The Effect of Active Principles of Lyophilized Garlic and Chlorella on Reduction of Tissue Bioaccumulation and Lead Antagonism to Zinc in *Carassius gibelio*

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In living organisms lead is classified as potential toxic metal, and in high concentration can produce intoxication with the alteration of some vital organs, especially liver and kidney. In aquatic environment lead can be absorbed by fishes and other organisms, with different distribution in various tissues. Our aim of experiment was to verify and demonstrate the protective effect of lyophilized garlic and chlorella against bioaccumulation of lead in fishes living in aquatic environment deliberated polluted with lead. Thus, lyophilized garlic and chlorella administrated as supplements in fodder for fishes (Carassius gibelio) diminished the antagonistic effect of lead against zinc in all tested tissues: liver, kidney, heart, brain, ovary, testis, muscles myotome – epaxial, skin – with scales, gills, and intestine.

Keywords: fish, lead intoxication, garlic, chlorella, zinc, tissue

For aquatic ecosystems, heavy metals are one of the five major types of toxic pollutants [1], having all the necessary characteristics to be included in this classification: tend to accumulate in living organisms [2, 3], have chemical stability, are poorly biodegradable, readily and easy solubilized [4].

In physiological amounts, some heavy metals are essential nutrients for metabolism; but once exceeded these values, they can bio-accumulate and reach toxic levels. The non-essential minerals does not hold important biological role and are typically highly toxic, their concentration tissue leading to poisoning, reduced fertility, major tissue damage and organ dysfunction.

Lead is the most prevalent heavy metal contaminant in the aquatic environment and also in the industrialized areas, being three times more concentrated than in preindustrial areas [5, 6]. Synthetic chelation or natural agent chelation of heavy metals in the cells is the common treatment for heavy metal toxicity [7]. There are advantages of synthetic chelating agents (such as DMPS, DMSA, EDTA, etc. with strong ability to capture and then removing metals from the body), there are also disadvantages for their use [8]. Natural chelates presented in vegetal sources, if used properly, have proved to be highly effective for removing heavy metals from the body [9-13].

Even for mother and their new born baby the lead and selenium from fetal or baby serum is significant influenced by the lead and selenium concentration in mother serum. Concentration of some bio-metals or potential toxic metals is also influenced by the environment and the life style of the mother, one of the negative factors is mother direct or passive smoking [14].

The bioaccumulation factors for metals are depending on the plant and animal variety of the ecosystem. Along the trophic chain some plant accumulates different metals from water and soil, and if these plants become the main feed for animals, the animals can accumulate also the metals in their tissues. Thus, there are different studies that aimed to find special bio-indicators for soil, water and generally ecosystem quality [15-17].

Our research objectives were focused towards highlighting the lead toxicity on some tissues from *Carassius gibelio Bloch* (carp – Cyprinidae family) after induction of chronic intoxication with lead nitrate, hydrated with ½ water molecules – $Pb(NO_3)_2$ x ½H_2O. Also, by this exploratory study we wanted to research the detoxifying potential of the active principles consist of lyophilized garlic and lyophilized chlorella in experimental contamination with lead, to *Carassius gibelio*, and the effect of garlic and chlorella on antagonism of lead to zinc.

Experimental part

For choosing the experimental organisms we took into consideration the accessibility and ecologically representativeness criteria, and for these reasons we chose *Carassius gibelio Bloch* (known as Prussian carp, silver Prussian carp or Gibel carp), family Cyprinidae, genus *Carassius*.

After gravimetric measurements, we selected 120 individual fishes, weighing 22-25g each, which were *accommodated* in 4 aquariums of 60L capacity (i.e. 30 fishes/aquarium), with aeration systems. The experimental fishes were acclimatized for 2 weeks in laboratory conditions, eliminating the individuals suspicious regarding health.

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During the accommodation and experimental period the fishes were fed 2 times daily with granulated commercial product, ensuring 12 h light regime : 12 h darkness regime, giving a special attention to maintain relatively constant values of water quality indices (temperature, pH, dissolved oxygen, hardness, nitrates, nitrites), avoiding the situation where the water quality changes may potentiate the toxic action of the tested substances.

Thus, the water temperature and dissolved oxygen were measured using Oxygen processor waterproof Hanna HI 9145, and for the determination of *p*H, NO_2^-, NO_3^-, hardness of the water, we used the TERMATEST kit.

Lead as the investigated metal – was administered at a concentration of 75ppm (in water) circulation of lead in water being provided by an aquarium compressor. The dose we used was determined based on the average lethal concentration for fish LC50 for a period of 3 weeks [18]. Once a week we refreshed the water in each aquarium and to the amount of replaced water we added the correspondent concentration of lead according to the experiment protocol.

The tested products were:

- lead as $Pb(NO_3)_2$ x ½H_2O (Merck), administered as 75ppm in water;
- lyophilized chlorella (Medica Laboratories Bucharest, *Chlorella pyrenoidosa* species – fig. 1) integrated in feed in proportion of 2%;
- lyophilized garlic (fig. 2) laboratory made, and making the powder of lyophilized garlic (fig. 3), then incorporated in feed in proportion of 2%.

Fig. 1. Lyophilized Chlorella

Fig.2. Lyophilized garlic

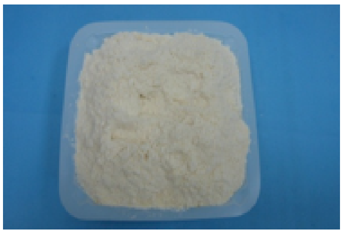

Fig.3. Powder of lyophilized garlic

Incorporating the lyophilized garlic and chlorella, incorporated in fish feed involved the following steps:

- bringing the fish feed granules to very fine particle size;
- incorporating the lyophilized material in the proportion determined by the experimental protocol and establish the quantity of feed necessary to every individual fish/day, according to the model of a fish feed ration proposed by Oprea and Georgescu [19];
- homogenization and wetting the obtained mixture feed;
- re-granulation of the feed for experimental administration;
- drying the obtained granules.

At the end of the 21 days of experiment we sampled the biological tissue (gills, muscles myotome – epaxial, heart, skin and scales, intestine – sampling after 12 hours starving for emptying the intestine, liver, brain, gonads of both sexes, kidney). The sampling was performed after fish euthanasia (using clove oil) of both control and the two experimental groups subjected to lead action – with and without the addition of lyophilized product in the feed. After sampling the tissues we performed the analytical determination in order to determine zinc concentrations, using atomic absorption spectrometer (AAS – VARIAN). Data statistical evaluation an interpretation was performed using ANOVA program, with two factors out replication, taking into account two factors: the tested tissue and the feed adopted treatment.

Results and discussions

Administration (in water) for 21 days of lead (in 75ppm dose) resulted in the mobilization of trace elements from tissues, which were recognize as essential nutrients for the development of biological functions in the organism [20, 21].

Zinc is widespread in the animal organism, his presence being essential for catalytic activity of over 170 enzymes. Gills and gastrointestinal tract are responsible for concentrating the zinc from tissues, gills having a major role in its excretion [22].

Our experimental results showed zinc is mobilized by lead, regardless of tissue (p <0.001), the most affected tissue being intestine, kidney, heart and gonads. The levels of zinc in intestine decreases from 62.24mg/kg to 15.2mg/kg), in kidney decreases from 58.45mg/kg to 20.22mg/kg), in heart also decreases from 46.2mg/kg to 12.18mg/kg, and in gonads we demonstrated a decrease from 46.28mg/kg to 18.51mg/kg in the case of the ovary, and for testicle we registered a decreases of 47.01mg/kg to 16.06mg/kg, fig. 4).

Statistical evaluation of our result data were evaluated using ANOVA statistical program, and in table 1 are presented the statistical significance having in view lead intoxication for the tested tissue and the fishes' fodder: un-supplemented feed for control (C) and lead intoxicated group (Pb); garlic supplemented feed for lead intoxicated group (Pb + garlic); chlorella supplemented feed for lead intoxicated group (Pb + chlorella).

By the literature data the initial effects of heavy metal contamination are evident first in the tissue and cell, before

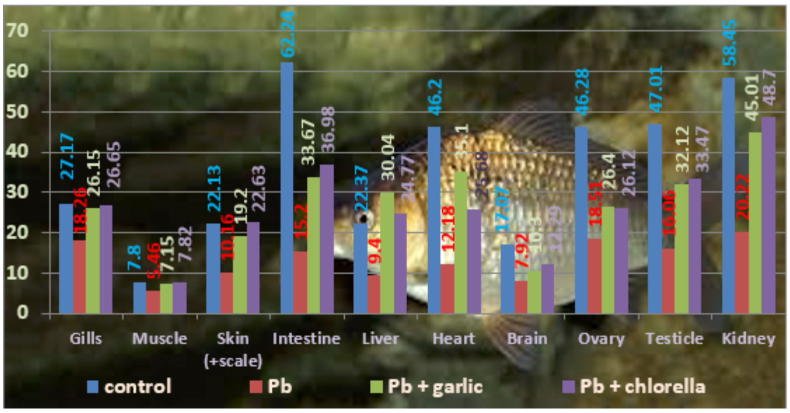

Fig. 4. Graphical representation of zinc (mg/Kg) in various tissues in fish (*Carassius gibelio*)

TISSUE	Number	Sum	Average	Variance	SD
Zn (mg/kg wet weight)					
Gills	4	98.23	24.5575	17.79942	3.65371
Muscle	4	28.23	7.0575	1.231092	0.96089
Skin (and scales)	4	74.12	18.53	33.42527	5.00689
Intestine	4	148.09	37.0225	374.4616	16.75847
Liver	4	86.58	21.645	76.90243	7.59453
Heart	4	119.16	29.79	208.1628	12.49488
Brain	4	47.58	11.895	15.09377	3.36457
Ovary	4	117.31	29.3275	141.088	10.28669
Testicle	4	128.66	32.165	160.4834	10.97099
Kidney	4	172.38	43.095	264.7083	14.09011
Group					
C (Control)	10	356.72	35.672	348.0123	17.69777
Pb	10	133.37	13.337	25.28667	18.41435
Pb + Garlic	10	265.14	26.514	133.5987	16.26958
Pb + Chlorella	10	265,11	26,511	135,6584	15,78267
Source of variance					*p*
Between tissues					$p < 0.001$
Between treatments					$p < 0.05$

Table 1
STATISTICAL INDICES AND THEIR STATISTICAL SIGNIFICATION FOR ZINC CONCENTRATION IN TISSUES

the identification of significant changes related to the behavior of the fish in their aquatic environment.

The presence of high concentration of lead in intestine, leads to diminishes zinc absorption and bioavailability. Also, lead can disturb the zinc metabolism, liver actively acting in this process. If zinc intake decreases, this slows the synthesis of metallothionein in hepatocytes and as a second result, in hepatocytes is accumulating less zinc. Thus, we can say that lead acts as an antagonist of zinc. Also, lead occupies the binding site of zinc on metallothionein, reduces the kidney leucine-aminopeptidase activity in vivo, process that may be responsible for proteinuria, or may compete with zinc for binding sites on the enzymes involved in gametogenesis.

The lyophilized garlic and chlorella administrated as supplement in fishes' fodder, by its active principles, immobilizes lead, diminishing the antagonistic effect on zinc. Thereby, tissue concentrations of zinc it remains relatively low compared to individuals form the control group, but increase compared to the intoxicated group, reaching values of 33.67mg/kg and 36.98mg/kg for gut, 45.01mg/kg and 45.01mg/kg for kidney, 32.12mg/kg and 32.12mg/kg in testis, 26.40mg/kg and 26.12mg/kg for ovary.

Conclusions

Regarding to our experimental results we can formulate the conclusion that lead intoxication can be attenuated by some nutritional supplements added to fodder formulas in fish.

Lead absorbed from various aquatic resources manifests cumulative effect in tissues of *Carassius gibelio*. Also, lead acts as antagonist for zinc in living organisms, but fish intoxicated with lead and fed with supplemented fodder can diminish the antagonistic effect of lead against zinc.

Garlic and chlorella lyophilized supplement added to fish fodder present significant improvement for zinc concentration in experimental tissues compared to control tissues.

The quantum of zinc in liver, heart, and ovary was higher for lead intoxicated fishes fed with garlic supplements (Pb + Garlic group), but for gills, muscles, skin (and scales), intestine, brain, testis and kidney zinc concentration was higher for lead intoxicated fish fed with chlorella supplements (Pb + Chlorella group).

Natural nutritional supplements can potent or diminish the antagonistic or synergic effect of a mineral against some other minerals, fact that can be used to potent the toxic effect of a potential metal contamination in aquatic environment.

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