Histological Aspects Regarding the Antioxidant and Chelating Potential of Chlorella in Experimental Pb Contamination of *Carassius gibelio* Bloch species. II

Marioara Nicula[1], Gabi Dumitrescu[1], Diana Berzovan[1], Ioan Peț[1], Lavinia Ștef[1], Tiberiu Iancu[1], Isidora Radulov[1], Monica Dragomirescu[1], Camelia Tulcan[1], Dorel Dronca[1], Mirela Ahmadi[1], Liliana Petculescu-Ciochină[1], Adela Marcu[1], Silvia Erina[1*]

[1]Banat University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, 300645-Timisoara, Aradului, 119, Romania

Abstract

The purpose of this study was to highlight the histological aspect of some tissue of Prussian carp's specimens, subjected to sub lethal Pb intoxication with and without chlorella dietary supplementation. 90 Prussian carps, with weighing between 10 and 12 g were divided according to the following treatments for 21 days: C (without treatment), E1 (75 ppm Pb into water), E2 (75 ppm Pb into water+2% lyophilized chlorella in feed). Samples of gill, intestine, liver and kidney were removed at the end of experimental period and analyzed in light microscopy. QuickPHOTO Micro 2.2 software has been used for the histological study. Our findings are: obvious histological alterations have been observed in experimental Pb-poisoned group; active chlorella compounds cannot counteract the effects of Pb-induced oxidative stress on intestinal tissue; chlorella has been shown to be a weak Pb chelator on liver tissue; chlorella lyophilisate reduces to a small extent the toxic effects of the contaminant in the kidneys; respiratory barrier slightly affected in the group receiving chlorella in feed.

Keywords: chlorella, experimental lead intoxication, fish, gill, histological lesions, kidney, liver, intestine

1. Introduction

The development of industry and agriculture promotes the rapid growth of metal pollution of the environment. Aquatic pollution with heavy metals is usually represented by high levels of Hg, Cr, Pb, Cd, Cu, Zn, Ni etc. in the aquatic system [1, 2]. Anthropogenic activities such as heavy metal wastewater discharge, is an important cause of heavy metal pollution [3, 4]. Waters residues mainly result from mining activities, mineral testing, metallurgy, galvanizing, chemical industry, paper industry.

* Corresponding author: Silvia Erina
Tel. +40745507418
Email: silviaerina@animalsci-tm.ro

There are microalgae that can remove heavy metals from wastewater [5-7] and promote heavy metal detoxification [8-10].

Fish in the upper position of the food chain are likely to accumulate larger amounts of metals and develop more severe biological consequences. In the same time, fish are important "vehicles" in the process of contaminants agents transferring to the human consumers, which may indicate the potential exposure to pollutants [11].

Histopathological studies performed in the laboratory have shown that they can be effective tools for direct detection of toxic effects of chemical compounds on the internal organs of fish [12, 13].

A wide range of histopathological changes in fish exposed to pollution with heavy metals have been studied [14-16].

The purpose of this study was to highlight the histological aspect of some tissue of Prussian carp's specimens, subjected to sub lethal lead intoxication with and without chlorella dietary supplementation.

2. Materials and methods

Materials and methods are the same with those presented in a previous paper [12] except: the heavy metal investigated, in this case - Pb in dose of 75 ppm as $Pb(NO_3)_2$ x $½H_2O_2$, feed supplementation (2% chlorella lyophilisate) and the tissue sampled - in this paper - gill, kidney, liver, intestine [17].

3. Results and discussion

Gill

The morphological aspects observed in the optical microscopy suggest the presence of an unaffected respiratory barrier in the case of individuals belonging to the control group. As can be seen in figure 1, primary gill lamellae are uniformly distributed on one side and on the other side of the arches; secondary gill lamellae arranged bilaterally on primary gill lamellae are dense and relatively uniform in size, on the largest surface of them (Figure 1).

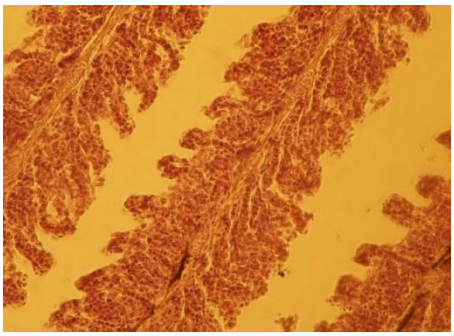

Figure 1. Gill-C-detail
normal appearance; (H-E staining, x10)

In the present study, the microscopic analysis of the sections made on the gill tissue of the Pb-intoxicated specimens, highlights the disorganization of the secondary gill lamellae architecture: hypertrophic lamellar epithelial cells, with vacuolated cytoplasm; alteration of the conjunctival structure in the lamellar axis; gill lamella fusion (Figure 2). Moreover, massive epithelial and conjunctival degenerations appear, with the shortening to the destruction of the secondary gill lamellae and vascular congestion (Figure 3).

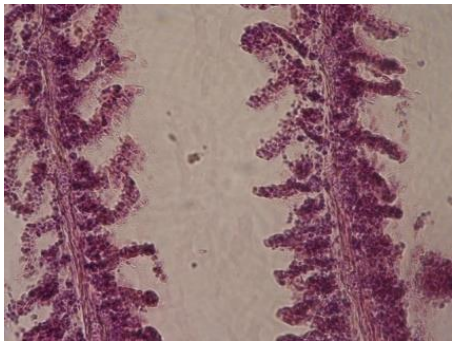

Figure 2. Gill-E1
hypertrophic lamellar epithelium;
vacuolated cytoplasm
(H-E staining, 10x)

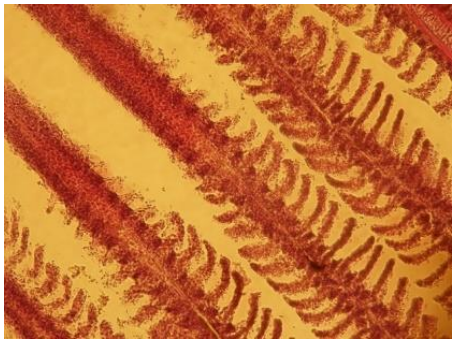

Figure 3. Gill-E1
epithelial and conjunctival degenerations;
shortening / destruction of the secondary gill lamellae;
vascular congestion (H-E staining, 10x)

In individuals who received 2% chlorella in feed, the secondary gill lamellae, although thin, retain their uniform appearance; vascular hypertrophies appear at the top of the primary gill lamellae; hyperplastic territories are also present (Figure 4 and Figure 5). The morphological aspects observed in these specimens suggest the presence of a slightly affected respiratory barrier, a consequence of epithelial cell hyperplasia.

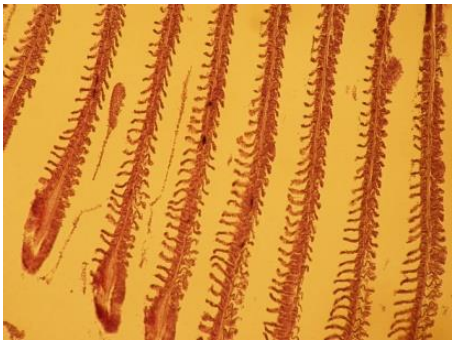

Figure 4. Gill-E3
thin secondary gill lamellae;
vascular hypertrophies; hyperplastic territories
(H-E staining, 10x)

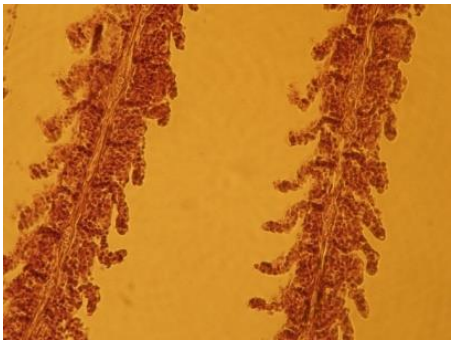

Figure 5. Gill-E3-detail
(H-E staining, 40x)

Intestine

In figure 6, the normal histoarchitecture of the intestinal wall belonging to the control group is observed. The intestinal mucosa has dense, rectangular villi. The intestinal epithelium is intact consisting of enterocytes and goblet cells; villous chorion that incorporates blood capillaries has normal appearance (Figure 7).

Figure 6. Intestine-C**;** cross section
(H-E stain, 10x)

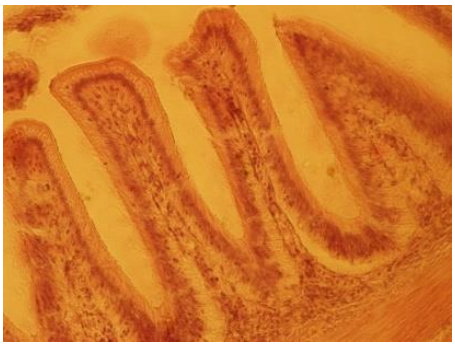

Figure 7. Intestine-C; cross section
blood capillaries
(H-E stain, 40x)

The intestinal mucosa of individuals intoxicated with Pb has widened-looking villi, reduced in height (Figure 8). Also, villous territories with altered epithelium are evident, until the disappearance of cells and microvilli. Leukocyte infiltrative cells are present in the chorion (Figure 9). In individuals of the LE2 group, the intestinal mucosa has high villi, with a rectangular or triangular appearance. Frequent epithelial alterations are observed at the apical portion of the villi, but also microvilli alteration (Figure 10 and Figure 11), which illustrates the intensification of the destructive action of the contaminant on the intestinal epithelium; active chlorella compounds cannot counteract in this case the effects of Pb-induced oxidative stress on the intestinal tissue.

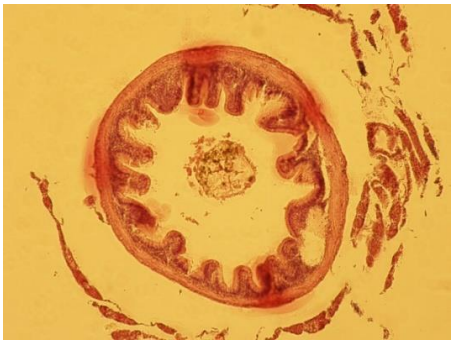

Figure 8. Intestine-E1; cross section
widened-looking villi, reduced in height
(H-E stain, 10x)

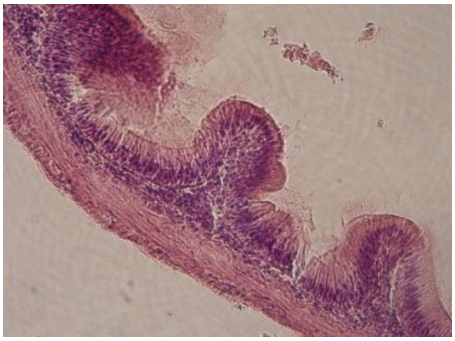

Figure 9. Intestine-E1; cross section
altered epithelium
cells and microvilli disappearance;
leukocyte infiltrative cells
(H-E stain, 10x)

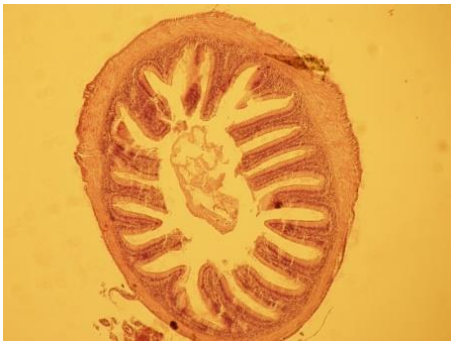

Figure 10. Intestine-E2
cross section
apical epithelial alterations;
microvilli alteration
(H-E stain, 10x)

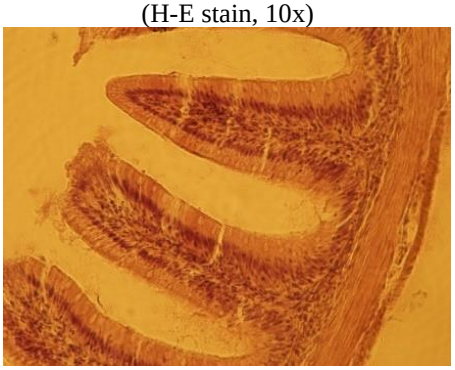

Figure 11. Intestine-E2-detail
cross section
(H-E stain, 10x)

Liver

Microscopic examination of the sections made on the liver of the control group specimens reveals that the liver parenchyma consists of liver cells arranged in hepatocyte cords with an ordered appearance. Polygonal liver cells have a spherical nucleus with an obvious nucleolus and heterochromatin on the inner face of the nuclear envelope. Sinusoidal capillaries with a normal appearance are arranged between the hepatocyte cords (Figure 12).

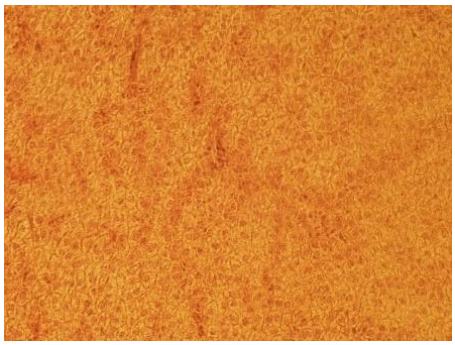

Figure 12. C-Liver
(H-E stain, 10x)

The liver of individuals subjected to chronic lead poisoning loses its normal architecture; disorganized aspect of the hepatocyte cords (Figure 13) is observed on the sections made on this organ, the hepatocytes are hypertrophic, with clear vacuolated cytoplasm (hepatocellular necrosis are considered biomarkers for the evaluation of chronic intoxications in fish) and pycnotic nucleus (morphology characteristic of cell death); in them, pericentrolubular necrosis with hypertrophies of sinusoidal capillaries is frequently manifested (Figure 13). Figure 14 shows the disorganized appearance of hepatocyte cords and centrolobular vein hypertrophy, and pericentrolobular hepatocyte necrosis with sinusoidal hypertrophies in Figure 15.

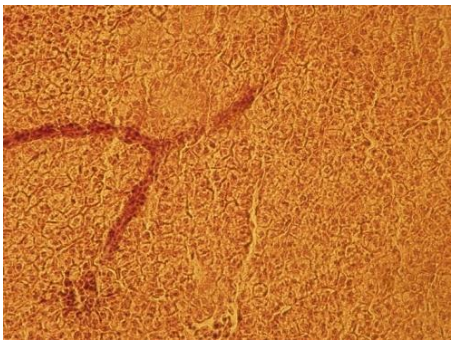

Figure 13. Liver-E1
hypertrophic hepatocytes; clear vacuolated cytoplasm; pycnotic nucleus;
hypertrophies of sinusoidal capillaries
(H-E stain, 10x)

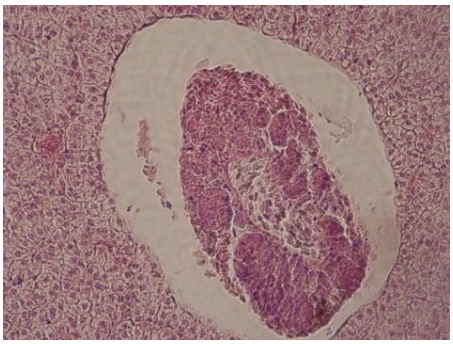

Figure 14. Liver-E1
centrolobular vein hypertrophy
(H-E stain, 40x)

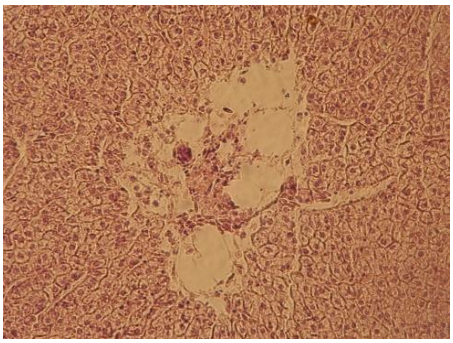

Figure 15. Liver-E1
pericentrolobular hepatocyte necrosis; sinusoidal hypertrophies
(H-E stain, 10x)

Histopathological changes in individuals intoxicated with Pb are also present in those who received additional chlorella in the daily diet, namely: hepatocyte cords are disordered, hepatocytes have clear cytoplasm and pycnotic nucleus, arranged pericentrolobular, centrolobular and interportal vascular hypertrophies (Figure 16 and Figure 17).

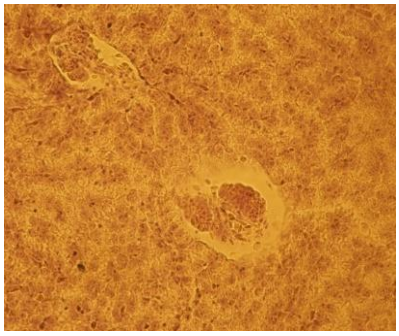

Figure 16. Liver-LE1
disordered hepatocyte cords;
clear cytoplasm and pycnotic nucleus;
centrolobular and interportal vascular hypertrophies
(H-E stain, 10x)

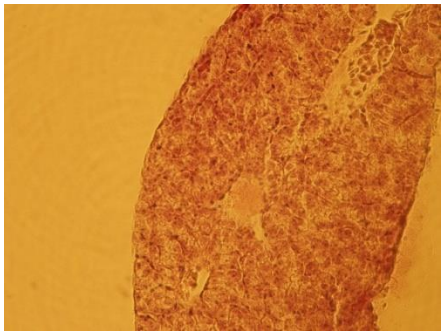

Figure 17. Liver-E1-detail
(H-E stain, 40x)

Kidney

Renal corpuscles are homogeneous in size and appearance in individuals of the control group.

The urinary tubes are lined with cubic or prismatic nephrotic epithelium, provided at the apical pole with brush border cell (Figure 18).

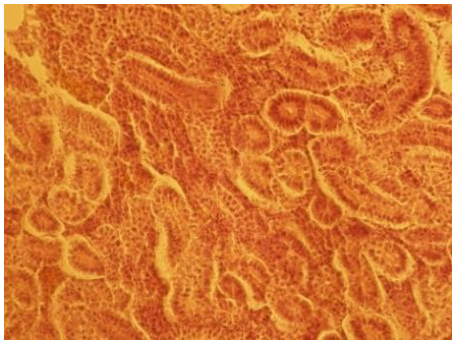

Figure 18. Kidney-C
normal structure of the renal parenchyma (H-E stain, 40x)

The sections made on the kidneys of experimentally contaminated individuals, highlight a series of changes in the renal parenchyma, but also in the stroma: the renal corpuscle is hypertrophic, with lesions in the glomerular capillaries and enlarged capsular space; peritubular edema (Figure 19), nephrocyte and interstitial necrosis (Figure 20 and Figure 21).

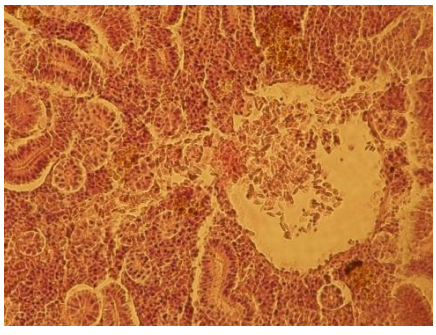

Figure 19. Kidney-E1
hypertrophic renal corpuscle;
lesions in the glomerular capillaries;
enlarged capsular space; peritubular edema
(H-E stain, 10x)

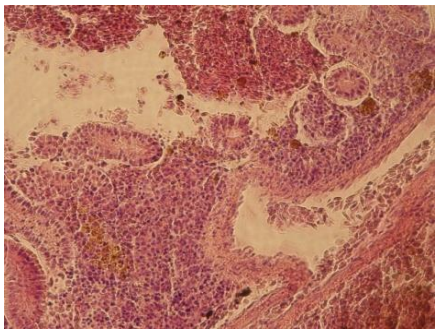

Figure 20. Kidney- E1
peritubular edema; vascular hypertrophy;
nephrocyte and interstitial necrosis (H-E stain, 10x)

The renal glomeruli appear compressed, the Bowman's capsular space widens and the peritubular edema persist in the individuals

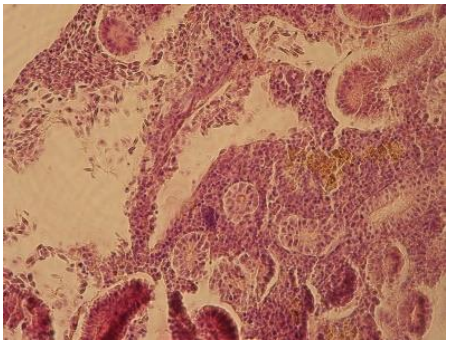

Figure 21. Kidney-E1
nephrocyte degenerative processes;
cytoplasm vacuolation
(H-E stain, 10x)

of the experimental group E2; instead, hemorrhagic lesions are reduced, and in some areas are even absent (Figure 22).

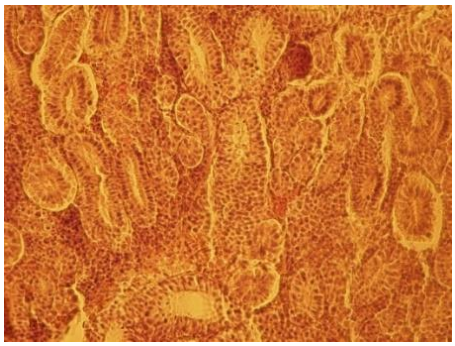

Figure 22. Kidney- E2
peritubular edema
(H-E stain, 10x)

As such, chlorella lyophilisate, through its active principles, manages only to some extent to diminish the toxic effects of the contaminant.

Our histopathological results are in agreement with those of Khalesi et al., (2017) [18], Saili et al., 2019 [19], Mladin et al., (2021) [20].

Pollutants enter the body of fish through their gills and manifest the toxic effect primarily in the gill epithelium. Therefore, the gills are the first organs that show changes in the action of physico-chemical stressors [21].

Gastrointestinal tract is another major route of entry fish body of a wide variety of toxic substances from the feed or the aquatic environment in which they live.

Even if the liver has the ability to degrade toxic compounds, its regulatory mechanisms can be overcome by increasing the concentration of these compounds, resulting in structural alterations [22, 23].

The predominant function of the kidney is excretion [24] and is considered one of a target organ in heavy metal toxicity [21]. But when its excretory ability is overwhelmed, kidney has capability to reabsorb and concentrate divalent ions and large amounts of heavy metals such as cadmium (Cd), iron (Fe), lead (Pb), zinc (Zn), etc. [25, 26], leading to impair their activity.

Chlorella detoxifying ability is due to its unique cell wall extremely resistant to breakage and the material associated with it, sporopollenin (a carotene-like polymer that is resistant to degradation) (Atkinson et al., 1972) [27].

Sporopollein is more effective in binding toxic metals than any other natural substance [28]. In the same time, it seems that porphyrins (active part of chlorella's chlorophyll have a strong heavy metal binding effect [28], helping detoxification.

4. Conclusions

- histological alterations found in tissue of fish expose to sub lethal dose of lead nitrate, even in those belonging to specimens receiving chlorella dietary supplementation, conclude that, Pb has bioaccumulated in gills, intestine, liver and kidney, causing physiological changes in their structure and function,
- respiratory barrier slightly affected by lead intoxication in the group receiving chlorella in feed;
- active chlorella compounds cannot counteract the effects of Pb-induced oxidative stress on intestinal tissue;
- chlorella has been shown to be a weak Pb chelator on liver tissue;
- chlorella lyophilisate reduces to a small extent the toxic effects of the contaminant in the kidneys;

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