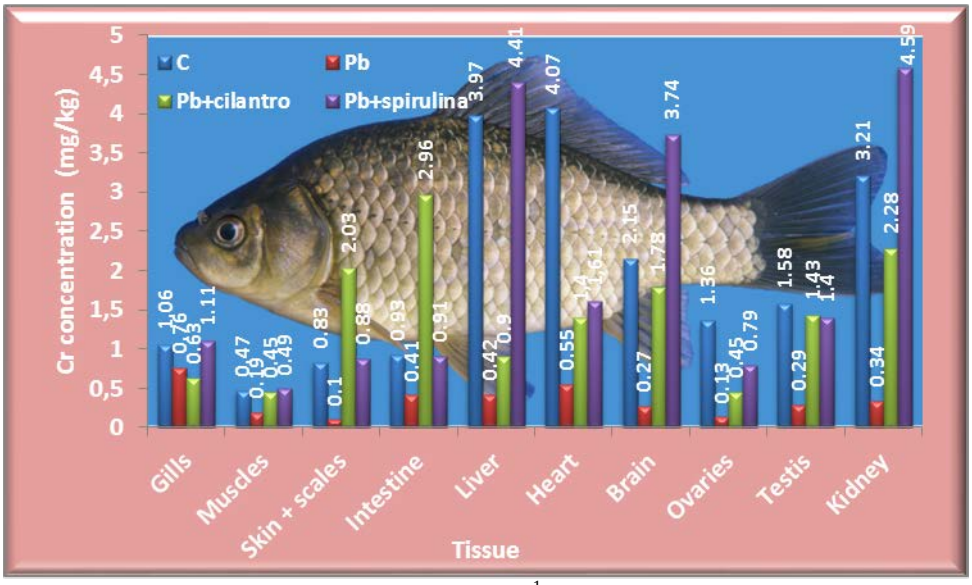

Figure 2. Graphical representation of Cr (mg kg^{-1}) in various tissues in *Carassius gibelio*

4. Conclusions

The results obtained and presented in this paper allow the following conclusions to be drawn:

- Pb displaces causing deficiency or biounavailability of copper and chromium
- the active principles from lyophilized cilantro and spirulina diminish the Pb antagonistic effect on the essential minerals Cu and Cr.

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