

Ameliorative Effect of Active Principles from Cilantro and Spirulina Powder on Lead Antagonism to Calcium and Magnesium in *Carassius gibelio* Bloch

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Abstract

The aim of this work was to investigate the calcium and magnesium tissue distribution in selected organs of Prussian carp specimens before and after experimental lead intoxication, with and without cilantro and spirulina powder addition in fish diet.

The study involved 120 individuals with similar biometric measurements, caught from a local fish farm and equally divided in four experimental groups, as follows: C group (without treatment), E1 group (75 ppm Pb into water as $\text{Pb}(\text{NO}_3)_2 \times \frac{1}{2}\text{H}_2\text{O}$), E2 group (75 ppm Pb into water+2% lyophilized cilantro in feed), E3 group (75 ppm Pb into water+2% lyophilized spirulina in feed). Tissue samples (gills, muscle myotomers–epaxial, heart, skin and scales, intestine, liver, brain, gonads, kidney) were collected at the end of a 21 days experimental period.

Atomic absorption measurements of the analytes were carried out with a AAS-VARIAN model.

Data analyses were performed using a SPSS IBM 22 software package. Significance of differences in the means content of calcium and magnesium in fish tissues were statistically analysed by ANOVA completed with post-hoc Tukey's test.

The calcium and magnesium tissue concentrations were significantly reduced by sublethal lead-induced poisoning ($p < 0.001$). But, coriander and spirulina powder were able to attenuate much of the lead antagonistic effect with respect to calcium absorption and bioavailability, spirulina being more effective in the case of the gill and renal tissue, while coriander in the case of cardiac tissue. Instead, spirulina powder has improved magnesium distribution in all analyzed tissues.

Keywords: calcium, cilantro, fish, lead toxicity, magnesium, natural chelators, spirulina

1. Introduction

Industrial, economic and demographic development generates large quantities of waste with different chemical composition and variable content of toxic heavy metals. Poor management

of these wastes leads to environmental pollution and degradation.

Surface water contamination with heavy metals has disastrous effects on the environment for a long time.

Toxic pollutants such as heavy metals exert their effects on the various organs and systems of aquatic organisms, the effect being specific to the substance concerned.

Their toxicity is increased by the tendency of bioaccumulation in aquatic organisms [1] along the food chain, producing insidiously serious pathological alterations [2-4].

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Lead is second on the 2017 ATSDR Priority List of Hazardous Substances [5].

Toxicity of lead on fish has been reported previously by many authors including Taweel et al., (2012), [6], Mobarak et al., (2011) [7] or Bothaina et al. (2012) [8].

Synthetic amino acids (the most common one is EDTA-Ethylene Diamine Tetracetic Acid) pull accumulated heavy metals (usually lead or mercury) out of the body. But EDTA also can remove necessary minerals from the body in addition to lead [9].

There are natural solutions which actually facilitate lead removal [10-12]. Two of the best natural chelators are cilantro and micro-alga spirulina. Cilantro's lead removing ability was discovered by Dr. Yoshiaki Omura at the Heart Disease Research Foundation in New York in the 1990s [13]. The researchers concluded that cilantro contained substances that display a suppressive effect on lead deposition [14, 15].

Spirulina displays a metalloprotective effect acting as a cleansing and detoxifying phytonutrient against the toxic substances [16-18].

The aim of this work was to investigate the calcium and magnesium tissue distribution in selected organs of Prussian carp specimens before and after experimental lead intoxication, with and without cilantro and spirulina powder addition, in fish diet.

2. Materials and methods

Materials and methods are the same with those presented in a previous paper [19], except the minerals which were investigated, in this case, calcium and magnesium.

3. Results and discussion

Calcium is a bio mineral playing a vital role in the physiology and biochemistry of organisms by its involve in skeleton building, in muscle contraction, blood clotting, transmission of nervous impulses, maintenance of cellular integrity and acid-base balance, and activation of several important enzymatic systems [20]. Calcium uptake is ensured by gill epithelium, fins and oral epithelium, even if the gills are considered the major locus of calcium metabolism

regulation [21]. The calcium deposit (99%) of the fish body is almost entirely, present in bones, teeth and scales while extra skeletal calcium is widely distributed in organs and tissues (1%). In extracellular fluids and in the circulatory system, ionic calcium plays a major role in muscular activity and osmoregulation. Most fish are able to uptake dissolved calcium directly from the aquatic environment through gills, although natural food sources are rich in calcium.

In the control group, tissues showing high concentrations of Ca were: gills ($6222.14 \text{ mg kg}^{-1}$), cord ($2817.59 \text{ mg kg}^{-1}$), skin+scales ($3015.69 \text{ mg kg}^{-1}$), intestine ($1684.97 \text{ mg kg}^{-1}$) and kidney ($1675.72 \text{ mg kg}^{-1}$) (Figure 1).

There is a significant decrease in Ca tissue ($p < 0.001$) (Figure 1 and Table 1), in the specimens subjected to chronic lead poisoning, which proves the antagonistic relationship between the two elements.

Like all minerals having entry control, Ca has a carrier protein that takes it from the absorption mucosa and whose synthesis is vitamin D-dependent. Pb affects the homeostatic control of Ca tissue level mimicing calcium and substituting it in many major cell processes that depend on calcium and competing with it for the same carrier protein binding site [22]. It also enhances renal secretion of Ca due to its toxic effect on renal tubules generally, accompanied by disruption of Ca reabsorption.

Cilantro and spirulina powder manage to attenuate much of the Pb antagonistic effect with respect to the tissue Ca absorption and bioavailability, spirulina being more effective in gill ($5617.8 \text{ mg kg}^{-1}$) and renal (1290 mg kg^{-1}) tissue and cilantro in the case of cardiac tissue (2800 mg kg^{-1}).

Magnesium is an important cofactor in numerous enzymatic reactions of intermediate metabolism. The targeted enzymes are phosphokinase, thiokinase, phosphatase, pyrophosphatase, and amino acyl synthetase. It is also necessary in bone metabolism, osmoregulation and neuromuscular transmission [23]. Most of the Mg in fish (60% in carp) is distributed to the skeleton. Magnesium is slightly above 0.6% of bone ash compared to Ca (30%) and P (15%). The 40% difference is divided between muscle, viscera (where it plays a vital role as an enzyme cofactor and as an important structural component of the cell membrane) and extracellular fluid [24].

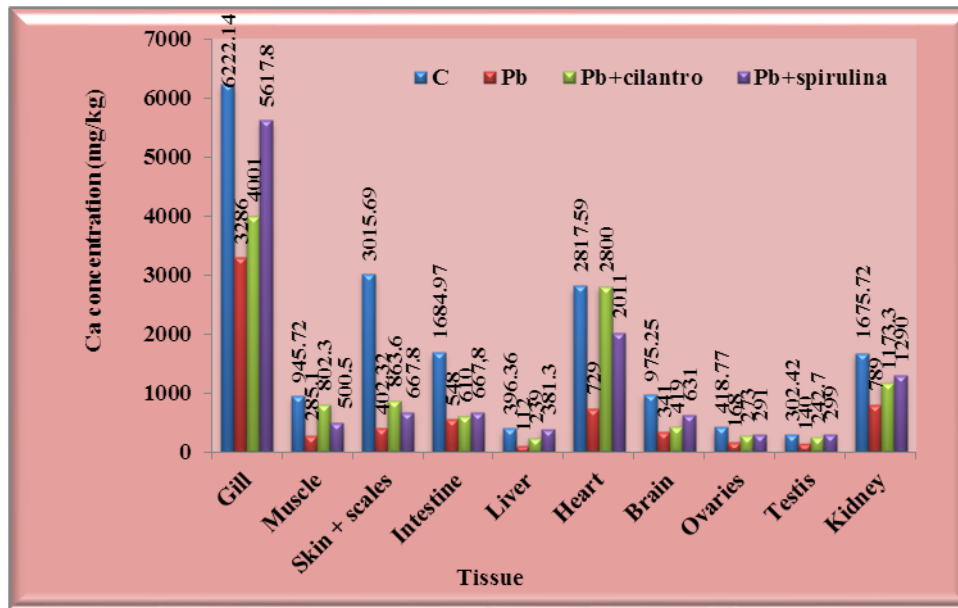


Figure 1. Graphical representation of Ca (mg kg⁻¹) in various tissues in *Carassius gibelio* Bloch

Table 1. Statistical significance regarding Ca tissue level

Tissue	Number
Gill	4
Muscle	4
Skin+scales	4
Intestine	4
Liver	4
Heart	4
Brain	4
Ovaries	4
Testis	4
Kidney	4
Group	
C	10
Pb	10
Pb+cilantro	10
Pb+spirulina	10
Source of variance	<i>p</i>
Between tissues	p<0.05
Between groups	p<0.001

Mg is a bio element present in all animal tissues. In the control group it was found to be higher amounts in gills (948.42 mg kg⁻¹), intestine (573.455 mg kg⁻¹), and kidney (488.18 mg kg⁻¹),

while in poisoned individuals with Pb, a severe decrease (p<0.001) of its concentration in all analyzed tissues (Figure 2 and Table 2) was recorded.

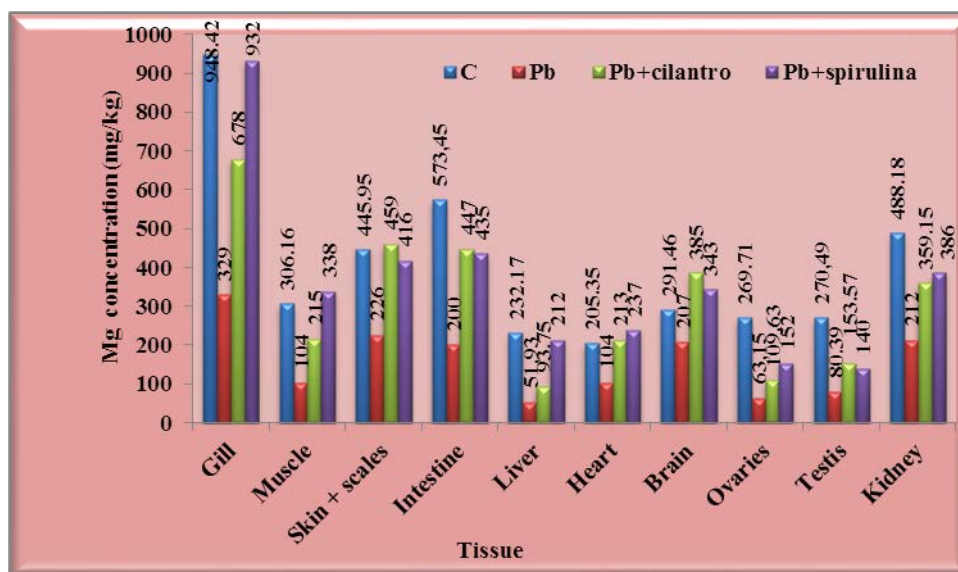


Figure 2. Graphical representation of Mg(mg kg⁻¹) in various tissues in *Carassius gibelio* Bloch

Table 2. Statistical significance regarding Mg tissue level

Tissue	Number
Gill	4
Muscle	4
Skin+scales	4
Intestine	4
Liver	4
Heart	4
Brain	4
Ovaries	4
Testis	4
Kidney	4
Group	
C	10
Pb	10
Pb+cilantro	10
Pb+spirulina	10
Source of variance	<i>p</i>
Between tissues	p<0.05
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As was the case for the calcium, the antagonistic effect of Pb on Mg translates into alteration of the absorption, transport and, apparently, of the tubular reabsorption. And because Mg is cofactors in hundreds of enzymatic reactions, it is possible that, due to a significant decrease in tissue bioavailability, the enzyme activity that operates with this element is severely disturbed. Besides their major role in diminishing the antagonistic effects of Pb on Mg tissue content, active principles from spirulina and cilantro powder improve the subsequent capture and distribution of Mg from fish diet, as evidenced by its higher

values measured in muscle tissue (338 mg kg⁻¹), and brain (343 mg kg⁻¹).

4. Conclusions

This study revealed that: 1) the two studied metals concentrations are diminished in the tissues of the intoxicated fish, Pb antagonizing them; 2) active principles from cilantro and spirulina powder act as powerful Pb chelating agents, attenuating its deleterious effect on tissue concentrations of essential bio-elements Ca and Mg.

References

- Sivakumar, R., Li, X., Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China, *Toxicology Reports*, 5, 2018, 288-295.
- Nicula, M., Dumitrescu, G., Pacala, N., Tulcan, C., Dragomirescu, M., Ștef, L., Dronca, D., Gherbon, A., Ciochina, L. P.; Garlic, cilantro and chlorella's effect on gill histoarchitecture changes in Cd-intoxicated Prussian carp (*Carassius gibelio*), *Romanian Biotechnological Letters*, 2016, 21(5), 11932-11940.
- Nicula, M., Dumitrescu, G., Pacala, N., Ștef, L., Tulcan, C., Dragomirescu, M., Dronca, D., Gherbon, A., Ciochina-Petculescu, L., Garlic, cilantro and chlorella's effect on liver histoarchitecture changes in Cd-intoxicated Prussian carp (*Carassius gibelio*), *Romanian Biotechnological Letters Journal*, 21(6), 2016, 12062-12070.
- Jia, Y., Wang, L., Qu, Z., Wang, C., Yang, Z., Effects on heavy metal accumulation in freshwater fishes: species, tissues, and sizes, *Environ Sci Pollut Res Int.*, 2017 24(10), 9379-9386.
- ATSDR (Agency for Toxic Substances and Disease Registry), ATSDR's Substance Priority List, 2017, <https://www.atsdr.cdc.gov/spl/>.
- Taweel, A. K. A., Shuhaimi-Othman M., Ahmad, A. K., Analysis of heavy metal concentrations in Tilapia fish (*Oreochromis niloticus*) from four selected markets in Selangor, Peninsular Malaysia. *J. Biol. Sci.*, 2012, 12, 138-145.
- Mobarak, Y. M. S., Sharaf, M. M., Lead acetate-induced histopathological changes in the gills and digestive system of silver sailfin molly (*Poecilia latipinna*), *Int. J. Zool. Res.*, 2011, 7, 1-18.
- Bothaina, M. K., Imam, A. A. M., Ahmed, S. A. H., Abdel Salam, M. I. O., Effect of Lead Nitrate on the Liver of the Cichlid Fish (*Oreochromis niloticus*): A Light Microscope Study. *Pakistan Journal of Biological Sciences*, 2012, 15, 854-862.
- Nicula, M., Gergen, I., Harmanescu, M., Banatean-Dunea, I., Marcu, A., Simiz, E., Polen, T. Lunca, M., Assessing the impact of EDTA chelating effect on some macro- and microminerals in Prussian carp (*Carassius Gibelio*) tissues, *Animal Sci. and Biotech.*, 2011, 44 (2), 40-44.
- Nicula, M., Pacala, N., Ștef, L., Pet, I., Dronca, D., Gherbon, A., Ahmadi, M., In vivo Experiments of Natural Products Protection of Antagonistic Effects of Lead on Iron, *Rev.Chim.*, 68(12), 2017, 2747-2751.
- Nicula, M., Păcală, N., Radulov, I., Ahmadi, M., Dronca, D., Gherbon, A., The Effect of Active Principles of Lyophilized Garlic and Chlorella on Reduction of Tissue Bioaccumulation and Lead Antagonism to Zinc in *Carassius gibelio*, *Rev.Chim.*, 2017, 68(9), 2006-2009.
- Nicula, M., Pacala, N., Ștef, L., Pet, I., Dronca, D., Ahmadi, M., Gherbon, A., Garlic and Chlorella Biomodulate Lead Toxicity on Manganese Homeostasis in *Carassius gibelio* Bloch, *Rev.Chim.*, 2018, 69(4), 986-989.
- Milner, C., The two best herbs for lead poisoning, 2016, https://www.theepochtimes.com/the-two-best-herbs-for-lead-poisoning_1957074.html
- Aga, M., Iwaki, K., Ueda, Y., Ushio, S., Masaki, N., Fukuda, S., Kimoto, T., Ikeda, M., Kurimoto, M., Preventive effect of Coriandrum sativum (*Chinese parsley*) on localized lead deposition in ICR mice, *J Ethnopharmacol.*, 2001, 77(2-3), 203-208.
- Winarti, S., Pertiwi, C. N., Hanani A. Z., Mujamil, S. I, Putra, K. A., Herlambang, K. C., Beneficial of Coriander Leaves (*Coriandrum sativum* L.) to Reduce Heavy Metals Contamination in Rod Shellfish, *Journal of Physics: Conference Series*, 953, conference 1.
- Bangeppagari, M., Gooty, J. M., Tirado, J. O., Mariadoss, S., Thangaswamy, S., Maddela, N. R., Ortiz, D. R., Therapeutic Efficiency of spirulina against Lead Acetate Toxicity on the Fresh Water Fish Labeo rohita, *American Journal of Life Sciences*, 2014, 2(6), 389-394.
- Upasani, C., Balaraman, R., Effect of vitamin E, vitamin C and spirulina on the levels of membrane bound enzymes and lipids in some organs of rats exposed to lead, *Indian J. Pharmacol.*, 2001, 33, 185-191.
- Hegazi, S. M., Fadl, S. E, Gad, D. M., El-Keredy, M. S., Abeerd, Effect of spirulina incorporated in diets of *Tilapia nilotica* fresh water fish exposed to lead toxicity , Conference: First Conference of Chemistry and Environmental Health, Animal Health Res. Inst. Agri. Res. Center, Egypt., 2014.
- Nicula, M., Pacala, N., Ștef, L., Pet, I. Bencsik, I. Radulov I., Iancu, T., Tulcan, C., Dragomirescu, M., Dronca, D., Berbecea, A., Dumitrescu, G., Simiz E., Ahmadi, M., Marcu, A., The Effect of Active Principles of Cilantro and Spirulina Powder on Lead Antagonism to Zinc and Iron in *Carassius gibelio*, *Animal Science and Biotechnologies*, 2017, 50(1), 265-271.
- Nutrient Requirements of Fish, National Academy Press Washington, D.C. 1993.
- Simkiss, K., Calcium metabolism of fish in relation to ageing. In: *The Proceedings of an International Symposium of the Ageing of Fish*, T. B. Bagenal, ed. Old Woking, U. K.: Unwin Brothers, 1974.
- Toxicity of lead, <http://users.ge.ibf.cnr.it/marchetti/piombo/toxicityoflead.html>
- Houston, A. H., Erythrocyte magnesium in freshwater fishes. *Magnesium*, PubMed, 1985, 4, 106-128.
- CHOW, K. W., SCHELL, W. R., The minerals. In: *FAO. Lectures presented at the FAO/UNDP Training Course in fish feed technology*, ACDP/REP/80/11, 198.