

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

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Abstract

Our research purpose was focused towards highlighting the lead toxicity on some tissues from *Carassius gibelio* Bloch (Prussian carp – Cyprinidae family) after induction of chronic intoxication with lead nitrate, hydrated with ½ water molecules – $Pb(NO_3)_2 \cdot \frac{1}{2}H_2O$. By this exploratory study, we wanted to research 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 iron and zinc.

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 $Pb(NO_3)_2 \cdot \frac{1}{2}H_2O$), 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. The analytical determination in order to establish zinc and iron concentrations, using atomic absorption spectrometer (AAS – VARIAN) was performed.

Cilantro and spirulina powder additionally administered in feed, has immobilized Pb through their active principles, thus decreasing its antagonistic effect expressed to Zn, even if Zn tissue level remained low. Pb antagonistic effect shown to Fe was diminished especially in tissue sampled from individuals receiving spirulina powder in feed.

Keywords: fish, iron, lead intoxication, lyophilized cilantro, lyophilized spirulina, zinc

1. Introduction

Mineral elements are inorganic substances that fulfill many vital functions absolutely essential to the very existence of the body. Fish need minerals because:

- they are the constitutive elements of certain tissues (mainly skeletal structures);
- they are molecules that serve as enzymatic co-factors;
- they participate in the intra- and extracellular ion balance;
- they interfere with the regulation of endocrine function;
- they are constitutive components of hormones and enzymes, even contributing to the activation of some enzymes [1, 2].

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Among these, there are 35 metals, of which 23 are considered heavy metals. In small quantities certain heavy metals (iron, copper, magnesium and zinc) are essential in the development of metabolic processes. Many heavy metals become toxic when the body cannot remove the surplus. Unlike other metals, lead has no physiological role in the body and there is no minimum level to be considered nontoxic.

The ionic mechanism of lead toxicity [3] mainly refers to the ability of metal ion to replace other bivalent and monovalent cations, ultimately disrupting the cell's biological metabolism. It displaces and can cause deficiency or bio unavailability of calcium, zinc, manganese, copper and iron [4].

This mechanism induces major disruptions in various biological processes, such as cell adhesion, intra and intercellular signaling, protein folding, maturation, apoptosis, ionic transport, enzymatic regulation, and neurotransmitters release [4].

Lead is a pernicious toxin [5]. Once it gets in your body, it resists leaving through normal elimination and detoxification processes. The conventional treatment for heavy metal poisoning is chelation. Beside synthetic chelators, two of the best natural ones (cilantro and spirulina), facilitate lead removal.

Cilantro known as a popular culinary and medicinal herb, In animals, it decreased lead absorption into bone and inhibition of the delta-aminolevulinic acid dehydratase (ALAD) enzyme [6].

Results of animal studies on lead suggest that coriander achieves this by chelating lead in the gut and reducing absorption as well as chelating lead systematically, with enhanced lead excretion in the kidneys.

Spirulina is a cyanobacterium classified as blue green algae. It has also been found to reduce the toxic load of lead [7, 8] by its chelating an antioxidant properties.

The purpose of our work was to:

- investigate the antagonistic interactions between lead and the essential elements zinc and iron in fish tissue exposed to experimental lead intoxication.
- to assess the efficacy of *Coriandrum sativum* (cilantro) and spirulina (*Arthrospira platensis*) powder on the

decreasing the lead antagonism to iron and zinc.

2. Materials and methods

Biological material

Prussian carp fingerlings (*Carassius gibelio* Bloch) were chosen as test species.

Fresh fish samples were caught from a local farm and gravimetrically selected. Thus, individuals of 22-25 g weight were acclimated for two weeks under laboratory conditions prior to experiment, removing those with suspicions on their health. Then, 120 Prussian carp's fingerlings were transferred in 4 separate glass aquariums equipped with aeration system and a 60 L capacity at a stocking density of 30 fishes per aquarium.

Experimental design

The control and test organisms were randomly distributed in test solutions according to doses of lead, cilantro, spirulina, and their combinations as follows: the control ones were maintained in Pb-free freshwater, the others three groups receiving 75 ppm Pb into the water as $Pb(NO_3)_2 \times \frac{1}{2}H_2O$ (E1), 75 ppm Pb into water+2% lyophilized cilantro in feed (E2), (75 ppm Pb into the water+2% lyophilized spirulina in feed (E3), respectively.

Chemicals

Fish in groups E1, E2, and E3 were exposed for 21 days to chronic lead intoxication as $Pb(NO_3)_2 \times \frac{1}{2}H_2O$ in concentration of 75 ppm from a stock solution. The sub lethal treatment was calculated from percentage mortalities of fish as described by Veena et al. [9].

Fish nutrition

Fish were fed twice a day with commercial dry pellets. Freeze-dried cilantro and chlorella's incorporation (Figure 1 and 2) in fish's feed has involved the following steps:

- bringing the dry pellets to fine size particles;
- adding the lyophilized material in the proportion determined by the experimental protocol setting the daily feed amount needed by a specimen, according to the calculation model in fish proposed by Oprea and Georgescu [10];
- wetting and homogenization of the mixture obtained;

- regranulation and drying of the final pallets (Figure 3).

Spirulina (*Arthrospira platensis*) powder was produced by Bucharest Medical Laboratories; coriander (Figure 2) powders was obtained by lyophilization to Interdisciplinary Training and

Research Platform "Sustainable Ecological Agriculture, and Food Safety" from USAMVB Timisoara, Romania. Lyophilization was carried out at a temperature of -53°C and a pressure of 0.05 mTorr.



Figure 1. Lyophilized spirulina and transformed into fine powder



Figure 2. Lyophilized cilantro and transformed into fine powder



Figure 3. Final regranulated pallets designed to Prussian carps' feeding

Monitoring of physical-chemical parameters of the water in the aquariums

Physical-chemical indicators of water were daily measured throughout the exposure: water temperature and dissolved oxygen with a movable oxygen-meter with water resisting microprocessor Hanna HI 9145; pH, NO_2^- , NO_3^- , pH, hardness of water with a photoFlex colorimeter and a Germany termatest kit.

The controlling conditions included (T: $19-21^{\circ}\text{C}$; pH: 7.44-8; total hardness: 120 mg/l; DO: 7.5-8 mg $\text{O}_2 \text{ L}^{-1}$; nitrites: $<0.3 \text{ mg/l}$; nitrates: $<12.5 \text{ mg/l}$).

The medium was weekly renewed by adding $\text{Pb}(\text{NO}_3)_2$ from a stock solution to maintain the constant concentration of Pb and a 12 h light/12 h dark photoperiod was assured.

Sampled and Assay

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.

The analytical determination in order to establish tissue zinc and iron concentrations, using atomic absorption spectrometer (AAS – VARIAN) was performed.

Statistical Analysis

Statistical analysis was performed using SPSS IBM 22 software. Data were reported as a significant level of $p < 0.05$. Testing differences

between means was realized by ANOVA completed with post-hoc Tukey test.

3. Results and discussion

Pb administration (75 ppm in water) for 21 days resulted in the mobilization of trace elements Fe and Zn from tissues, which were recognize as essential nutrients for the development of biological functions in the organism.

Zinc is widely distributed in the animal body, its presence being essential for the catalytic activity of more than 170 enzymes. It is involved in the synthesis of nucleic acids [2, 11] and serves as a specific cofactor for several metalloenzymes, including dehydrogenase, aldolase, peptidases and phosphatases. In addition, zinc is connected with the metabolism of prostaglandins [2].

Gills and gastrointestinal tract are responsible for absorption of this element from water environment, gills having a major role in Pb excretion as well [12].

In our work, Pb has mobilized Zn regardless of considered tissue ($p < 0.001$), the most affected being intestine (Zn level diminish from 62.24 mg kg^{-1} to 15.2 mg kg^{-1}), kidney (Zn level fall from 58.45 mg kg^{-1} to 20.22 mg kg^{-1}), heart (Zn level diminishes from 46.2 mg kg^{-1} to 12.18 mg kg^{-1}) and gonads (Zn level fall from 46, 28, mg kg^{-1} to 18.51 mg kg^{-1} for ovaries and from 47.01 mg kg^{-1} to 16.06 mg kg^{-1} for testicle) (Table 1, Figure 4).

Table 1. Statistical indices and statistical significance regarding Zn tissue level

Tissue	Number	Sum	Average	Variance	SD
Zn (mg kg ⁻¹ wet weight)					
Gill	4	96.7	24.175	18.7803	3.75303
Muscle	4	30.79	7.6975	4.302425	1.79633
Skin+ scales	4	68.8	17.2	34.319	5.07339
Intestine	4	170.68	42.67	428.51	17.92714
Liver	4	86.51	21.6275	72.00949	7.34895
Heart	4	109.16	27.29	202.859	12.33468
Brain	4	56.58	14.145	17.85257	3.65916
Ovaries	4	106.48	26.62	195.0763	12.09575
Testis	4	122.2	30.55	189.6769	11.92718
Kidney	4	179.45	44.8625	285.1479	14.62398
Group					
C	10	356.72	35.672	348.0123	17.69777
Pb	10	133.37	13.337	25.28667	18.41435
Pb+cilantro	10	241.33	24.133	142.9241	16.26958
Pb+spirulina	10	295.93	29.593	213.7813	15.78267
Source of variation					p
Between tissues					p<0.05
Between treatments					p<0.001

The presence in high level of lead in the intestine leads to the decrease of absorption and Zn bioavailability implicitly. Also, Pb may disrupt the Zn metabolism, liver involving actively in this regulation: when Zn intake decreases, also metallothionein synthesis in hepatocytes is reduced; less zinc will accumulate in hepatocytes.

Hence, the Pb - description as an antimetabolite of Zn. Pb occupies the binding site of Zn to metallothionein, reduces renal leucinaminopeptidase activity in vivo, activity that may be responsible for proteinuria, or competes with Zn for occupy the binding sites on enzymes involved in gametogenesis.

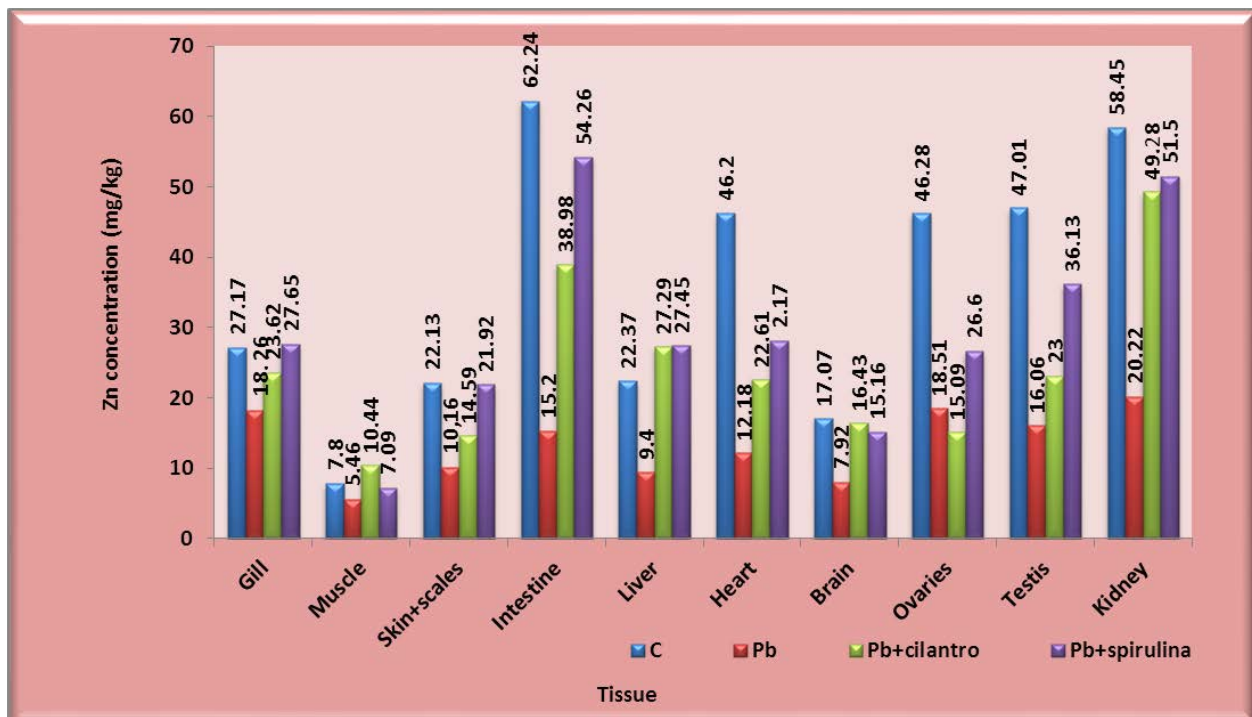


Figure 4. Graphical representation of Zn (mg kg⁻¹) in various tissues in *Carassius gibelio*

Cilantro and spirulina lyophilisate administered additionally in feed, by their active principles have immobilized, Pb, diminishing its antagonistic effect against Zn. As a result, the tissue level of Zn, has registered relatively low levels compared with control group. But when it was compared with poisoned group, Zn tissue level has increased, reaching values of 38.98 mg kg⁻¹ and 54.26 mg kg⁻¹ for the intestine, 49.28 mg kg⁻¹ and 51.5 mg kg⁻¹ for kidney, 23.00 mg kg⁻¹, 36.13 mg kg⁻¹ for testicle and 26.6 mg kg⁻¹ respectively for the ovary.

Regard to Fe-absorption in fish, although gill epithelia is permeable in some degree to iron, intestinal mucosa is considered to be the major site of absorption [2, 13].

In liver, heart, intestine and kidney specimens of the control group, we found the highest concentrations of Fe (85.51 mg kg⁻¹, 81.78 mg kg⁻¹, 77.82 mg kg⁻¹ and 68.59 mg kg⁻¹ respectively) (Table 2, Figure 5). After the exposure to the toxic metal, the iron has decreased significantly, reaching 15.62 mg kg⁻¹, 59.12 mg kg⁻¹, 20.14 mg kg⁻¹ and 33.65 mg kg⁻¹, respectively.

In this situation, Pb exerts its inhibitory effect on the absorption and metabolism of Fe by its possible binding of ferritin on the intestinal mucosa and that of transferrin (involved in the Fe-transport) in the blood, proteins that seem to have high affinity for Pb. As such, the liver deposit of Fe reduces and the Fe concentration in the post hepatic blood as well. Besides, heme production necessary to erythrocyte hemoglobin synthesis will be affected by and anemia will install, as Shalaby (2001) [14] and Karuppasamy et al. (2005) [15] found. Moreover in human individuals, the anemia installation is one of Pb intoxication-associated symptoms [16] (Peraza et al., 1998).

Cilantro and spirulina powder has diminished the antagonism shown to Fe, especially regarding the tissues sampled from individuals receiving additional spirulina in feed, the recorded values approaching and even exceeding those of the control specimens: 95.85 mg kg⁻¹, 72.75 mg kg⁻¹, 78.84 mg kg⁻¹ and 58.38 mg kg⁻¹. But cilantro powder fails to counteract the Fe mobilization from cardiac tissue, while spirulina powder - from testicle.

Table 2. Statistical indices and statistical significance regarding Fe tissue level

Tissue	Number	Sum	Average	Variance	SD
Fe (mg kg ⁻¹ wet weight)					
Gill	4	112.8	28.2	196.2506	12.13210
Muscle	4	122.14	30.535	364.1518	16.52616
Skin+ scales	4	43.82	10.955	19.75983	3.84966
Intestine	4	239.39	59.8475	755.9776	23.81141
Liver	4	279.23	69.8075	1338.627	31.68549
Heart	4	240.9	60.225	570.0218	20.67647
Brain	4	109.723	27.43075	398.5854	17.28986
Ovaries	4	109.97	27.4925	286.5186	14.65909
Testis	4	104.8	26.2	267.2379	14.15728
Kidney	4	218.97	54.7425	220.9641	12.87335
Group					
C	10	585.63	58.563	441.3055	19.92925
Pb	10	212.12	21.212	234.5095	20.29722
Pb+cilantro	10	362.64	36.264	543.3489	21.44939
Pb+spirulina	10	421.353	42.1353	1007.257	13.82252
Spource of variation					p
Between tissues					p<0.05
Between treatments					p<0.001

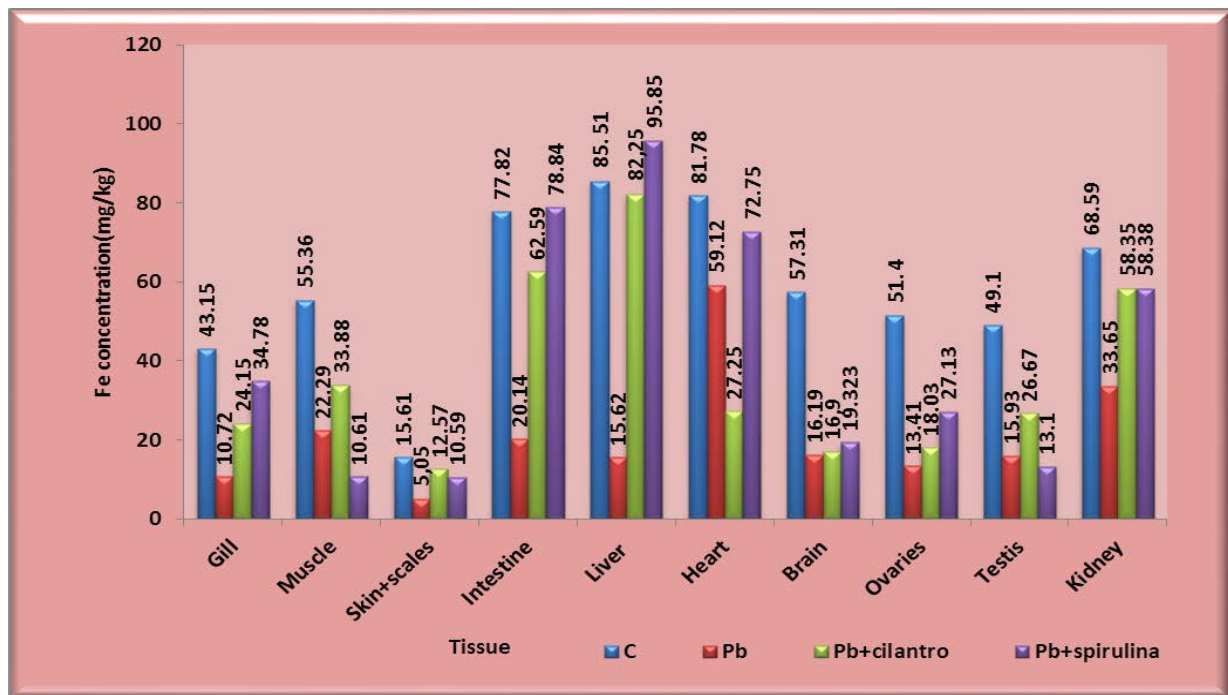


Figure 5. Graphical representation of Fe (mg kg^{-1}) in various tissues in *Carassius gibelio*

4. Conclusions

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

- Pb taken on various ways from aquatic environment, mobilizes essential elements like Fe and Zinc from fish tissues
- The active principles from lyophilized cilantro and spirulina diminish the Pb antagonistic effect on the essential minerals Fe and Zn
- At the same time, they improve the subsequent capture and distribution of micronutrients in feed, as evidenced by their values in the analyzed tissues.

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