Determination of mercury fraction linked to protein of muscle tissue and liver of fish from Amazon region-Brazil

José Cavalcante S. Vieira¹ (PG), Bruna Cavecci¹ (PG), João V. Quiroz² (PG), Alis C. Bittarello² (PG), Camila P. Braga¹ (PG), Cilene C. F. Padiha¹ (PG), Luis Fabricio Zara³ (PG), Pedro M. Padiha¹ (PG)*. *padiha@ibb.unesp.br

¹Institute of Bioscience, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil
²College of Veterinary and Animal Science, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil
³University of Brasilia, College of Planaltina, Distrito Federal, Brazil

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Introduction

The fresh water fish fauna of the Amazon is considered the richest in the world, with more than 1,300 described species. This diversity of fish from different regions with in the Brazilian Amazon is still difficult to assess because the existing studies from several research institutes are sparse. Commercial fishing is concentrated on approximately 40 exploited species, and among the most commonly caught are the following: dourada (Brachyplatystoma rousseauxi); pacu (Mylossoma sp, sp Myleus); jaraqui (Semaprochilodus spp.); tucunaré (Cichla spp.) and filhote (Brachyplatystoma filamentosum). These species represent 75% of the production of freshwater fish from Amazon region.

Results and Discussion

This study utilized metalloproteomic techniques to characterize mercury-bound proteins in muscle and liver tissue of dourada and tucunaré collected in the AHE JIRAU - Madeira River basin-Brazil. The proteome of the muscle and liver tissue was obtained after two steps of fractional precipitation and the steps of the separating the proteins by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Mercury was identified and quantified in the protein spots by graphite furnace atomic absorption spectrometry (GFAAS) after acid mineralization in an ultrasound bath. GFAAS determinations indicated the presence of mercury in the protein spots with a molecular weight less than 20 kDa. The mercury concentrations in the spots in which this protein fraction was present were in the range of 11.40 – 35.10 μg kg⁻¹. Based on the mercury concentrations, it was possible to estimate that the protein spots contained approximately 1–3 mercury atoms per protein molecule and also presented stoichiometric ratios for mercury atoms per protein molecules. These protein spots were characterized by electrospray ionization tandem mass spectrometry (ESI-MS/MS) after trypsin digestion. From a total of 20 analyzed spots, 4 proteins showing Hg biomarker characteristics were identified:

<table>
<thead>
<tr>
<th>PROTEIN</th>
<th>CHARACTERISTICS</th>
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<tbody>
<tr>
<td>Parvalbumin and its isoforms</td>
<td>this protein has divalent metal-binding sites available that can bind to Hg²⁺ ions</td>
</tr>
<tr>
<td>Ubiquitin-40S ribosomal protein S27a</td>
<td>zinc-finger metal binding domains. The zinc has many characteristics similar to those of mercury (weak acid)</td>
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<tr>
<td>Zinc finger and BTB domain containing protein 24</td>
<td>The presence of cysteine (weak base) in the peptide sequence may promote Hg²⁺ (weak acid) binding</td>
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<td>Dual specificity protein phosphatase 22-B</td>
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Conclusions

Using 2D-PAGE as a selective step in protein separation was confirmed to be very efficient, effectively separating many protein spots per gel. GFAAS could quantify the mercury present in the protein extracts, pellets and protein spots from both muscle and liver tissue. The identified protein spots by ESI-MS/MS that show biomarker characteristics can be used for monitoring, at a protein level, the toxic concentrations of mercury in fish species from the Amazon region.

Acknowledgments
