

# MULLET CHRONIC EXPOSURE TO Cu AND Zn: LIVER HISTOPATHOLOGICAL ASSESSMENT AND METABOLIC ENZYME ACTIVITIES



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## Abstract:

The relation between Cu and Zn in liver of wild mullets (*Liza saliens*) living in a polluted coastal lagoon and their hepatic lesions as well as plasma enzyme activities are pointed out in this study. Heterogeneous parenchyma (HP) was the most prevalent hepatic alteration (34%). Fish exhibiting this lesion were also the ones with higher Cu concentrations suggesting that this pathology could depend on Cu and Zn accumulation in liver. Compared to mullets collected in the sea, lagoon mullets showed higher AST activity indicating a change in protein metabolism.

## Materials and methods:

❖ Mulletts, from lagoon and from sea (14 Km northwards), were captured by gill net, anaesthetised and the blood drawn from their caudal vessels. Livers from lagoon fish were randomly taken and immediately fixed in 10% buffered formalin.

❖ Livers were routinely prepared for histological observations, and sections stained with hematoxylin and eosin (HE). Alterations were classified in general diagnostic categories: non-neoplastic lesions (NNL); foci of necrosis (FN); granuloma and melanomacrophage centres (MMC) [4]. Additionally an extensive area of heterogeneous parenchyma (HP), composed of hepatocytes with different spectrum of vacuolization, was classified.

❖ Plasma was obtained by centrifugation (5 min, 10,000 g, 4 °C) and enzyme activities were colorimetric determined using an automatic dry chemistry system analyzer (Clinical Diagnostics, VITROS 950).

❖ Cu and Zn levels in livers of the lagoon fish were analysed elsewhere [3].

❖ Mann-Whitney U-Test was used to test significant differences between metals levels in fish with and without a specific liver lesion, and to test differences between plasma parameters in fish from the two environments. The significance level ( $\alpha$ ) was set at 0.05.

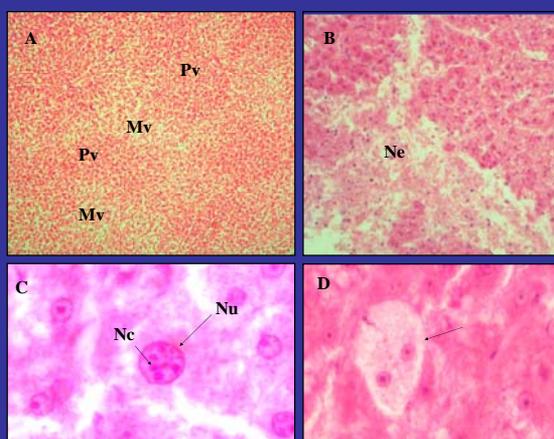
## Results & Discussions

Lagoon mullets with 23 to 49 cm had high metal concentrations in liver ranging from 51 to 547 mgCu·kg<sup>-1</sup> d.w. and 26 to 190 mgZn·kg<sup>-1</sup> d.w. [3].

Livers with no cell alterations (43%) showed a similar structure to that described in *Liza saliens* juveniles living in non polluted environments [5].

## Introduction:

Toxicological studies of acute exposure have shown that the concentration of pollutants can change enzyme activities and often directly induce cell damage in specific organs [1]. Histopathology used as indicator of environmental stress provides a definitive biological end-point of historical exposure [2]. High metal concentrations in the liver of *Liza saliens* from a portuguese coastal lagoon were reported [3] and their relation with fish hepatic alterations and plasma enzyme activities Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were examined.



**Fig 1** - Histological sections of *Liza saliens* liver: (A) extensive heterogeneous parenchyma with large spectrum of vacuolization (Mv) and poorly vacuolated (Pv) 40x; (B) large area of lytic necrosis (Ne) 100x; (C) nuclei hypertrofia (Nu) and simultaneous nucleolus (Nc) 250x; (D) focal hepatocyte death (arrow) 250x;

The prevalence of finding 1 type of lesion (34%) was higher than the prevalence of 2, 3 or 4 different types of lesions (Tab 1).

Heterogeneous parenchyma (HP), was the most prevalent hepatic alteration (34%), characterized by areas of heterogeneous parenchyma vacuolization, consisting of hepatocytes either with a large spectrum of vacuolization contributing to parenchyma heterogeneity, or poorly vacuolated showing moderate to strong basophilia (Tab 1, Fig 1A).

Livers showing HP had significantly higher metal contents accumulated (337±128 mg·Cu and 107±31 mg·Zn) when compared with fish without HP (223 ±133 mg·Cu and 79±29 mg·Zn) suggesting that this could be a response to Cu and Zn accumulation in this organ.

Some FNs spread over large areas (Tab 1, Fig 1B). NNLs included cell and nuclear polymorphism, focal hepato and nuclear hypertrophy (Fig 1C) and frequent focal cell death (Fig 1D). These lesions reflect the stress condition of the habitat however fish seem to be able to develop compensating enzyme mechanisms to cope with it.

It is generally accepted that changes in enzyme activity in plasma is a sensitive indicator of even minor cellular damage, since the level of these enzymes will be higher than normal [6].

The AST levels were higher in mullet from lagoon than in mullet from sea (Tab 2), an indication of change in protein metabolism, rather than a consequence of cell permeability and integrity alterations, since ALT and ALP activities were not affected.

## Conclusions:

- ❖ The lesion Heterogeneous Parenchyma was associated with Cu and Zn accumulation in fish liver.
- ❖ Increase of AST activity in mullets from the lagoon indicates metabolic changes as an adaptive response to metal exposure.
- ❖ Mulletts from the lagoon seem to be well adapted to stress conditions since no pre-neoplastic nor neoplastic lesions were found.

**Tab. 1** - Prevalence of the number and categories of hepatic alterations in the lagoon mullets (N = 35).

| Parameters        |                           | Prevalence (%) |
|-------------------|---------------------------|----------------|
| Number of lesions | 4                         | 3              |
|                   | 3                         | 6              |
|                   | 2                         | 14             |
|                   | 1                         | 34             |
|                   | 0                         | 43             |
| Categories        | Heterogenous parenchyma   | 34             |
|                   | Foci of necrosis          | 31             |
|                   | Granulomas                | 14             |
|                   | Non-neoplastic lesions    | 9              |
|                   | Melanomacrophagic centres | 6              |

Number of lesions = number of different types of liver lesions found.  
 Prevalence = % of fish presenting a number of lesions or a specific lesion.

**Tab. 2** - Plasma parameters in mullets of similar size sampled in the lagoon and in the sea.

| Variables               | Lagoon          | Sea         |
|-------------------------|-----------------|-------------|
| Length (cm)             | 36.9 ± 4.6      | 32.4 ± 6.4  |
| ALP (UL <sup>-1</sup> ) | 33.3 ± 17.5     | 25.1 ± 18.4 |
| ALT (UL <sup>-1</sup> ) | 4.3 ± 2.6       | 12.6 ± 9.2  |
| AST (UL <sup>-1</sup> ) | 263.2 ± 137.4** | 95.7 ± 44.9 |

N=9; Data are presented as mean ±sd \*\*<0.01.

## References

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