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# Elemental characterization of injuries in fish liver

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ABSTRACT

Fish liver is the primary organ related to the biotransformation of organic contaminants and metals. This organ is very sensitive to organic and inorganic contaminants and can accumulate them in higher amounts relative to the environment itself and to other organs. One of the most common injuries is a histopathology called melanomacrophage centers, characterized as modifications of the cellular structure of the tissue and usually accompanied by pigmented cells. The aim of this study is to apply micro-PIXE in combination with conventional PIXE as a qualitative and quantitative analysis of elements to characterize histopathologies in the liver of fishes. Micro-PIXE results show that there is a higher concentration of Fe, P, K, Ti, Cr, Ni, Cu and Zn in melanomacrophage centers. On healthy tissue, the distribution of these elements is homogeneous. In cases where the histopathological study showed injuries without melanomacrophage centers, the micro-PIXE analysis showed much smaller clusters with higher concentrations of these elements, suggesting the presence of melanomacrophage centers which are too small to be detected by histopathological conventional methods. Broad PIXE results showed that the concentration of Si, Cl, K, Ti, Fe and Cu are directly related to the presence of melanomacrophage centers. Moreover, it could be observed that the concentration of Cr, Mn and Ni is directly related to the injuries but not to melanomacrophage centers.



## 1. Introduction

Coastal environments have been transformed by human activity, especially with increasing addition of substances in marine and estuarine environments characterizing contamination [1]. Fish can be used as monitors of environmental quality, both, in terms of the biodiversity of species in the community [2], as well as the healthiness of individuals [3]. Fish biomarkers are biological responses measured on organization levels below individual, namely cells, body fluids, tissues or organs. In this way, they provide an indirect measure of exposure and/or effects of contaminants [4].

Histopathological biomarkers can detect modifications or injuries in several tissues and organs and constitute a very important tool because they present a relatively fast response to the sublethal stressors. These biomarkers are highly sensitive and ecologically relevant, but have low specificities to contaminants, since certain injuries may be derived from different sources. Therefore, the diagnostics is not reliable and useful for resolving questions about the causative agents of injuries [1].

The liver is considered the first organ to identify histological biomarkers due to its central role in many metabolic functions like protein synthesis, gall secretion, metabolites accumulation, intoxification and detoxification. These functions make the liver bioaccumulate higher levels of toxic substances up to several orders of magnitude higher than the environment itself or even other organs [1]. One of the tissue's most characteristic alterations are the melanomacrophage centers (MMCs), commonly associate with chronic inflammatory injuries and cell degeneration. The MMCs are clusters of monocytes containing melanosomes, lysosomes, and an accumulation of ceroid and lipofuscin. Several studies have demonstrated positive correlations between liver injuries and bottom-living fishes exposed to contaminated areas [5].

As histological studies are time-consuming and present low specificity, the present study was focused on using a new tool to evaluate modifications on micro-structures of fish liver with micro-PIXE along with broad PIXE as techniques to determine the presence of heavy metals on MMCs and their respective distribution on the liver tissue [6].

The micro-PIXE technique, among other ion beam analysis techniques, has some potentialities for environmental studies. Particularly in this case, besides the non-destructive character of the

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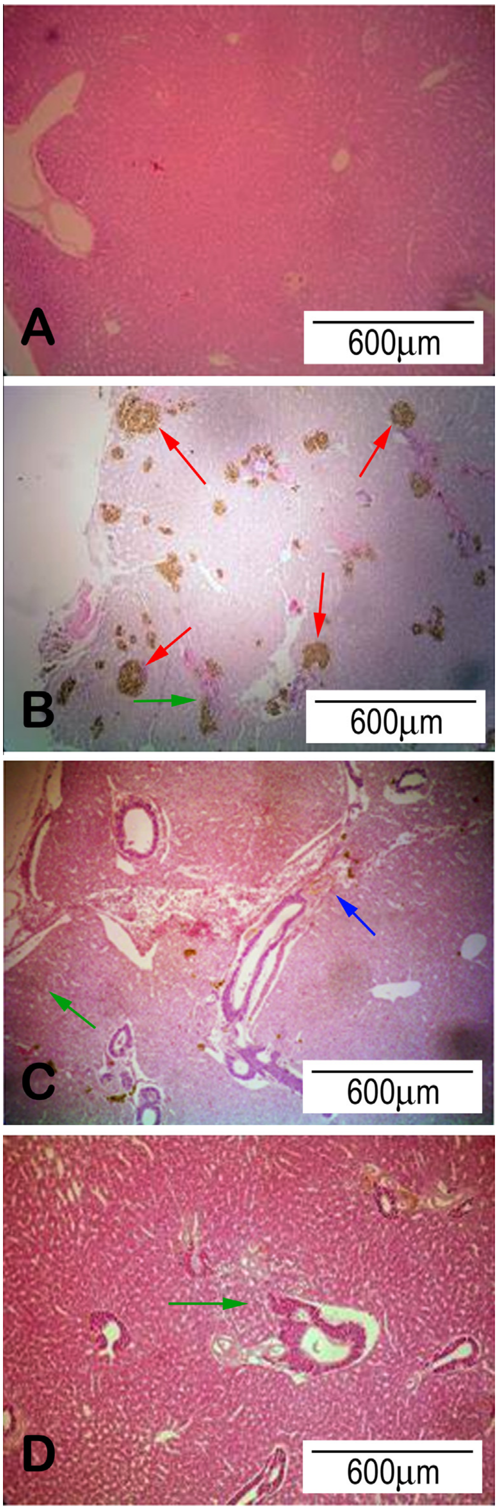


**Fig. 1.** Photomicrography of fish liver. Panels A–D represent liver tissues in the following conditions: (A) healthy liver with its morphology preserved and without apparent injuries or MMCs; (B) liver with injuries with MMCs (red arrows), showing disorganization and small focus steatosis (green arrows); (C) liver with injuries and no apparent MMCs, showing small focus of necrosis (blue arrows) and steatosis (green arrows); (D) liver with injuries but without apparent MMCs, showing focus of steatosis (green arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

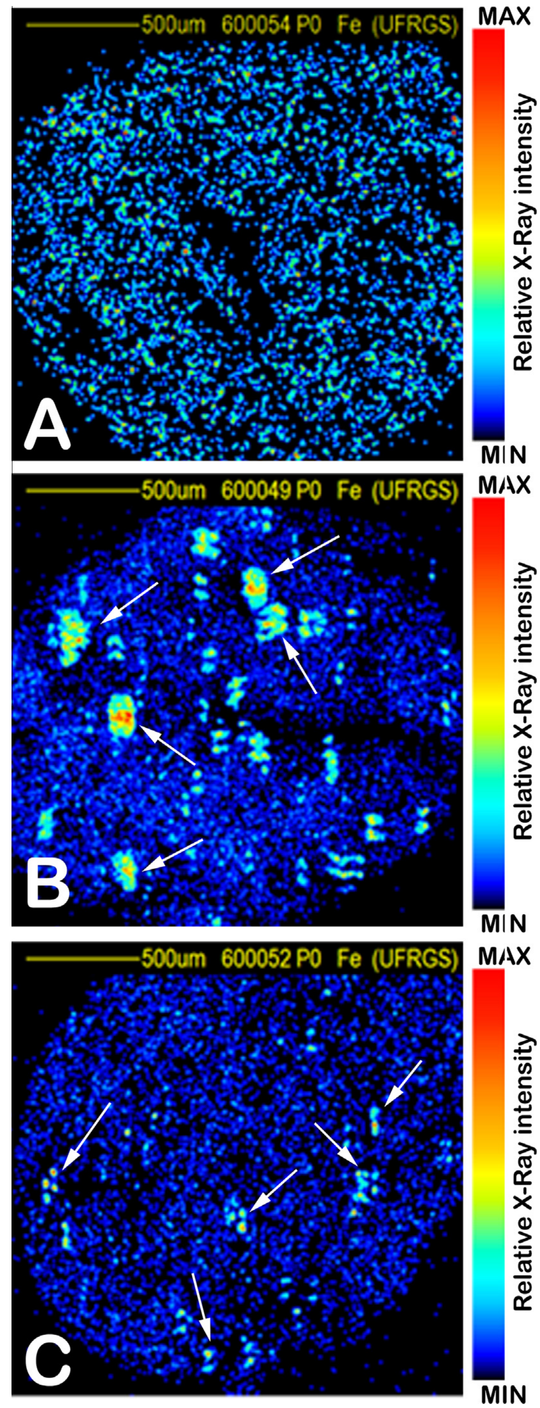


**Fig. 2.** Elemental maps showing the distribution of Fe in the liver tissues. The scanned area is $1500 \times 1500\ \mu m^2$. The panels show liver tissues in the following condition: (A) healthy liver; (B) liver with injuries and MMCs; (C) liver with injuries but no apparent MMCs as revealed by the histopathological study. The MMCs both in panels B and C are indicated with white arrows.

analysis, it can indicate in which way some elements are distributed along the modifications. Moreover, the microscopic characteristic of the microprobe together with the capability of scanning certain areas of the sample open the possibility of correlating different elements by producing maps of each one of them [7]. This enables a deep analysis on which elements are present on healthy tissues, on MMCs and on other organ alterations.

## 2. Materials and methods

Specimens used in this study were caught in the Santos-São Vicente estuarine system, located in a tropical area of Southeastern